

## Setor 04. Inflamação/Inflammation

### 04.001

Effects of tacrolimus, a calcineurin inhibitor, in experimental periodontitis in rat. Coimbra LS<sup>1</sup>, Herrera BS<sup>1</sup>, Figueiredo MN<sup>1</sup>, Guimarães MR<sup>1</sup>, Nassar PO<sup>1</sup>, Andia DC<sup>1</sup>, Nassar CA<sup>1</sup>, Spolidório DMP<sup>1</sup>, Rossa Jr C<sup>2</sup>, Spolidório LC<sup>1</sup> <sup>1</sup>FOA-UNESP - Fisiologia e Patologia, <sup>2</sup>FOA-UNESP - Diagnóstico e Cirurgia

**Introduction:** Periodontitis is a leukocyte-mediated bone loss and inflammation with pathogenic features similar to those observed in other inflammatory diseases, such as arthritis. Since Tacrolimus, is an immunosuppressive drug used for the treatment of some cases of rheumatoid arthritis, we hypothesized that it may modulate periodontal disease. **Methods:** All the experimental protocols were approved by the local Ethics Committee for Animal Experimentation (number 26\2004). Periodontitis was induced in Wistar rats (male, 180-220 g) by placing a cotton ligature around the first lower molar tooth, half of the animals received Tacrolimus dissolved in the drinking water (1 mg/kg body weight) until being sacrificed, 5, 10, 15 and 30 days after the ligature procedure. Were collected, jaws for radiological evaluation, gingiva for activity of myeloperoxidase (MPO; a biochemical marker for neutrophil infiltration), and blood samples for differential white blood cells counts and levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ . **Results:** Tacrolimus treatment induced a significant decrease of alveolar bone loss and MPO activity in comparison with the control group in all time periods studied ( $p < 0.05$ ). Specifically, Tacrolimus suppressed the expression of serum interleukin (IL-1b), tumor necrosis factor (TNF- $\alpha$ ) and IL-6 ( $p < 0.001$ ) usually observed after the induction of periodontium inflammation. **Conclusions:** Tacrolimus treatment in periodontitis-induced in rats conferred protection against the inflammation-induced tissue and bone loss associated with periodontitis, through a mechanism involving IL-1b, TNF- $\alpha$  and IL-6. The mechanisms by which Tacrolimus prevented the increase in cytokine expression and minimized progression of periodontal disease will be explored in ongoing studies and may provide clues for host modulation therapies aiming at the prevention of tissue destruction associated with periodontal disease. Supported by FAPESP (04/09851-4)

#### 04.002

Effect of atorvastatin on 5-fluorouracil-induced oral mucositis in hamsters. Medeiros. C. ACX<sup>1</sup>, Figueiredo JG<sup>2</sup>, Macedo RN<sup>1</sup>, Bitencourt FS<sup>1</sup>, Barboza, DRMM.<sup>1</sup>, Castro MOO<sup>6</sup>, Leitão RFC<sup>3</sup>, Nogueira NAP<sup>6</sup>, Alencar NMN de<sup>1</sup>, Brito GAC<sup>1</sup> <sup>1</sup>UFC - Farmacologia, <sup>2</sup>UFC - Bioquímica e Biologia Molecular, <sup>6</sup>UFC - Análises Clínicas e Toxicológicas

**Introduction:** Oral mucositis (OM) is a frequent side effect in patients undergoing chemotherapy. It is characterized by intense inflammatory mucosal reaction and formation of ulcers in oropharyngeal cavity. We investigated the effect of atorvastatin (ATV) – cholesterol-lowering drug with anti-inflammatory activity - in oral mucositis induced by 5-fluorouracil (5-FU) in Golden hamsters. **Methods:** Surgical procedures were approved by local ethics committee for experimental animal use under number 016/07. Oral mucositis (OM) was induced by intraperitoneal (i.p.) administration of 5-FU in the first and second days of experiment (60 and 40 mg/kg i.p., respectively), with subsequent excoriations of the cheek pouch mucosa on the fourth day. The animals were pre-treated with i.p. ATV 1, 5 or 10 mg/kg or vehicle (saline and 5% vol/vol ethanol). In the fifth or tenth days, they were sacrificed. Samples of cheek pouches were removed for histopathological analysis, determination of cytokines level and for myeloperoxidase (MPO) assay. To verify a possible systemic repercussion, the animals were analyzed for the following: leukogram, biochemical parameters, bacteremia and the cytokine levels in the serum. **Results:** Our results showed that ATV reduced local inflammation, assessed by macroscopic and microscopic tissue analysis, as well as by levels of TNF- $\alpha$  and myeloperoxidase enzyme on the fifth day of OM. However, the association of ATV and 5-FU amplified the leukopenia of animals with OM, and increased aspartate-aminotransferase and alanine-aminotransferase serum levels. Along with these changes, we detected presence of Gram negative bacillus in the blood of the animals treated with ATV + 5-FU, and increased the serum level of IL-1  $\beta$  and IL-10 followed by reduction in their survival rates on the tenth day. **Discussion:** The protective effect of ATV on oral mucositis is consistent with literature which suggests anti-inflammatory effect for this drug. However, ATV amplified the leucopenia induced by 5-FU and the association ATV 5 or 10 mg/kg with 5-FU caused 100% mortality of animals on the tenth day of the experiment. This mortality is probably linked to septicemia and hepatotoxicity. These results suggest that the association of ATV and 5-FU deserves attention and further research in humans. **Supported by:** Funcap, Capes and CNPq.

#### 04.003

Diclofenaco não altera a hiporreatividade vascular induzida pela ETX à All e revertida pelo N $\omega$ NLA em ratos. Fracasso JF<sup>1</sup>, Rodrigues LA<sup>2</sup>, Dias Junior PP<sup>3</sup> <sup>1</sup>UNIFEB/PANT-FCFAR, <sup>2</sup>UNIFEB - Ciências Fisiológicas, <sup>3</sup>UNIFEB - Farmacologia

A sepsis é caracterizada por sinais e sintomas de hipotensão e hiporreatividade determinados pela liberação de fatores endoteliais endógenos em resposta a endotoxina (Etx) liberada das paredes bacterianas que podem levar à morte. No presente estudo avaliamos o efeito do Diclofenaco na redução dos efeitos hipertensores da angiotensina II (All) após tratamento com Etx, revertidos pelo N $\omega$ NLA em ratos. **Métodos:** Ratos Wistar (220-250 g), anestesiados com Nembutal (40 mg/kg, i.p.), tendo sido canulados: traquéia, veia jugular esquerda para injeção de drogas, carótida direita conectada a um transdutor de pressão e ligada a um fisiógrafo ANAMED para registro da Pressão Arterial Média (PAM). A PAM (mm de Hg) foi medida após injeção de 0,5 e 1,0 ( $\mu$ g/kg i.v.) antes (controle), 60 min. após Etx (5 mg/kg i.v.) e 60 min. após N $\omega$ NLA (25 mg/kg i.v.) em ratos com e sem pré-tratamento com Diclofenaco sódico [5 mg/kg] (i.p.) 60 min. antes dos experimentos. Os resultados estão apresentados como média  $\pm$  epm, analisados estatisticamente por ANOVA para medidas repetidas onde  $P \leq 0,05$  foram considerados significativos. **Resultados:** *Controle:* Basal [mm de Hg] (122,2  $\pm$  4,5); All (0,5  $\mu$ g/kg) (141,3  $\pm$  4,1); All (1,0  $\mu$ g/kg) (172,5  $\pm$  5,2) Após Etx: Basal (72,4  $\pm$  5,1); All (0,5  $\mu$ g/kg) (83,8  $\pm$  6,4); All (1,0  $\mu$ g/kg) (96,7  $\pm$  3,0) Após N $\omega$ NLA: Basal (118,4  $\pm$  4,8); All (0,5  $\mu$ g/kg) (142,9  $\pm$  5,3); All (1,0  $\mu$ g/kg) (171,5  $\pm$  6,1). *Após Diclofenaco:* Basal (124,1  $\pm$  6,0); All (0,5  $\mu$ g/kg) (142,2  $\pm$  5,9); All (1,0  $\mu$ g/kg) (176,2  $\pm$  4,1) Após Etx: Basal (75,4  $\pm$  3,2); All (0,5  $\mu$ g/kg) (78,3  $\pm$  4,1); All (1,0  $\mu$ g/kg) (94,1  $\pm$  5,5) Após N $\omega$ NLA: Basal (114,2  $\pm$  4,9); All (0,5  $\mu$ g/kg) (139,7  $\pm$  5,1); All (1,0  $\mu$ g/kg) (182,3  $\pm$  5,7). **Conclusão:** A hiporreatividade vascular à All causada pela Etx foi completamente revertida pelo N $\omega$ NLA, um inibidor da NO-sintase em animais controle. Nos animais pré-tratados com Diclofenaco (Anti-inflamatório não-esteroidal) a reversão da reatividade da All pelo N $\omega$ NLA ocorreu sem a interferência do mesmo na dose empregada, sugerindo o envolvimento da via L-arginina-NO no mecanismo de hiporreatividade vascular, não-afetado pelo Diclofenaco. **Apoio Financeiro:** FCF - Araraquara/UNESP

#### 04.004

The essential oil of *Croton zehntneri* and its main constituent, anethole, inhibit paw edema induced by bradykinin and serotonin in mice. Ponte EL<sup>1</sup>, Andrade ACS<sup>2</sup>, Pires AF<sup>2</sup>, Sousa PL<sup>2</sup>, Sousa AAS<sup>2</sup>, Pereira MG<sup>2</sup>, Oliveira AC<sup>3</sup>, Leal-Cardoso JH<sup>2</sup>, Coelho-de-Sousa, A. N.<sup>2</sup>, Assreuy AMS<sup>2</sup> <sup>1</sup>Faculdade Christus - Ciências Biomédicas, <sup>2</sup>UECE - Ciências Biomédicas, <sup>3</sup>UECE - Ciências da Saúde

**Introduction:** The genus *Croton* (Euphorbiaceae) is widely distributed, with main occurrence in tropical areas. Essential oils from this genus are sources of bioactive molecules, from which are obtained several compounds with diverse chemical groups of great therapeutic potential and use in folk medicine. The essential oil of *Croton zehntneri* (EOCz) possesses several constituents, including anethole and estragole. The EOCz chemical analysis revealed that anethole is its major constituent (78%). In our laboratory, it was demonstrated that EOCz and anethole inhibit the first phase of paw edema induced by carrageenan in mice, whose mediation occurs mainly by histamine, serotonin and bradykinin. Serotonin, released by activated mast cells and platelets, and bradykinin, a preformed mediator, also released by platelets, promote relaxation of vascular smooth muscles. It was investigated if the anti-inflammatory effect of EOCz and anethole involve release/action of bradykinin and serotonin in mice.

**Methods:** EOCz was obtained via hydrodistillation for it drags of steam of water and anethole was commercially obtained (Sigma<sup>®</sup>). Male Swiss mice (25-30g) were manipulated in accordance with principles recommended by our Institutional Ethical Committee (UECE N<sup>o</sup>. 0559924-4). Paw edema was induced by subcutaneous (s.c.) injection of bradykinin (3 mol/paw) or serotonin (100 g/paw) and measured immediately before subcutaneous injection of stimuli (zero time) and at selected time intervals (30min., 1-3h) thereafter by hydroplethysmometry. Control groups received sterile saline (NaCl 0.9%, 100 mL/100g; s.c.). Animals were orally treated with EOCz or anethole (10mg/kg), 1 hour before edema induction. Results were expressed as Mean  $\pm$  S.E.M. (n = 6-8) of the increase in paw volume (mL) calculated by subtracting the basal volume measured at zero time or in arbitrary units (area under the time-course curve - AUC). Data were analyzed by ANOVA followed by Bonferroni's test and p values less than 0.05 were considered significant. **Results and Discussion:** The paw edema induced by bradykinin was inhibited by EOCz (BK x EOCz AUC: 1762.5  $\pm$  223.9 x 750.0  $\pm$  164.3) about 57%, and anethole (BK X anethole AUC: 2231.2  $\pm$  364.0 x 900  $\pm$  157.8) in 59%. However, the paw edema induced by serotonin (AUC: 16693.7  $\pm$  1173.0) was inhibited only by anethole (AUC: 9150.0  $\pm$  844.2) in 45%. In conclusion, EOCz and anethole present anti-inflammatory activity. The EOCz activity occurs via inhibition of bradykinin (EOCz) and that of anethole probably involves participation of bradykinin and serotonin. **FUNDING:** CAPES, FUNCAP and CNPq.

#### 04.005

Late effects of intestinal I/R in lung inflammatory response. Putative involvement of VEGF and lymphatic system. Vitoretti LB, Breithaupt-Faloppa AC, Domingos HV, Sudo-Hayashi LS, Oliveira-Filho RM, Tavares de Lima W ICB-USP - Farmacologia

Abdominal trauma associated with intestinal ischemia/reperfusion (I/R) may affect distal organs, particularly the lungs, suggesting a causal link between injury-released mediators and the pulmonary dysfunction of Adult Respiratory Distress Syndrome (ARDS). In this context, the lymphatic system might constitute a path for such inflammatory mediators generated in the injured gut to approach the pulmonary microcirculation. Among the mediators, vascular endothelial growth factor (VEGF) was identified by its properties to increase permeability and act as a cellular growth factor, hence its potential for a key role in the pathogenesis of ARDS. Here we studied the profile of lung inflammation and the role of intestinal lymph after prolonged reperfusion periods in an intestinal I/R rat model. Upon anesthesia, male rats were subjected to occlusion of the superior mesenteric artery for 45 min and kept under reperfusion for 24, 72 or 120 h. In parallel, the thoracic lymphatic duct was obstructed before the ischemic procedure. Lung and intestinal myeloperoxidase activity (MPO) and the Evans Blue dye extravasation were determined. IL-10 and VEGF levels were also quantified in lung explant. We observed that, only after 120 h of intestinal I/R was found an increased lung MPO activity and lung and intestinal leakage compared with 24 and 72 h of reperfusion. In addition, the obstruction of the lymphatic duct after 120 h of intestinal I/R significantly decreased the lung microvascular permeability. IL-10 levels increased 24 and 72 h of intestinal I/R, with or not intact lymphatic duct. However, after 120 h of intestinal reperfusion the levels of IL-10 were not different of basal. VEGF levels 24 and 72 h after intestinal reperfusion were similar to basal, but significantly increased at 120 h of reperfusion, which were decreased by lymphatic duct obstruction. In conclusion, gut inflammatory mediators generated by intestinal I/R cause an acute and delayed inflammatory response. Endogenous control regulates the acute lung injury but fail to maintain the normal lung immunity that in turn mediates the decreased IL-10 and the increased VEGF levels, leading to the delayed lung inflammation. CEEA: Certificate n° 080/05. Supported by: FAPESP 07/56872-5; 05/02271-5; 07/07139-3 and CNPq 306526/2006-9

#### 04.006

Paradoxical effect of formaldehyde exposure on allergic lung inflammation. Lino dos Santos Franco A, Domingos HV, Ligeiro de Oliveira AP, Oliveira-Filho RM, Tavares de Lima W ICB-USP - Farmacologia

**Introduction:** Formaldehyde (FA) is a common indoor and outdoor pollutant that is found in many products including particle board, plywood, floor coverings, office furniture. FA cause lung inflammation and may impair the lung cell recruitment after an allergic stimulus. Considering the positive relationship between exposure to FA and asthma, we evaluated the release of cytokines from lung explants of allergic rats exposed earlier to FA inhalation. **Methods:** The rats were subdivided in 4 groups: FA, as rats subjected to FA inhalation (1%, 90min, 3days), FA/OVA, rats sensitized to Ovalbumin (OVA) after the last FA inhalation, OVA/OVA, rats OVA sensitized but not exposed to FA and Naive. The lung explants were carried out 24 h after FA or OVA challenge. Cytokines levels were quantified in samples of supernatants of lung explants in culture. **Results:** FA inhalation ( $17.1 \pm 2.0$ ) or the OVA-induced ( $20.9 \pm 2.8$ ) lung inflammation when compared to naïve group ( $6.1 \pm 1.1$ ). However, the interaction FA-OVA decreased the number of cells in BAL ( $7.39 \pm 0.9$ ). We observed increased levels of IL-1  $\beta$  and IL-6 in FA/OVA group ( $205.0 \pm 5.5$ ;  $1194.0 \pm 55.5$ ) when compared to FA ( $71.1 \pm 2.7$ ;  $544.0 \pm 30.5$ ) and OVA/OVA groups ( $175.0 \pm 13.4$ ;  $640.0 \pm 44.4$ ). **Conclusions:** In conclusion, the findings could suggest that the FA interferes with the cellular migration into lung after allergic challenge and modifies the functional status of alveolar macrophages and other cells present into the lungs. We put forward the view that FA changes the functional activity of lung cells contributing to the impaired cellular recruitment and to the increased generation of inflammatory mediators. **Acknowledgments:** FAPESP (2008/50766-1)

#### 04.007

Role of leukotriene B<sub>4</sub> and nitric oxide on tracheal hyperresponsiveness induced by formaldehyde exposure. Lino dos Santos Franco A, Breithaupt-Faloppa AC, Ligeiro de Oliveira AP, Domingos HV, Oliveira-Filho RM, Vargaftig BB, Tavares de Lima W ICB-USP - Farmacologia

**Introduction:** Formaldehyde (FA) use is widely disseminated in the society and a putative risk of its inhalation is a growing concern. FA exposure elicits direct irritation of upper and low airways and asthma deterioration. We reported that FA inhalation increased the lung inflammatory-cell influx and caused a bronchial hyporeactivity to methacholine (Lino dos Santos Franco *et al.*, 2006). Considering a wide high level of pollutants such as FA generated in industrialized countries and the putative risk of its exposure to airway homeostasis, in this study, we investigated the ex-vivo rat tracheal responsiveness, caused by methacholine (MCh) after distinct periods of FA-exposure (30min, 6h, 24-72h; at 0,1ppm). **Methods:** Tracheal ring was transferred to glass Petri dish containing DMEM (2ml) and FA (10 µl; 0.1 ppm) were added (FA group). As a control, isolated tracheal rings incubated with DMEM (naïve group) were used. The tracheal rings were maintained in humidified atmosphere (37°C, 5% CO<sub>2</sub> and 95% O<sub>2</sub>) for 30 min, 6, 24, 36, 48 and 72 hours. Tracheal rings were suspended in organ bath system and the isometric contractions to MCh were assessed. Next, cumulative dose-response curves to MCh were constructed according to Van Rossum (1963). **Results:** FA-exposure for 48 h caused an increased tracheal responsiveness to MCh not related to epithelium. Moreover, short-term (30 min and 6h) and 72 h of FA exposure elicited a tracheal responsiveness to MCh, similar to that one found in the trachea of the control group. Levels of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) significantly increased in the supernatant of FA-cultured trachea. Moreover, LTB<sub>4</sub>-antagonist receptor MK886 (1 µM), but not the TNF-α inhibitor pentoxiphylline (PTX, 1 µM) added to tracheal culture was able to prevent the FA-induced tracheal hyperresponsiveness. Yet, L-arginine, the precursor of nitric oxide generation, blunted the FA-induced tracheal hyperresponsiveness. **Conclusions:** Our data suggest that FA exposure triggers an increased airways contraction after the cholinergic response, which is mediated by activating the connective tissue mast cells that releases LTB<sub>4</sub> from a non-epithelial source. Aside, from that it might impair the NO generation. This study adds a new contribution to the understanding of FA-induced mechanisms related to activation structural upper airways cell. **Acknowledgments:** FAPESP (2008/50766-1).

#### 04.008

Lipopolysaccharide downregulates integrin necessary for hepatic neutrophil adhesion in a CD44/IL-10 dependent manner. Menezes GB<sup>1</sup>, Lee, WY<sup>2</sup>, Waterhouse, C<sup>2</sup>, Cara DC<sup>1</sup>, Kubes, P<sup>2</sup> <sup>1</sup>UFMG - Patologia Geral, <sup>2</sup>University of Calgary - Imunologia

**Introduction:** Hepatic neutrophil recruitment is a key feature in liver damage during infection and inflammation, and liver failure is one of the major causes of death during sepsis. Neutrophil response to local inflammatory stimulus in a previously inflamed liver is not well described in the literature and little is known about why neutrophil accumulation in the liver microvasculature during systemic inflammation occurs independently of integrins. **Methods:** In order to investigate mechanisms of hepatic neutrophil recruitment, we developed a novel surgical approach employing a fMLP-impregnated filter (1mm<sup>2</sup>) placed onto the liver surface, compared to intraperitoneal (IP) administration of LPS. Genetically manipulated mice strains (and blocking antibodies) were used to study the role of adhesion molecules and cytokines. Adhesion, crawling and morphology of GFP-expressing neutrophils within hepatic sinusoids were studied by using spinning disk confocal intravital microscopy. Neutrophils were harvested from blood and liver for flow cytometry studies and *in vitro* incubations of neutrophils with LPS and cytokines were performed to distinguish the differential effects on expression of adhesion molecules. **Results and Discussion:** Local administration of fMLP (2mcg/filter) caused neutrophil adhesion within 30 minutes of exposure with continued cell recruitment up to 2 hours. Highest numbers of cells were present immediately adjacent to the filter. 90% of neutrophils exhibited crawling movement and polarized cell shape. Adherent and crawling neutrophils were markedly reduced in ICAM1 and MAC1-deficient mice, and administration of anti-ICAM1 antibody one hour after fMLP exposure prevented any further increase in neutrophil adherence, indicating that this recruitment is dependent on ICAM1-MAC1 interaction. CD44<sup>-/-</sup> mice displayed no difference in adherence, suggesting that this molecule is not involved in local fMLP-induced neutrophil recruitment. However, systemic LPS injection (0.5mg/kg; 4h) induced a greater accumulation of neutrophils in sinusoids that was dependent on CD44. Integrins were not important in this model and no crawling was observed. Strikingly, local fMLP could not induce any new adhesion in CD44<sup>-/-</sup> mice treated IP with LPS, indicating that integrin dependent neutrophil adhesion was inhibited by LPS. Flow cytometry revealed that during LPS systemic inflammation, while MAC1 is upregulated in circulating neutrophils, it is downregulated in liver neutrophils. Downregulation of MAC1 in liver neutrophils was not observed in IL10<sup>-/-</sup> nor in CD44<sup>-/-</sup> mice. ELISA confirmed that IL-10 is highly expressed in liver and additionally, *in vitro* neutrophil incubation with IL-10 induced downregulation of MAC1 expression in LPS-stimulated neutrophils. **Conclusions:** We propose a novel mechanism by which neutrophils can use different adhesion molecules during local and systemic inflammation. Systemic LPS can prevent integrin-dependent neutrophil adhesion triggered by local inflammation possibly due to MAC1 downregulation driven by high IL-10 levels expressed during inflammation. **Support:** FAPEMIG/CIHR/AHFMR. **Animal Care Protocol:** MO8031 (UofC).



#### 04.009

Leukocytes increase the inhibitory effect of lipopolysaccharide (LPS) on platelet aggregation. Menuzzo CD, Pires MEL, Casarin AL, Cardelli NJA, Antunes E, Marcondes S FCM-UNICAMP - Farmacologia

**Introduction:** Sepsis is a condition associated with significant morbidity and mortality. Lipopolysaccharide (LPS) is a cell wall component of gram-negative bacteria and reproduces many of the manifestations of sepsis. The physiopathological responses to LPS involve activation of a number of cell types such as lymphocytes, neutrophils and platelets, causing the release of different inflammatory mediators and production of reactive oxygen and nitrogen species. Some works show that leukocytes interact and cross-talk with platelets in many settings including inflammation and vascular disorders. Therefore, the objective of the present study was to investigate the role of leukocytes as modulator of platelet reactivity in presence of LPS. **Methods:** The present study was approved by the Human Ethics Committee of State University of Campinas (UNICAMP) n° CEAA1711-1. Blood from abdominal aorta of male Wistar rats (250-320g) was collected in 3.8% sodium citrate (1:9 v/v). Two different experimental protocols have been carried out, as follows. In the first protocol, blood was centrifuged at 200g for 15 min to obtain platelet-rich plasma (PRP). The number of platelets was adjusted to  $2 \times 10^8$  platelets/ml with Krebs's solution, and platelet suspension was incubated with saline or LPS (300 mg/ml) for 1 or 4 h. Next, PRP was centrifuged (200 g, 15 min), and aggregation assays were carried out using ADP-activated platelets. In the second experimental protocol, blood was centrifuged at 50g for 15 min to obtain leukocytes. The leukocyte layer was separated, and the remaining blood was centrifuged at 200 g for 15 min to obtain PRP. Next, the leukocyte suspension was added to the PRP, and incubated with saline or LPS (300 mg/ml) for 1 or 4 h. The cell suspension was centrifuged again at 200 g for 15 min, and the supernatant (PRP) was used to aggregation assays using ADP-activated platelets. **Results:** Pre-incubation of platelets with leukocytes ( $5.0 \times 10^5$  cells/ml) for 1 or 4 h slightly increased ADP (5  $\mu$ M)-induced platelet aggregation (1 h:  $84 \pm 2\%$  and  $67 \pm 3\%$ ; at 4 h:  $61 \pm 7\%$  and  $52 \pm 1\%$  aggregation, when the platelets were pre-incubated with leukocytes or not, respectively). Incubation of platelets with LPS for 1 h reduced by 57% the platelet aggregation ( $P < 0.05$ ); however, at 4-h incubation, the inhibitory effect of LPS was significantly smaller (11% reduction). In the second experimental protocol, pre-incubation of platelets with LPS and leukocytes for 4 h markedly inhibited ADP (5 mM)-induced platelet aggregation (52% reduction). In addition, ADP (5 mM)-induced platelet aggregation was abolished by LPS when platelets were pre-incubated with leukocytes for 1 h. **Conclusion:** Our studies show that incubation of platelets with LPS and leukocytes increases the inhibitory effect of LPS on platelet aggregation. These results indicate that LPS induces the formation of substance(s) in leukocytes that inhibits platelet activation. The contribution of nitric oxide for this effect is under current investigation. **Supported by:** CAPES

#### 04.010

Anti-inflammatory effects of pioglitazone in a murine model of pleurisy induced by carrageenan. Reis G.O<sup>1</sup>, Buss ZS<sup>1</sup>, Medeiros YS<sup>2</sup>, Frode TS<sup>1</sup> <sup>1</sup>UFSC - Análises Clínicas, <sup>2</sup>UFSC - Farmacologia

**Introduction:** Peroxisome proliferator-activated receptors are ligand-activated transcription factors of the nuclear receptor family related to glucose homeostasis, lipid metabolism, cell proliferation, and inflammatory responses. This study investigated the effects of Pioglitazone (PIO) upon: leukocyte migration, exudation, myeloperoxidase(MPO) and adenosine-deaminase(ADA) activities in the mouse model of pleurisy induced by carrageenan. **Methods:** Non-fasted adult Swiss mice, 18-20 g, were used throughout the experiments. Pleurisy was induced by intrapleural injection of 0.1mL of carrageenan(Cg, 1%) (Saleh, *Br. J. Pharmacol*, v. 118, p.811, 1996). The inflammatory response was analyzed after 4h and 48h. In the first phase (4h) different groups of animals were treated 0.5h prior pleurisy with PIO (10-50 mg/kg) administered by oral route (p.o.). Another group of animals were pre-treated with one dose of PIO (20mg/kg) administered at different intervals point (0.5-4h) before Cg. The inflammation was analyzed after 4h. In the second phase (48h) three groups of mice were separated as follows: **1)** mice treated with one dose of PIO (20mg/kg) administered 0.5h prior to Cg; **2)** mice treated with two doses of PIO (20 mg/kg) administered 0.5h prior to Cg and the second dose at 24h of interval time after the first dose; **3)** mice treated with four doses of PIO (20mg/kg) administered at 0.5h prior to Cg followed by other three doses administered at 12h of interval time after the first dose. The inflammatory parameters were evaluated after 48h. Animals were previously challenged (1h prior) with Evans blue dye (25mg/kg) administered by intravenous route (i.v.) in order to evaluate the exudation. Statistical analysis of the data was performed by two-way analysis of variance (ANOVA) followed by Student's t test. A level of  $P < 0.05$  was accepted as statistical significant. The study was approved by Ethical Committee for use of Animals in Research (**CEUA-UFSC: protocol-PP00181**). **Results and Discussion:** In the first phase (4h) PIO (20 and 50mg/kg) decreased leukocytes by  $44.5 \pm 5.8\%$  and  $44.4 \pm 6.6\%$ , and neutrophils by  $46.5 \pm 6.0\%$  and  $45.4 \pm 7.6\%$ , respectively ( $P < 0.01$ ). The time course profile study demonstrated that PIO (20 mg/kg) was effective in inhibited the inflammation only when it was given 0.5h before Cg. PIO (20mg/kg) inhibited MPO by  $42.6 \pm 12.6\%$  and ADA activities  $34.7 \pm 7.9\%$  ( $P < 0.05$ ). On the late phase (48h) PIO (20mg/kg) administered with two or four doses reduced leukocytes by  $47.5 \pm 2.2\%$  and  $44.5 \pm 6.3\%$ , respectively ( $P < 0.01$ ) and mononuclears by  $49.6 \pm 1.2\%$  and  $45.9 \pm 4.0\%$ , respectively ( $P < 0.05$ ). PIO (20mg/kg) administered twice at 24h of interval time also inhibited ADA activity by  $45.2 \pm 6.2\%$  ( $P < 0.01$ ). PIO didn't inhibited exudation in this inflammatory response induced by carrageenan ( $P > 0.05$ ). **Conclusion:** PIO demonstrated important anti-inflammatory effect by inhibiting leukocyte migration. This effect was associated with the MPO and ADA activities decrease once these enzymes are considered markers of activated neutrophils and mononuclears, respectively. **Financial Support:** CAPES, CNPq and PIBIC-UFSC.

#### 04.011

Involvement of cytokines on central nervous system during *Tityus serrulatus* venom (Tsv)-induced fever. Malvar DC, Pessini AC, Martins JM, Figueiredo MJ, Souza GEP FCFRP-USP - Física e Química

**Introduction:** *Tityus serrulatus* scorpion is considered one of the most dangerous species for humans in Brazil, responsible for many clinical cases of envenomation in the southern region of the country. Its venom contains several basic proteins of low molecular weight with neurotoxic activity<sup>1</sup>. It has been shown that Tsv causes an integrated febrile response which was inhibited by peripheral treatment with nitric oxide synthase inhibitors, B<sub>1</sub> and B<sub>2</sub> kinin receptors antagonist and IL-1 receptors antagonist (IL-1ra)<sup>2</sup>. Furthermore, intraperitoneal (i.p.) injection of Tsv induces increase of important pyrogenic cytokines in serum, IL-1 $\alpha$  and  $\beta$ , IL-6 and TNF- $\alpha$ <sup>3</sup>. However, the roles of these cytokines on central nervous system were not evaluated yet. Thus, the aim of this study was to evaluate the involvement of IL-1, IL-6 and TNF- $\alpha$  on central nervous system during Tsv-induced fever. For that, Tsv-induced fever was evaluated on animals pre-treated with IL-1ra, anti-IL-6 antibody or soluble receptor of TNF- $\alpha$  (srTNF- $\alpha$ ). **Methods:** Desiccated Tsv was purchased from Phoneutria Biotechnology and Services (Belo Horizonte, Brazil) and kept at -20 °C until use. Rectal temperature (°C) of male Wistar rats (180-200g, n=4-7) was measured every 30 minutes for up 6h by teletelemetry. Rats received vehicle (saline), IL-1ra (200  $\mu$ g), anti-IL-6 (5  $\mu$ g) or srTNF- $\alpha$  (500ng) intracerebroventricular (i.c.v.) 15 min before the i.p. injection (0.5ml) of Tsv (150mg kg<sup>-1</sup>). Control animals received vehicle (saline), IL-1ra, anti-IL-6 or srTNF- $\alpha$ , at the same route and doses, 15 min before the i.c.v. injection of IL-1 $\beta$  (3,2ng), IL-6 (300ng) or TNF- $\alpha$  (250ng), respectively, or i.p. saline (ethical commission protocol n° 200/2008 – CETEA/FMRP-USP). **Result:** Tsv increased body temperature from 30min, peaked after 2h by nearly 2.5°C, and decreased thereafter, reaching basal values after approximately 4.5h. Pre-treatment with anti-IL-6 (2h, Saline/Tsv: 2.2  $\pm$  0.1 °C; anti-IL-6/Tsv: 1.4  $\pm$  0.2 °C), but not IL-1ra (2h, Saline/Tsv: 2.8  $\pm$  0.1 °C; IL-1ra/Tsv: 2.7  $\pm$  0.3 °C) or srTNF- $\alpha$  (2h, Saline/Tsv: 2.8  $\pm$  0.1 °C; srTNF- $\alpha$ /Tsv: 3.1  $\pm$  0.1 °C), showed a significantly reduction on Tsv-induced fever. In the control animals, IL-1ra, anti-IL-6 or srTNF- $\alpha$  abolished the fever induced by IL-1 $\beta$ , IL-6 or TNF- $\alpha$ , respectively. The i.c.v. administration of IL-1ra, anti-IL-6, srTNF- $\alpha$  or saline did not alter the basal rectal temperature. **Discussion.** These results indicate that IL-6, but not IL-1 and TNF- $\alpha$ , had an important role on central nervous system during the Tsv-induced fever. Probably, the relevant role of IL-1 on Tsv-induced fever observed in our previous study<sup>1</sup> result from its peripheral effect. 1. Becerril B. *Toxicon* 35:821, 1997. 2. Pessini AC. *Toxicon*. 48:556, 2006. 3. Pessini AC. *Toxicon*. 3:765, 2003. **Financial support:** CNPq, FAPESP.

#### 04.012

Hydrogen sulfide reduces carrageenan-dependent joint inflammation through a caspase-1 deficiency and increased levels of IL-10 dependent mechanism. Ekundi-Valentim E, Teixeira SA, Barreto MAA, Moreira, DF, Belizário JE, Muscará MN, Costa SKP USP-ICB - Farmacologia

**Introduction:** we showed that exogenous supply of H<sub>2</sub>S in the rat knee joint allowed a significant reduction of carrageenan (CGN)-induced acute arthritis signs and symptoms, although did not prevent the upregulation of iNOS, which may contribute to the pathogenesis of arthritis<sup>1</sup>. We have now furthered this study in order to investigate the mechanisms possibly involved in the protective effect of H<sub>2</sub>S in the CGN-induced arthritis in the rat knee joint. **Methods:** male Wistar rats (180 - 200 g) were used in this study. Experimental procedures were in accordance with our Institutional Ethics Committee (protocol n° 64, page n° 46, book 2/2007). Animals were subjected, under halothane anesthesia, to intra articular injection (i.art.) of 3% CGN or saline (50µl; control group). Prior CGN injection ( -60 min), the inhibitor of H<sub>2</sub>S formation, DL-propargylglycine (PAG; 53 µmol/knee joint) or an H<sub>2</sub>S donor Lawson's reagent (LR; 3.6 µmol/knee joint) was injected i.art. in the rat knee. A control group was treated with the non-selective cyclooxygenase inhibitor indomethacin (Indo; 6 mg/kg; i.p.; - 60 min). Four hours after CGN injection, functional assays in addition to inflammatory biomarkers (e.g. leukocyte influx, cytokines) and both IL-1b converting enzyme (caspase-1) and inducible nitric oxide synthase (iNOS) activities were performed in the rat knee. Data are mean ± SEM. Stats were performed by ANOVA *plus* Bonferroni's *t*-test; P<0.05 was taken as significant. **Results:** functional assays revealed that the ipsilateral (IPSI) knee of vehicle-treated rats exhibited a potent oedema associated with pain scored behavior, high contents of myeloperoxidase (MPO) and iNOS. These effects were associated with a significant influx of leukocytes ( $4 \pm 1$  cells  $\times 10^6$ /cavity), increased production of IL-1b ( $16.3 \pm 3.8$  pg/ml), reduced levels of IL-10 ( $41 \pm 6$  pg/ml) and increased activity of caspase-1 ( $0.026 \pm 0.003$  Arbitrary U/sec-) as compared to non-arthritic rats ( $0.010 \pm 0.001$  Arbitrary U/sec-). Treatments with LR or Indo caused a significant reduction of oedema, pain score, MPO activity, leukocyte influx ( $0.7 \pm 0.1^{***}$  and  $1.0 \pm 0.2^{**}$  cells  $\times 10^6$ /cavity for LR and indometacin respectively; n=7-8), IL-1b production ( $7.16 \pm 1.15^*$  and  $6.75 \pm 30^*$  pg/ml for LR and Indo respectively; n=5-7) and caspase-1 activity ( $0.0160 \pm 0.0008^*$  and  $0.0180 \pm 0.0006$  Arbitrary Unit/sec- for LR and indo respectively; n= 5-6). In contrast, increased concentration of IL-10 was found in synovial fluid of treated animals ( $86 \pm 10^*$  and  $214 \pm 19^{***}$  pg/ml for LR and Indo, respectively; n=5-6). PAG treatment had no effect on CGN-induced inflammation and biomarkers, but potentiated the activity of synovial iNOS (not shown; n= 4). **Discussion:** These results reveal for the first time a role for caspase-1 inhibition in parallel with increased levels of IL-10 as major components of the mechanism by which exogenous supply of H<sub>2</sub>S protect against CGN-induced arthritis in the rat knee. **References:** 1. Ekundi-Valentim, E. *et al.*, In: EPHAR, Fundamental & Clinical Pharmacology, 2008. v. 22. p. 37-45. **Acknowledgments:** \*recipient of a grant from University Agostinho Neto, Angola. We thank FAPESP and CNPq (Brazil) for financial support.

#### 04.013

The apoptosis clearance signal phosphatidylserine inhibits leukocyte migration and promotes inflammation resolution *in vivo*. Sordi R, Bet AC, Della Justina, AM, Ramos GC, Balsanelli, LS, Assreuy J UFSC - Farmacologia

Phagocytosis of apoptotic leukocytes appears to be a *sine qua non* condition to trigger inflammation resolution and it takes place mainly by the recognition of phosphatidylserine (PS) residues on the external surface of apoptotic cells. For this reason, herein we explored the effects of PS-containing liposomes, an apoptosis clearance signal, in the leukocyte migration profile in a mouse air pouch model of inflammation induced by carrageenan. We studied the murine air pouch model of inflammation induced by carrageenan. Total and differential cell counts were performed kinetically in order to evaluate the effects of PS-containing liposomes (100 mg/kg, i.p.). Furthermore, we investigate if PS treatment could be triggering other anti-inflammatory mediator release. Thus, the peritoneal cavity fluid of PS treated, non-inflamed, animals were collected and injected in animals with an ongoing inflammatory process. The nature of such possible liposome-induced soluble mediators (LISM) was also investigated. Phosphatidylcholine (PC) liposomes were also evaluated. All procedures were approved by our Institutional Ethics Committee (PP003/CEUA-UFSC) and are in accordance with NIH Animal Care Guidelines. Administration of PS, but not PC, liposomes reduced the PMN and MN leukocyte influx into inflamed pouches in a dose-dependent manner. The PS treatment also abbreviated the inflammation length. Interestingly, PS may act indirectly, triggering the secretion of soluble mediators that could be transferred from donor mice. The LISM treatment is directly dependent on the presence of peritoneal macrophages and is susceptible to heat or trypsin degradation or cycloheximide treatment. PS-containing liposomes promote reduction of PMN influx by triggering anti-inflammatory autacoids release, which seems to be of protein nature and are produced *de novo* after PS exposition. **Financial support:** CAPES, CNPq, FAPESC and PRONEX.

#### 04.014

Lectin from *Canavalia boliviana* reduces rolling and adhesion of neutrophils: involvement of cytokines. Luz PB<sup>1</sup>, Figueiredo JG<sup>2</sup>, Bitencourt FS<sup>1</sup>, Carmo JRF<sup>1</sup>, Gonçalves IB<sup>1</sup>, Talbot J<sup>3</sup>, Teixeira CS<sup>2</sup>, Cunha FQ<sup>3</sup>, Cavada BS<sup>2</sup>, Alencar NMN de<sup>1</sup>  
<sup>1</sup>UFC - Fisiologia e Farmacologia, <sup>2</sup>UFC - Bioquímica e Biologia Molecular, <sup>3</sup>FMRP-USP - Farmacologia

**Introduction:** Lectins are (glyco)proteins that can recognize and reversibly bind to carbohydrates or other substances derived from sugars and are encountered throughout animal and plant kingdoms. Several biological activities of plant lectins have been described, including pro-inflammatory and anti-inflammatory effects. *Canavalia boliviana* (Leguminosae – Papilionoideae) was collected from the Amazon Rain forest located in the Amazon state - Brazil. The aim of the present work was to investigate the anti-inflammatory activity, not exploited yet, of a lectin isolated from the seeds *Canavalia boliviana* (CboL) in peritonitis model through its role on leucocyte-endothelium interaction and production/involvement of cytokines (interleukin-1 $\beta$ : IL-1 $\beta$ ; tumor necrosis factor-alpha: TNF- $\alpha$ ; interleukin-10: IL-10) *in vivo*. **Methods:** Animal handling and experimental protocols were registered on the Institutional Ethics Committee under number 57/2009. Mice (n=6) were treated by saline (i.v.) or CboL (1, 5 and 10 mg/kg; i.v.) after 30 minutes peritonitis was induced by injection of carrageenan (Cg; 500 $\mu$ g). Four hours after the peritonitis induction, cells in peritoneal cavity were counted and expressed as mean  $\pm$  S.E.M cells $\times 10^6$ /ml. The leukocyte rolling (2<sup>nd</sup> hour) and adhesion (4<sup>th</sup> hour) were examined by intravital microscopy and cytokines production (TNF- $\alpha$ , IL- $\beta$  and IL-10) were determined in the serum - 4<sup>th</sup> hour; the results were expressed as picograms/ml of cytokine. **Results and Discussion:** CboL reduced the number of neutrophils to 1.69 $\pm$ 0.6, 1.64 $\pm$ 0.4 and 1.16 $\pm$ 0.5 at doses 1, 5 and 10 mg/kg, respectively (control group=3.71 $\pm$ 0.9). CboL (1, 5 and 10mg/kg) decreased rolling and adhesion (27.18 $\pm$ 5.2; 10.47 $\pm$ 3.2 and 6.9 $\pm$ 2.2 leukocytes rolling/10 $\mu$ m/min, 1.80 $\pm$ 0.1; 0.86 $\pm$ 0.2 and 0.67 $\pm$ 0.2 adherent cells/100  $\mu^2$ , respectively) - control group=44.5 $\pm$ 8.9 and 2.6 $\pm$ 0.6, respectively. The lectin decreased the production of TNF- $\alpha$  (68.9 $\pm$ 10.4), IL-1 $\beta$  (92.5 $\pm$ 9.4) and IL-10 (290 $\pm$ 16.4) when compared to control (116.5 $\pm$ 7.0, 137.3 $\pm$ 12.0 and 201.4 $\pm$ 41.9 pg/mL per group respectively). CboL presents anti-inflammatory activity which could be explained by decrease rolling and adhesion of the leukocytes on endothelium with also via inhibition of cytokines production.

#### 04.015

Phagocytic and microbicidal activity of alveolar macrophages from rats with allergic lung inflammation - modulation by leukotrienes. Silva RC<sup>1</sup>, Câmara NOS<sup>2</sup>, Landgraf RG<sup>2</sup> <sup>1</sup>UNIFESP - Nefrologia, <sup>2</sup>ICB-USP - Imunologia

**Introduction/Objective:** It is well documented that leukotrienes (LTs) are released in allergic lung inflammation and that they participate in the physiopathology of asthma. A role for LTs in innate immunity has emerged more recently: LTB<sub>4</sub> and cysLTs were shown to enhance the FcγR -mediated phagocytosis by alveolar macrophages (AMs) (Mancuso et al., *Infect Immun* 66:5140-6, 1998). Thus, using a rat model of asthma we evaluated the AMs for FcγR -mediated phagocytosis and killing of *Klebsiella pneumoniae* in comparison with AMs from normal rats. The effect of treatment with a cys-LTs antagonist (montelukast) on AMs function was also investigated. **Methods:** Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Biomedical Sciences Institute/USP–Ethical Committee for Animal Research (CEEA – 141/2006). Male Wistar rats (6 animals/group) were immunized twice with OVA/alumen intraperitoneally, and challenged with OVA aerosol. After 24h, the animals were killed and the AMs obtained by bronchoalveolar lavage. AMs were cultured with IgG opsonized red blood cells (50:1) and phagocytosis was evaluated after 2 h. AMs were cultured with IgG opsonized *Klebsiella pneumoniae* (1:30) and bacteria viability evaluated after 4 h. Leukotriene C<sub>4</sub> and nitric oxide were quantified by ELISA and Griess method respectively. Montelukast was given 30 min before the antigen challenge. **Results:** The results obtained showed that alveolar macrophages taken from sensitized and challenged rats, present markedly increased phagocytic capacity via Fcγ R (10X compared to controls) as well as killing of *K.pneumoniae* (4X higher than controls). The increased phagocytosis was inhibited 15X and killing 3X by treatment of the rats with montelukast in comparison with the non-treated group. Addition of LTB<sub>4</sub> or cys-LTs increased phagocytosis in control AMs but has no effect on AMs from allergic lungs. The BAL levels of LTB<sub>4</sub> and LTC<sub>4</sub> were 30 ng/ml and 8 ng/ml, respectively and of nitric oxide was 5 μmol/ml . Montelukast reduced nitric oxide and LTC<sub>4</sub> in 39% and 73%, respectively. **Conclusion** These results suggest that LTs produced during allergic lung inflammation potentiate the capacity of alveolar macrophages to phagocyte and to kill *K. pneumonia* via FcγR. **Financial support:** FAPESP and CNPq

#### 04.016

Intrauterine undernourishment reduced lymphocyte migration and pulmonary fibrosis in rats. Landgraf MAV<sup>1</sup>, Landgraf RG<sup>2</sup>, Silva RC<sup>3</sup>, Câmara NOS<sup>2</sup>, Fortes ZB<sup>1</sup> <sup>1</sup>ICB-USP - Farmacologia, <sup>2</sup>ICB-USP - Imunologia, <sup>3</sup>UNIFESP - Nefrologia

**Introduction/Objectives:** Experimental and epidemiological dates have show that malnutrition predisposes individuals to infections. Immune responses are compromised, particularly in undernourished children. Asthma is a chronic inflammatory disease which involves structural changes in the lung, which are collectively called airway remodeling. One of the major events in this process is the local activation of fibroblasts to myofibroblasts. These cells synthesize type I collagen which accumulates around bronchi and small blood vessels. The present study aimed to investigate the lymphocyte migration, collagen tissue deposition and mucus production in undernourishment rats submitted to allergic lung inflammation. **Methods:** Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Biomedical Sciences Institute/USP–Ethical Committee for Animal Research (CEEA – 129/2007). Male Wistar rats (8-9 wk of age) that had been undernourished *in utero* by feeding their dams 50% less food than that consumed by control dams and were immunized intraperitoneally with ovalbumin in alumen and challenged with ovalbumin given by aerosol (5 animals/group). Bronchoalveolar lavage was performed and the lungs were examined one day after the last challenge. Lung sections were stained with Picrosirius or PAS to determine the levels of collagen or mucus, respectively, by morphometric analysis and expressed as the mean of the area. **Results:** Intrauterine undernourishment rats immunized and challenge were presented significantly reduced in total cell (40%), eosinophils (43%), lymphocytes TCD<sub>4</sub> (57%) and Tγδ (41%) in bronchoalveolar lavage fluid. Besides, collagen deposition in the peribronchial area (mm<sup>2</sup>x10<sup>3</sup>) was also significantly reduced (31%) and mucus production was reduced in 62%. **Conclusion:** These results indicate that intrauterine undernourishment rats presented reduced allergic lung inflammation and pulmonary fibrosis. **Financial support:** FAPESP and CNPq.



#### 04.017

*In vitro* evaluation of superoxide radicals scavenging activity of rutin and metal-rutin complexes. Miyashiro CAHV UNIBAN - Farmacologia Experimental

**Introduction:** The flavonoids have a high antioxidant and anti-inflammatory activities. Besides, recent researches demonstrate that the antioxidant activity of flavonoids is believed to increase when they are coordinated with transition metal ions. Reactive oxygen species (ROS) are implicated in the etiology of several inflammatory processes. **Methods:** The antioxidant effect of the transition metal-Rutin complex can be evaluated by an assay *in vitro*, which measures the activity of radical superoxide scavenging, dispersed in the environment. Among the evaluated composites are Cu-Rutin, Fe-Rutin, Ni-Rutin complexes and Rutin itself, and they are tested in the concentrations of 1, 10 and 100  $\mu\text{M}$ . This assay was submitted to the spectrophotometric monitoring of the reduction of nitrobluetetrazolium (NBT), by means of the observation of formazan crystals formation on the presence of the superoxide radicals ( $\text{O}_2^-$ ), one of a kind reactive oxygen species (ROS). **Results:** The results showed that the percentage of superoxide radicals scavenging by Rutin, Cu-Rutin, Fe-Rutin, Ni-Rutin complexes are, respectively: 9.7%, 0.9%, 0.5%, 0.0%, at the concentration of 1 $\mu\text{M}$ ; 7.8%, 46.4%, 11.0%, 16.4% at the concentration of 10 $\mu\text{M}$ ; and 51.8%, 71.3%, 52.6%, 51.8%, at the concentration of 100  $\mu\text{M}$ . **Discussion:** The results demonstrated that the Cu-Rutin complex presented the highest superoxide radicals scavenging effect, on the proposed assay. It presented a significantly high activity ( $P < 0.05$ ) at 10 and 100 $\mu\text{M}$  concentrations, when compared to the Rutin itself. These findings give support to news *in vitro* and *in vivo* researches including anti-inflammatory activities. **Financial Support:** UNIBAN and FAPESP.

#### 04.018

Kinins and nitric oxide, but not prostaglandins, are involved in the oedematogenic responses induced by *tityus serrulatus* scorpion venom in rat paws. Pessini AC<sup>1</sup>; Kanashiro A<sup>1</sup>; Malvar DC<sup>2</sup>; Machado RR<sup>1</sup>; Soares DM<sup>4</sup>; Figueiredo, M. M.<sup>1</sup>; Kalapothakis E<sup>6</sup>; Souza GEP<sup>1</sup> <sup>1</sup>FCFRP - USP Física e Química; <sup>2</sup>UFRRJ - Ciências Fisiológicas; <sup>4</sup>USP - Farmacologia; <sup>5</sup>UFMG - Biologia Geral

**Introduction:** *Tityus serrulatus* venom (Tsv) is a mixture of short neurotoxic proteins that affect the ion permeability of excitable cells through specific interaction with Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>+</sup> or Cl<sup>-</sup> channels<sup>(1)</sup>. We have found that intraperitoneal injection of Tsv was able to induce a characteristic systemic and local inflammatory reaction, which is revealed by an integrated thermoregulatory response, i.e. fever, that seems to depend on kinins (via B<sub>1</sub> and B<sub>2</sub> receptors), IL-1, nitric oxide and vagal neurotransmission<sup>(2)</sup>. The present study investigated the role of kinins, prostaglandins (PGs) and nitric oxide (NO) in paw oedema after intraplantar (ipl) injection of Tsv in rats. **Methods:** Desiccated *T. serrulatus* scorpion venom was purchased from Phoneutria Biotechnology and Services (Belo Horizonte, Brazil) and kept at -20 °C until use. The paw oedema induced by Tsv was evaluated in male Wistar rats by plethysmometer method during 6 h. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations in conscious animals set by the Ethical Committee of University of São Paulo, Campus of Ribeirão Preto, SP, protocol number 07.1.90.53.9. **Results:** The ipl. injection of Tsv (0.1 to 10 mg/paw) elicited a dose-and time-dependent paw oedema that started at 15 min, peaked at the 30 min and decreased thereafter, being absent 5 h later. The maximal oedema formation was observed 15 min after Tsv injection of 10 mg/paw and this dose was selected for the remaining experiments. The prior treatment of animals with B<sub>1</sub> (DALBK, 100 nmol/paw) or B<sub>2</sub> receptor antagonist, (icatibant, 10 nmol/paw) significantly inhibited Tsv-induced paw oedema (DALBK: 53.0±0.95%; 59.1±1.1%; 40.7±1.0 %; 55.2±1.0 % at 15, 30, 60 and 120 min, respectively). Icatibant 26.3±0.7%; 37.4±1.2% at 15 and 30 min time-points, respectively). Celecoxib, a selective COX-2 inhibitor (5 mg/kg, p.o. 1 h prior) significantly inhibited at 30 and 60 min (percent of inhibition: 35.3±1.0%; 82.4±1.0 %, respectively) while indomethacin, a non-selective COX inhibitor (5 mg/kg, i.p. 30 min prior) does not affect the Tsv-induced paw oedema. L-NAME (50 mg/kg, i.p.30 min prior), a non-selective nitric oxide synthase inhibitor, significantly reduced the initial phase of Tsv-induced paw oedema (percent of inhibition: 67.3±1.0% and 63.4±1.0 % at 15 and 30 min, respectively). **Discussion:** Tsv injected ipl into the rat paw, causes dose-dependent paw oedema in which kinins (via B<sub>1</sub> and B<sub>2</sub> receptors) and NO are substantially involved. The COX-2 blockage by celecoxib resulting in oedema reduction cannot be discarded; however, the lack of effect of indomethacin mismatches this hypothesis. So, it is possible that inhibition of NFκB transmigration<sup>(3)</sup> may be involved in the anti-oedematogenic effect of celecoxib. <sup>(1)</sup>Becerril. B., *Toxicon*, v. **35**, pp. 821–835, 1997. <sup>(2)</sup> Pessini, AC, *Toxicon*, v.48(5), p.556, 2006. <sup>(3)</sup> Takada, Y., *Oncogene* v.23 (57), p.9247, 2004.

#### 04.019

CINC-1 is involved in fever induced by LPS in rats. Yamashiro LH<sup>1</sup>, Soares DM<sup>2</sup>, Bertozzi G<sup>2</sup>, Cunha FQ<sup>2</sup>, Teixeira MM<sup>3</sup>, Souza GEP<sup>1</sup> <sup>1</sup>FCFRP - Física e Química, <sup>2</sup>FMRP-USP - Farmacologia, <sup>3</sup>UFMG – Imunologia

**Introduction:** We showed before that CINC (cytokine induced neutrophil chemokine)-1, a CXC chemokine, centrally administered promotes an integrated febrile response along with an increase in the prostaglandin PGE<sub>2</sub> content in the cerebrospinal fluid (CSF) of rats. Moreover, treatment of animals with different antipyretic drugs (indomethacin, ibuprofen and celecoxib) or with anti-CINC antibody reduced or even abolished the febrile response induced by CINC-1<sup>(1)</sup>. Therefore, the aim of this study was to investigate the involvement of CINC-1 in fever induced by LPS and the effectiveness of reparixin, a rat CXCR2 inhibitor in this response. **Methods:** LPS (5 µg/kg) was injected intravenously in male Wistar rats (200g). Control animals received saline (Sal). CSF, hypothalamus, liver and blood (plasma) were collected from the animals at 1, 2.5 and 5<sup>th</sup> hour for CINC-1 dosage by ELISA. Rectal temperature was measured before the LPS injection and prior the collection time. Reparixin 25, 75, 150 and 300 ng per animal, was intracerebroventricularly (i.c.v.) injected just before LPS (5 µg/kg, i.v.). Rectal temperature was measured by telethermometry every 30 min for up 6h. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations in conscious animals set by the Ethical Committee of University of São Paulo, Campus of Ribeirão Preto, SP, protocol number 050/2008. **Results:** The i.v. injection of LPS induced fever starting at the 2.5<sup>nd</sup> hour and increased the CINC-1 in all investigated sites (see table 1). Reparixin, at dose of 300 ng, i.c.v. injected diminished the febrile response induced by i.v. injected LPS (3.5<sup>rd</sup> h: LPS + Sal= 1.69 ± 0.06; LPS + reparixin 300ng= 1.19 ± 0.12).

**Table 1. Fever and CINC-1 concentration at different sites after i.v. injection of LPS in rats.**

Time		1h	2.5h	5h
ΔT (°C)	Sal	0.07 ± 0.07	0.13 ± 0.24	0.1 ± 0.21
	LPS	0.02 ± 0.08	1.5 ± 0.07	1.39 ± 0.1
Hypothalamus (pg/g)	Sal	139.8 ± 29.3	58.4 ± 58.4	41.2 ± 41.2
	LPS	2713.9 ± 441.2	2329.1 ± 187.8	1426.0 ± 95.1
CSF (pg/ml)	Sal	0 ± 0	7.5 ± 7.5	0 ± 0
	LPS	720.9 ± 11.4	846.8 ± 6.8	613.9 ± 43.4
Liver (pg/g)	Sal	1907.0 ± 610.4	3345.0 ± 420.0	1978.7 ± 316.2
	LPS	5596.4 ± 579.1	6735.0 ± 471.2	6522.5 ± 485.9
Plasma (pg/ml)	Sal	183.0 ± 60.0	302.8 ± 131.0	210.5 ± 136.5
	LPS	847.6 ± 54.2	796.9 ± 32.5	327.9 ± 54.9

**Discussion:** These results show that febrile response induced by i.v. injection of LPS was accompanied by an increase of CINC-1 in the hypothalamus, CSF, liver and plasma and that reparixin, a rat CXCR2 inhibitor, reduced the fever induced by this stimulus. These findings support our previous hypothesis that CINC-1 acts as an endogenous pyrogen in the rat<sup>(1)</sup>. **Support:** FAPESP. (1) Soares et al., *Brain Research*, v.1233, p.79, 2008.

#### 04.020

Improved micro assay for glycosaminoglycans by use of dimethylmethylene blue assay in patellar cartilage in murine antigen- and zymosan-induced arthritis. Nascimento PGBD, Grespan R., Lemos HP, Cunha FQ FMRP - Farmacologia

**Introduction:** The dimethylmethylene blue (DMMB) assay for sulphated glycosaminoglycans (GAGs) has found wide acceptance as a simple technique of measuring the GAGs content of tissues and fluids. This article describes a modified form of the DMMB assay to quantitative the GAGs presented in patellar cartilage in a microtiter plate, useful as a quick method to evaluate efficacy of treatments in rheumatoid arthritis. The pharmacological efficacy of non-steroidal anti-inflammatory drugs (diclofenac and indomethacin) to prevent the GAG loss by matrix degradation in a model of articular arthritic lesion is also shown. **Methods:** *Animals:* BALB/c mice weighing 18-22g were housed in temperature-controlled rooms (22-25°C) and received water and food *ad libitum*. *Induction of antigen-induced arthritis:* BALB/c mice were sensitized with 500 mg of methylated bovine serum albumin (mBSA – Sigma Chemical Co., St Louis, MO) in 0.2 ml of an emulsion containing 0.1 ml phosphate-buffered saline (PBS) and 0.1 ml complete Freud's adjuvant (CFA; Sigma) through subcutaneous (s.c.) injection. Booster injections of mBSA dissolved in incomplete Freud's adjuvant (IFA; Sigma) were given seven and fourteen days after the first immunization. Twenty-one days after the initial injection, arthritis was induced in the immunized animals by intra-articular (i.a.) injection of mBSA (100 mg/cavity) dissolved in 10ml PBS. Five days later, the mice were re-challenged with the same dose of mBSA. The patellas were collected 5 days later to measure GAGs as described below. (Licença da Comissão de Ética em Experimentação Animal: 158/2005). *Induction of zymosan-induced arthritis:* BALB/c mice were challenged with 180 mg of zymosan (Sigma Chemical Co., St Louis, MO) dissolved in 10ml PBS. Two days later, the patellas were collected to measure GAGs as described below. *Preparation of DMMB solution:* 1,9-Dimethylmethylene blue was purchased from Polysciences, Inc. (no. 03610). Chondroitin 4-sulphate sodium salt utilized as standard was purchased from Biochemika (no. 27045). Other reagents were of analytic grade. The color reagent was prepared adding 64 mg of DMMB in 1 L of water containing 3.04 g of glycine, 3.04 g of NaCl and 95 mL of 0.1 M HCl, to give a solution at pH 3.0 and  $A_{525}=0.31$ . *Preparation of biological samples for the DMMB assay:* Patellae were fixed in 10% formalin overnight, decalcified in formic acid (5%) for 4 hours, and subsequently digested overnight at 60 °C in 60 µl of 10 mg/ml papain (type IV; Sigma) in 0.1 M sodium acetate, pH 6.5, 10 mM L-cysteine, 50 mM disodium EDTA per patella. After the digestion, the samples were centrifuged at 1500 rpm for 10 s and were ready for the GAG measurement. *Spectroscopic determination of sulfated GAG:* The essay was calibrated by use of reagent blanks, and standards containing up to 5 µg of chondroitin sulfate in the same solvent as the samples. 50 µL of each sample, was placed in a 96-well microplate (n=3) and 250 µL of DMMB solution was added to each well with a multi-channel pipette. The measurement was realized 30 s after the color reagent addition, at 525 nm in a Microplate Spectrophotometer System - SpectraMax (Molecular Devices). *Results & Discussion:* We were able to induce a femur-tibial inflammatory lesion that led to GAG content loss from the extra-cellular matrix (experimental groups that received saline as inflammatory challenge had 1.6-2.4 µg of GAG as average values, the groups challenged with mBSA had 0.4-1.0 µg of GAG) and revert the lesion extend by daily treatment with potassium diclofenac and indomethacin, anti-inflammatory drugs (the treatment was able to prevent about 50% of the GAG loss). The linear range of absorbance for GAG is 1-10 µg. We employed the Chondroitin-6-sulphate as GAG standard. **Financial Support:** FAPESP, CNPq (pnpd 151681/2008-2)

#### 04.021

Endogenous ATP via P2X7 receptors mediates mechanical hyperalgesia induced by TNF- $\alpha$ , IL-6, CINC-1 and dopamine, but not by bradykinin, IL-1 $\beta$  and PGE<sub>2</sub>. Teixeira JM<sup>1</sup>, Oliveira MC<sup>2</sup>, Parada CA<sup>2</sup>, Tambeli CH<sup>1</sup> <sup>1</sup>FOP-UNICAMP - Physiology, <sup>2</sup>IB-UNICAMP - Physiology and Biophysics

**Introduction:** Recently we demonstrated the involvement of P2X7 receptors in the development of inflammatory hyperalgesia induced by carrageenan (unpublished data). The aim of this study was to verify whether the activation of P2X7 receptors by endogenous ATP contributes to mechanical hyperalgesia induced by the main inflammatory mediators involved in the mechanical hyperalgesia induced by carrageenan (Ferreira SH, Agents Actions, 38 Spec No:C7-9, 1993). For this, the antagonists of the P2X7 receptor, oATP and A-438079, were co-administered with each of the inflammatory mediators: bradykinin, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, CINC-1, PGE<sub>2</sub> or dopamine. **Methods:** All experimental procedures were previously approved by the Ethics Committee in animal research at the State University of Campinas (protocol number: 1389-1). The hyperalgesic responses were quantified by a pressure analgesimeter Randal Sellito (Oliveira MC, Pain, 141(1-2):127, 2009) 3 h after the administrations of each inflammatory mediator in the subcutaneous tissue of rat's hind paw. **Results:** Bradykinin (1.5 $\mu$ g/paw, Means $\pm$ SEM: 35.5 $\pm$ 3.6 n=6), TNF- $\alpha$  (0.8pg/paw, 26.1 $\pm$ 4.1 n=6), IL-1 $\beta$  (0.15 e 1.5pg/paw, 22.2 $\pm$ 3.3 n=6 e 25.0 $\pm$ 3.7 n=6, respectively), IL-6 (0.1ng/paw, 31.1 $\pm$ 3.8 n=6), CINC-1 (1.0pg/paw, 26.0 $\pm$ 2.5 n=5), PGE<sub>2</sub> (0.1 $\mu$ g/paw, 27.3 $\pm$ 2.3 n=5) or dopamine (10 $\mu$ g/paw, 34.4 $\pm$ 3.0 n=6) induced a dose-related mechanical hyperalgesia 3 h after its administration in the subcutaneous tissue of rat's paw (p<0.05, Tukey test). The co-administration of oATP (6.0 $\mu$ g/paw) or A-438079 (300 $\mu$ g/paw) significantly reduced (p<0.05, Tukey test) the mechanical hyperalgesia induced by TNF- $\alpha$  (0.8pg/paw) (10.6 $\pm$ 1.9 n=5; 8.0 $\pm$ 2.0 n=5, respectively), IL-6 (0.1ng/paw) (2.0 $\pm$ 1.1 n=5; 1.3 $\pm$ 2.0 n=5, respectively), CINC-1 (1.0pg/paw) (7.3 $\pm$ 2.8 n=5; 0.6 $\pm$ 0.6 n=5, respectively) or dopamine (10 $\mu$ g/paw) (1.3 $\pm$ 0.7 n=5; 2.0 $\pm$ 1.1 n=5, respectively) however, it did not affect (p>0.05, Tukey test) the hyperalgesic response induced by bradykinin (1.5 $\mu$ g/paw) (29.1 $\pm$ 3.8 n=5; 24.6 $\pm$ 4.1 n=5, respectively), IL-1 $\beta$  (1.5pg/paw) (24.0 $\pm$ 4.7 n=5; 22.0 $\pm$ 2.0 n=5, respectively) or PGE<sub>2</sub> (0.1 $\mu$ g/paw) (24.6 $\pm$ 4.4 n=5; 21.3 $\pm$ 3.8 n=5, respectively). **Discussion:** These results indicate that the mechanical hyperalgesia induced by the inflammatory mediators TNF- $\alpha$ , IL-6, CINC-1 and dopamine, but not by bradykinin, IL-1 $\beta$  and PGE<sub>2</sub> in the subcutaneous tissue of the rat's paw is mediated by activation of P2X7 receptors. **Financial Support:** CNPq

#### 04.022

Activation of P2X7 receptors induces mechanical hyperalgesia by indirect mechanisms. Teixeira JM<sup>1</sup>, Oliveira MC<sup>2</sup>, Parada CA<sup>2</sup>, Tambeli CH<sup>1</sup> <sup>1</sup>FOP-UNICAMP - Physiology, <sup>2</sup>IB-UNICAMP - Physiology and Biophysics,

**Introduction:** Considering the involvement of P2X7 receptors in inflammatory pain (Fulgenzi A, *Int J Immunopathol Pharmacol*, 21(1): 61, 2008), the aim of this study was to verify whether the administration of the P2X7 receptor agonist BzATP in the subcutaneous tissue of rat's hind paw induces mechanical hyperalgesia, and if so, to study the mechanism by which this hyperalgesic response is developed. **Methods:** All experimental procedures were previously approved by the Ethics Committee in animal research at the State University of Campinas (protocol number: 1389-1). BzATP was administered in the subcutaneous tissue of rat's hind paw and behavioral responses were quantified after  $1/2$ , 1, 2, 3, 6 and 24h by a pressure analgesimeter Randal Sellito (Oliveira MC, *Pain*, 141(1-2):127, 2009). To assess whether the mechanical hyperalgesia induced by BzATP is mediated by bradykinin, by sympathomimetic amines, by prostaglandin and/or by the neutrophils migration, the bradykinin B1 (DALBK) or B2 (Bradyzide) receptor antagonists, the  $\beta$ 1- (Atenolol) or  $\beta$ 2- (ICI 118,551) adrenoceptor antagonists, the cyclooxygenase inhibitor (indomethacin) or the nonspecific selectin inhibitor (fucoidan), respectively, was co-administered with BzATP. **Results:** Subcutaneous administration of BzATP (25 $\mu$ g/paw) in the dorsum of the rat's hind paw induced a significant mechanical hyperalgesia  $1/2$  and 1h (Means $\pm$ SEM: 25.3 $\pm$ 4.6 n=5; 40.0 $\pm$ 5.8 n=5, respectively, p<0.05, Tukey test), but not 2, 3, 6 and 24h (10.6 $\pm$ 3.7 n=5; 5.0 $\pm$ 1.4 n=5; 3.3 $\pm$ 2.4 n=5; -0.6 $\pm$ 1.1 n=5, respectively, p>0.05, Tukey test) after its administration. BzATP (25, 75 e 225 $\mu$ g/paw, 1h) induced a dose-related mechanical hyperalgesia (40.0 $\pm$ 5.8 n=5; 32.0 $\pm$ 3.4 n=5; 34.1 $\pm$ 4.4 n=5, respectively, p<0.05, Tukey test). Co-administration of DALBK (3.0 $\mu$ g/paw), Bradyzide (0.5 $\mu$ g/ paw), Atenolol (6.0 $\mu$ g/ paw) or ICI 118,551 (1.5 $\mu$ g/ paw) significantly reduced the mechanical hyperalgesia induced by BzATP (0.8 $\pm$ 1.8, n=5; 6.6 $\pm$ 3.5, n=5; 6.6 $\pm$ 2.8, n=5; 2.6 $\pm$ 3.4, n=5, respectively, p<0.05, Tukey test). The pre-treatment with indomethacin (100 $\mu$ g/paw, s.c., 30 min) or fucoidan (25mg/kg, i.v., 20 min) significantly reduced the mechanical hyperalgesia induced by BzATP (4.1 $\pm$ 4.3, n=5; 10.6 $\pm$ 2.3, n=5; p<0.05, Tukey test and T test, respectively). DALBK (3.0 $\mu$ g/paw), Bradyzide (0.5 $\mu$ g/paw), Atenolol (6.0 $\mu$ g/paw), ICI 118,551 (1.5 $\mu$ g/paw) or indomethacin (100 $\mu$ g/paw) did not affect the mechanical hyperalgesia induced by BzATP (33.3 $\pm$ 3.5, n=4; 34.1 $\pm$ 4.7, n=4; 32.6 $\pm$ 1.7, n=5; 30.6 $\pm$ 3.4, n=5; 33.3 $\pm$ 3.9, n=4, respectively, p>0.05, T test) when administered in the contralateral paw. **Discussion:** These results suggest that the P2X7 receptors activation by BzATP induces mechanical hyperalgesia by an indirect action on the primary afferent nociceptors in the subcutaneous tissue of the rat's paw mediated by bradykinin, sympathomimetic amines, prostaglandins and neutrophils migration. **Financial Support:** CNPq

#### 04.023

Characterization of the anti-inflammatory properties from latex of *Hancornia speciosa* Gomes (Mangabeira). Marinho DG<sup>1</sup>, Alviano DS<sup>2</sup>, Alviano CS<sup>2</sup>, Matheus ME<sup>1</sup>, Fernandes PD<sup>1</sup> <sup>1</sup>ICB-UFRJ, <sup>2</sup>UFRJ - Microbiologia

**Introduction:** The mangabeira (*Hancornia speciosa* Gomes) is a tree belonging to the family Apocinaceae, with natural occurrence through Brazil, but with more incidences in litoral regions of Northeast. Its latex has a popular use and present medicinal properties, with several uses such as adjunct in the treatment of tuberculosis, ulcers, dermatoses and warts. The latex is also used as plaster to treat wry neck, contusions, and inflammatory process in joints. The objectives of this work were to evaluate and confirm the anti-inflammatory properties from the latex of *H. speciosa*. **Methods:** The use of animals in this work was approved by the ethical committee of animal experimentation from Centro de Ciências da Saúde (UFRJ), and received the number DFBC015. Male Swiss mice (20-25g, n=6-8) were used in the acetic acid (2%, intraperitoneal) induced abdominal contortions and in the licking response induced by formalin (2.5%, intraplantar). Wistar rats (150-180g, n=5-7) were used in paw edema induced by intraplantar injection of 0.1mL of serotonin (2.5µg), histamine (300µg), bradykinin (10µg), or carrageenan (1mg). Animals received oral administration of *H. speciosa* latex at doses of 0.01 to 1.3mg/kg 1h before experiments. **Results:** Pre-treatment of mice with 0.06, 0.6, and 1.3mg/kg of latex inhibited in 44%, 70%, and 72.5% the acetic acid-induced contortions (80.5±4.76 in vehicle-treated group vs 44.7±7.1; 24±9.3; and 22.2±6.2, respectively). Pre-treatment of mice with 0.1, 0.6, and 1.3mg/kg of latex inhibited in 0%, 38.2%, and 48.8% the 2<sup>nd</sup> phase of licking response induced by formalin (186.6±15.9 in vehicle-treated group vs 190.7±10.4; 115.3±3.1; and 95.6±3.9, respectively). Pre-treatment of rats with 0.1, 0.6, or 1.3mg/kg of the latex significantly and dose dependent inhibited the serotonin-induced paw edema (424±58.7 in vehicle-treated group vs 515.3±51.6; 314.4±29.5; and 281.9± 38.9), the bradykinin-induced paw edema (446.1±67.2 in vehicle-treated group vs 425.0±83.4; 441.0±76.1; and 246.9±90.5, respectively), and the 4<sup>th</sup> hour of carrageenan-induced paw edema (648.8±64.0 in vehicle-treated group vs 353.2±114.8; 479.4±100.2; and 268.3±83.6, respectively). But did not affected the histamine-induced paw edema in mice pre-treated with 0.6, and 1.3mg/kg of latex (343.0±51.0 in vehicle-treated group vs 301.4± 55.1; and 245.3±48.8, respectively). **Discussion:** Latex from *H. speciosa* showed a significant anti-inflammatory activity in doses smaller than 0.5 mg/kg. This effect was observed in all models tested. The results obtained suggest that part of the inhibitory effect observed with the latex was due to inhibition of receptors for serotonin and bradykinin. A more pronounced inhibitory effect was observed with edema induce by serotonin. However, the most important inhibitory activity could be noted 4h after carrageenan injection in rat paw, suggesting that the anti-edematogenic activity of *H. speciosa* latex may be acting through inhibition of cyclooxygenases pathway. **Financial support:** CAPES, CNPq e FAPERJ.

#### 04.024

The role of platelet-activating factor (PAF) in an experimental periodontal disease by *Aggregatibacter actinomycetemcomitans* in a murine model. Madeira MFM<sup>1</sup>, Silva TA<sup>2</sup>, Correa JD<sup>3</sup>, Mitre GC<sup>1</sup>, Campi PS<sup>1</sup>, Souza DG<sup>4</sup> <sup>1</sup>UFMG - Microbiologia, <sup>2</sup>UFMG - Patologia, Clínica e Cirurgia Odontológicas, <sup>3</sup>UFMG - Farmacologia, <sup>4</sup>UFMG - Bioquímica e Imunologia

**Introduction:** Periodontal disease (PD) is a chronic inflammatory disease of the attachment structures of the teeth. The bacterial biofilm attached to the surface of the tooth in close association with periodontal tissues, is the etiologic factor of this disease. *Aggregatibacter actinomycetemcomitans* is a small nonmotile periodontopathogen, a gram-negative coccobacillus with a range of potential virulence factors. PAF, or platelet-activating factor, possesses a wide spectrum of potent pro-inflammatory actions. The levels of PAF in mixed saliva or in gingival crevicular fluid and tissues are significantly increased during oral inflammatory conditions such as periodontitis, thus may participate in pathophysiologic events during the course of oral inflammation.

**Objective:** In the present work, our aim was to assess the role of PAF in the experimental model of periodontal disease induced by *A. actinomycetemcomitans*.

**Methods:** BALB/c wild type (WT) or PAFR deficient mice (PAFR<sup>-/-</sup>) received a direct injection of  $1 \times 10^9$  CFU/mL of *A. actinomycetemcomitans* strain FDC Y4 (diluted in 10  $\mu$ L of PBS) into palatal gingival tissue of second molar for the establishment of periodontal disease. Immediately after this injection, it was performed an oral administration of the same inoculum (diluted in 100  $\mu$ L of PBS with 1,5% of carboxymethylcellulose) and the administration was repeated 48 and 96 hours later. Negative controls received only PBS (NI). Tissues were analyzed by ELISA, myeloperoxidase content, and histology, in order to evaluate the levels of cytokines and chemokines, neutrophil influx, inflammatory infiltrate and alveolar bone loss.

**Results:** WT mice presented increase of TNF- $\alpha$  production after 15 days of infection (NI:  $17.59 \pm 5.05$ ;  $84.45 \pm 7.09$  pg/100mg of tissue) and of neutrophil recruitment after 45 days (NI:  $0.05 \pm 0.004$ ;  $0.17 \pm 0.016$  relative unit). Furthermore, this infection was followed by important alveolar bone loss, leukocytes migration to periodontal tissues and gingival epithelium hyperplasia. PAFR<sup>-/-</sup> mice presented a significant ( $p < 0,05$ ) increase of neutrophil influx and CXCL1 production on 30<sup>th</sup> days post-infection (pi) when compared to WT mice. Interestingly, the production of CXCL1 in PAFR<sup>-/-</sup> mice was higher on 60<sup>th</sup> days pi ( $p < 0,05$ ). TNF in PAFR<sup>-/-</sup> mice was as significant as in WT mice on 30<sup>th</sup> days pi ( $p < 0,05$ ). After 60 days of infection, the measure of bone loss showed a more significant bone loss in WT mice ( $p < 0,05$ ). **Conclusion:** PAF may play an important role in the modulation of the disease since PAFR<sup>-/-</sup> mice although had more neutrophil influx had no bone loss. **Supported by CNPq**



#### 04.025

Rosiglitazone prevents inflammatory periodontal bone loss by inhibiting RANKL expression. Da Silva-Filho VJ<sup>1</sup>, Campos-Júnior JC<sup>1</sup>, Hassumi MY<sup>1</sup>, Vieira SM<sup>2</sup>, Cunha FQ<sup>3</sup>, Alves JB<sup>1</sup>, Gonçalves RB<sup>4</sup>, Napimoga MH<sup>5</sup> <sup>1</sup>UNIUBE - Biopatologia e Biologia Molecular, <sup>2</sup>COPE-INPA, <sup>3</sup>FMRP-USP, <sup>4</sup>FOP-UNICAMP - Microbiologia e Imunologia, <sup>5</sup>UNIUBE - Biologia Celular e Molecular

**Introduction:** Rosiglitazone (RGZ), an oral anti-hyperglycemic agent used for non-insulin-dependent diabetes mellitus, is a high-affinity synthetic agonist for peroxisome proliferator-activated receptor-g (PPAR-g). Both *in vitro* and *in vivo* experiments have also revealed that RGZ possesses anti-inflammatory properties. Therefore, in the present study, we investigated the anti-inflammatory effects of RGZ in a rat model of periodontal disease induced by ligature placed around the mandible first molars of each animal. **Methods:** All experimental procedures were approved by the Ethical Committee for Animal Research of the University of Uberaba (#002/2008) Male Wister rats were divided into four groups: 1) animals without ligature placement receiving administration of empty vehicle (control); 2) animals with ligature receiving administration of empty vehicle; 3) animals with ligature receiving administration with oral RGZ (10 mg/kg/day); and 4) animals with ligature receiving administration of subcutaneous RGZ (10 mg/kg/day). Thirty days after induction of periodontal disease, the animals were sacrificed, and mandibles and gingival tissues were removed for further analysis. **Results:** Histomorphological and immunohistochemical analyses of periodontal tissue demonstrated that RGZ-treated animals presented decreased bone resorption, along with reduced RANKL expression, compared to those animals with ligature, but treated with empty vehicle. **Discussion/Conclusion:** These data indicated that RGZ may suppress the bone resorption by inhibiting RANKL-mediated osteoclastogenesis elicited during the course of experimental periodontitis in rats. **Financial Support.** PAPE/FAPEMIG 2008/002

#### 04.026

Avaliação da atividade anti-inflamatória do extrato etanólico obtido a partir do rizoma da *Typha domingensis Pers.* Ferreira JA, Oliveira C, Toma MA UNISANTA - Ciências e Tecnologia

**Introdução:** *typha domingensis Pers* é uma erva da família Typhaceae nativa da América do Sul que pode ser encontrada durante o ano inteiro na região de Ilha Comprida-SP. Conhecida como Taboa vem sendo utilizada popularmente como imunossupressora, antidiarréica e anti-inflamatória (Quin *et al.*, 2005). Estudos fitoquímicos qualitativos prévios demonstram a suposta presença de flavonóides no extrato etanólico do rizoma da Taboa, fato que pode conferir à mesma uma provável atividade anti-inflamatória. Esse trabalho objetiva avaliar a atividade anti-inflamatória aguda e crônica do extrato etanólico de *Typha domingensis Pers.* **Métodos:** Os rizomas de *Typha domingensis Pers* foram coletados na cidade de Ilha Comprida-SP, identificados no Herbário da Universidade Santa Cecília e submetidos à maceração com etanol absoluto de acordo com Baratelli, 2006. A identificação de flavonóides na amostra foi realizada através das reações com NaOH, FeCl<sub>3</sub> e Shinoda. Foram utilizados nos dois experimentos ratos Wistar, machos, pesando 200-220g. Grupos experimentais: *Controle negativo* (n=8; 10 ml/kg de salina 0.9%), *Controle positivo* (n=8; 100 mg/kg de Piroxicam) e *Extrato Thypha* (n=8; 100 ml/kg). Para a inflamação aguda foi utilizado o modelo de edema de pata induzido por administração de formol 1% de acordo com Henriques *et al.*, 1987. Na inflamação crônica foi realizado teste de granuloma cotton pellet segundo Dhananjayan, 2003. Tais procedimentos foram submetidos e autorizados pelo Comitê de Ética em Pesquisa Universidade Santa Cecília sob protocolo n° 42/09. **Resultados:** Os resultados do experimento mostraram que os animais do grupo extrato apresentaram uma diminuição significativa da inflamação aguda (\*\* $p < 0.001$ ) e crônica (\* $p < 0.05$ ) se comparados ao grupo controle negativo e sem diferença significativa ao grupo piroxicam ( $p > 0.05$ ). **Discussão:** Os resultados demonstraram que o extrato etanólico da *Typha domingensis Pers* obteve atividade anti-inflamatória. Postulam-se neste trabalho que tal atividade deva-se à presença de flavonóides, compostos com ampla atividade antioxidante e conseqüentemente anti-inflamatória (Silva, 2002). Tais resultados servem como suporte para a sequência futura do trabalho, na tentativa de elucidar os prováveis mecanismos envolvidos neste processo. **Bibliografia:** 1) Baratelli, Dissertação de mestrado curso de pós-graduação Química de Produtos Naturais da UFRJ, 2006; 2) Dhananjayan, *Indian J. Pharmacol*, 35: 388, 2003; 3) Henriques, *et al.*, *Braz. J. Méd. Biol. Res*, 20:243, 1987; 4) Qin *et al.*, *Ethnopharmacol*, 102, 424, 2005; 5) Silva, *Medicina, Ribeirão Preto*, 35: 127, 2002

#### 04.027

Short exposure of neonatal mice to 1,2-naphthoquinone activates CD11c<sup>+</sup> dendritic cells and amplifies the inflammatory effects of allergen sensitization at a late stage of life. Santos KT<sup>1</sup>, Florenzano J<sup>1</sup>, Peron JPS<sup>2</sup>, Ligeiro de Oliveira AP<sup>1</sup>, Favaro RR<sup>3</sup>, Rizzo LV<sup>2</sup>, Tavares de Lima W<sup>1</sup>, Muscará MN<sup>1</sup>, Costa SKP<sup>1</sup> <sup>1</sup>ICB-USP - Farmacologia, <sup>2</sup>ICB-USP - Imunologia, <sup>3</sup>ICB-USP - Biologia Celular e do Desenvolvimento

**Introduction:** In infants, exposure to particulate air pollution (e.g. diesel exhaust particles; DEP) is associated with lung illnesses<sup>1</sup>. Previously, we have attempted to model the environment of a child born in a polluted metropolitan area, in which DEP contaminant, 1,2-naphthoquinone (1,2-NQ), is one of the major contributors, and found that neonatal immune responses to 1,2-NQ increased susceptibility of mice to asthma at an adult stage<sup>2</sup>. We have now furthered this study in order to investigate the mechanisms possibly involved in the deterioration of asthma features by 1,2-NQ.

**Methods:** Neonatal mice were nebulized with 1,2-NQ (100 nM; 10 mL) or corresponding vehicle (PBS:Tween 80:DMSO) at days 6, 8 and 10 for 15 min. Eight weeks later, animals were sensitized/challenged with ovalbumin (OVA). Bronchial hyperresponsiveness (BHR) via enhanced pause (Penh) technique and inflammatory biomarkers were evaluated 24 h after last exposure to OVA. Data are mean  $\pm$  SEM. Stats were performed by ANOVA followed by Bonferroni's *t*-test or unpaired *t*-test.  $P < 0.05$  was taken as significant. **Results:** The short exposure of neonate mice to 1,2-NQ enhanced allergen-induced pulmonary and systemic inflammation as well as serum IgE at an adult stage, but failed to affect BHR when compared to mice exposed to OVA only. The exposure of mice to both 1,2-NQ and OVA increased pulmonary production of INF-g ( $142 \pm 16$  pg/ml;  $n=7$ ) and Th2 cytokines: IL-5 ( $13 \pm 1$  pg/ml;  $n=6$ ) and IL-13 ( $21 \pm 2$  pg/ml;  $n=5$ ), but significantly less of IL-5 ( $10 \pm 1$  pg/ml;  $n=6$ ), IL-13 ( $12 \pm 1$  pg/ml;  $n=5$ ) and INF-g ( $88 \pm 9$  pg/ml;  $n=4$ ) was observed in allergic mice only. Mice exposed to pollutant and OVA revealed a significant reduction in the pulmonary levels of pro-inflammatory cytokines TNF- $\alpha$  ( $112 \pm 10$  pg/ml;  $n=6$ ) and IL-1 $\beta$  ( $75 \pm 6$  pg/ml;  $n=7$ ) when compared to allergic group ( $180 \pm 20$  and  $140 \pm 10$  pg/ml for TNF- $\alpha$  and IL-1 $\beta$ , respectively;  $n=5-7$ ). Exposure to the pollutant 1,2-NQ also increased uptake of CD11c<sup>+</sup> dendritic cells (DCs;  $2.6 \pm 0.1$  % cells;  $n=7$ ) when compared to allergic group only ( $1.50 \pm 0.03$  % cells;  $n=6$ ). **Discussion:** Short exposure of neonate mice to 1,2-NQ is sufficient to promote increased susceptibility of asthma risk at an adult stage. This study provides the first report that pro-oxidative 1,2-NQ chemical can interfere with the immunological responses on CD11c<sup>+</sup> DCs activation and Th1 and Th2-promoting response pathways in the lung, thus suggesting a novel explanation for the adjuvant effect of 1,2-NQ on pulmonary illnesses (e.g. asthma) in children exposed to air pollution such as DEP. **References:** 1. Delfino, R.J. et al. *Ann Allergy Asthma Immunol.* 102(2):138-44;2009; 2. Santos, KT. et al. 40° Congresso SBFTE: p. 36;2008. **Ethics approval:** experiments were conducted under a protocol approved by our Institutional Ethics Committee (n. 113, book 2, page 51). **Acknowledgments:** CNPq and Fapesp for financial support. Mrs. Barreto MA for technical support.

#### 04.028

Heme oxygenase mediates the impairment of neutrophil to the lung during severe sepsis induced by pneumonia. Czaikoski PG<sup>1</sup>, Nascimento DCB<sup>2</sup>, Spiller F<sup>1</sup>, Sonogo F<sup>1</sup>, Cunha FQ<sup>1</sup> <sup>1</sup>FMRP-USP - Pharmacology, <sup>2</sup>FMRP-USP - Immunology

**Background:** Sepsis is the main cause of mortality in intensive care units and represents a critical health problem that continues to increase in importance. The primary sources of infection influence both the risk and the outcome of sepsis and pneumonia is a leading source of sepsis. *Klebsiella pneumoniae* is a gram negative bacteria responsible for a significant proportion of hospital-acquired infections and septicemias. This pathogen is clinically important because of high tendency to develop antibiotic resistance, such as resistance to carbapenems. The host defense against lung bacterial infection requires the efficient recruitment of neutrophils to infectious focus. In this context, heme oxygenase (HO) is an enzyme that catalyzes the degradation of heme into carbon monoxide (CO), biliverdin and free iron, and their activity is known to down-regulate the neutrophil migration. In the present study we evaluated the role of HO on neutrophil migration and infection control during severe sepsis induced by pneumonia. **Methods:** This experimental protocol was approved (n°. 027/2009) by COBEA of FMRP - USP. A sepsis model induced by pneumonia was standardized in our laboratory. C57BL/6 male mice (18-22g) were subjected to severe (SS) and mild sepsis (MS) by intratracheal administration of different number of *Klebsiella pneumoniae*. A SS mice group was pretreated with HO-1 specific inhibitor (ZnPP IX). The animals were killed 6 h after bacteria administration and alveolar neutrophil migration, pulmonar parenchyma neutrophil sequestration and bacterial count in lung and blood was evaluated. **Results:** We observed a failure of neutrophil migration towards alveoli and neutrophil sequestration into pulmonar parenchyma in SS mice, when compared to MS mice. As a consequence SS mice presented high levels of bacterial on alveoli and blood. However, the pre-treatment of the SS mice with ZnPP IX partially restored the neutrophil migration to the alveoli, decreased the lung neutrophil sequestration and improved the infection control. **Conclusion:** These results point to HO-1 could mediate the neutrophil migration failure to the lung and consequently impaired bacterial control during SS induced by pneumonia. Financial support: FAPESP, CAPES, FAEPA.

#### 04.029

Evaluation of the anti-inflammatory effect in rats treated with yogurt containing extract of mate tea (*Ilex paraguariensis* St. Hill.). Loch CR<sup>1</sup>, Ril TF<sup>2</sup>, Cichoski AJ<sup>3</sup>, Valduga AT<sup>4</sup>, Macedo SMD<sup>5</sup> <sup>1</sup>URI - Saúde Humana, <sup>2</sup>URI - Ciências da Saúde, <sup>3</sup>URI - Ciências Exatas e da Terra, <sup>4</sup>URI - Ciências Agrárias, <sup>5</sup>USP - Análises Clínicas e Toxicológicas

**Introduction:** When a tissue lesion happens, several reactions happen, in order to destroy or limit the spread of harmful agents. Inflammation can occur due to the lesion, which is considered to be a characteristic response of vascularized tissue to an aggressive agent, evidenced by the output of liquid and blood cells to the interstitium. Some plant extracts have therapeutic effect, and they are being used against the inflammation. Erva-mate has been studied in the last ten years because it promotes beneficial effects on the human health, being used as mate and tea. This study aimed to evaluate the anti-inflammatory action of yogurt containing extract of erva-mate, which was administered in rats submitted to peritonitis. **Methods:** Seventeen rats were divided into two groups; control group rats were treated with natural yogurt, whereas treated group received yogurt containing extract of erva-mate (2.5%). The animals were treated for 20 consecutive days by gavage with a single dose of 1mL per day. The recruitment of leukocytes to the peritoneum was evaluated by injection of 10mL of oyster glycogen in phosphate buffered saline (PBS) 1%, using the same procedure for both groups. After 4 hours, the cells that migrated to the peritoneum were collected by washing the peritoneal cavity with PBS. The inflammatory exudate had its cells quantified by an electronic cell counter (ABX Micros 60). This experimental protocol was approved by Ethics Committee of URI-Campus Erechim under number 024/PIA/09. **Results and Discussion:** The rats that received yogurt without extract (control group), presented  $2.9(\pm 0.8) \times 10^3$  mm<sup>3</sup> of exudate leukocytes, while the rats that received yogurt with erva-mate extract presented  $1.8(\pm 0.3) \times 10^3$  mm<sup>3</sup> of exudate leukocytes. The extract of erva-mate in the yogurt in 2.5% diminished ( $P < 0,05$ ) the cell recruitment to the peritoneum of rats inflamed by oyster glycogen. Reference: Browne MK, Leslie GB. Animal models of peritonitis. *Surg Gynecol Obstet* 1976; 143: 738-42. Ferraz EM, Ferraz AAB. Systemic inflammatory response syndrome. In: Ferraz AAB, Mathias CAC, Ferraz EM. Pipelines in general surgery. 1st ed. Rio de Janeiro: Medsi, 2003, 59: 629-636. Financial support: BioTécnica and URI-Campus Erechim

#### 04.030

Anti-inflammatory actions of lipoxin A<sub>4</sub> in zymosan-induced arthritis. Conte FP<sup>1</sup>, Menezes de Lima Jr O<sup>1</sup>, Verri Jr WA<sup>2</sup>, Ferraris FK<sup>1</sup>, Cunha FQ<sup>3</sup>, Penido C<sup>1</sup>, Henriques MGO<sup>1</sup> <sup>1</sup>FIOCRUZ - Farmacologia Aplicada, <sup>2</sup>UEL - Ciências Patológicas, <sup>3</sup>FMRP-USP

**Introduction** Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic joint inflammation, marked by cellular infiltration, edema formation, pain, among other events. Lipoxin A<sub>4</sub> (LXA<sub>4</sub>) is a lipid mediator that plays an important role in the resolution of inflammation. A recent study demonstrated LXA<sub>4</sub> increased levels in synovial fluid and enhanced expression of lipoxin A<sub>4</sub> receptor (ALX) in synovial tissues from RA patients (Hashimoto, *et al. J Rheumatol.*34(11):2144. 2007). The present study aims to evaluate the role LXA<sub>4</sub> on neutrophil migration and edema formation during the acute phase of zymosan-induced arthritis in mice. **Methods and Results** Stimulation of articular knee joints of C57BL/6 mice with zymosan (500 µg/25 µL; i.a.) was characterized by a pronounced edema formation and massive neutrophil influx into inflamed site within 6 h. Pre-treatment with exogenous LXA<sub>4</sub> (1-20 ng/cav; i.a.), 1 h prior i.a. injection of zymosan, reduced zymosan-induced edema formation at all doses used (maximum inhibition of 74%) and significantly reduced zymosan-induced total leukocyte and neutrophil influx (60 and 61%, respectively) only at the higher dose (20 ng/cav). In addition, LXA<sub>4</sub> (20 ng/cav) pre-treatment also significantly blocked zymosan-induced TNF-α production within 6 h (sal 0.25 ± 0.03; zy 0.40 ± 0.03; LXA<sub>4</sub> 0.25 ± 0.04 ng/ml, n=5). In agreement with previous reports, pre-treatment with the LXA<sub>4</sub> receptor (ALX) antagonist, BOC-1 (20 ng/cav; i.a., 90 min prior stimulation), reverted the anti-inflammatory effects of LXA<sub>4</sub> (60 min prior stimulation) observed on zymosan-stimulated mice, including edema formation (sal 0.01 ± 0.02; zy 0.67 ± 0.07; LXA<sub>4</sub> 0.29 ± 0.04; BOC-1 0.59 ± 0.06 x Δ mm, n=8) and neutrophil influx (sal 0.51 ± 0.05; zy 6.03 ± 1.45; LXA<sub>4</sub> 3.13 ± 0.63; BOC-1 6.98 ± 1.26 x 10<sup>5</sup> cells/knee, n=8). Corroborating with these results the pre-treatment with an agonist of ALX, BML-111 (200 ng/cav; i.a.), also reduced zymosan-induced edema formation, total leukocyte and neutrophil influx (47, 63 and 68%, respectively). In another set of experiments, acetylsalicylic acid (ASA; 300 mg/kg; p.o.), that produces endogenous aspirin triggered lipoxins (ATLs), was given 30 min before i.a. injection of zymosan and significantly reduced edema formation, total leukocyte and neutrophil influx (51, 67 and 67 % inhibition). Interestingly, BOC-1 (200 ng/knee) significantly reverted the inhibitory effect of ASA (300 mg/kg p.o.) on zymosan-induced cellular migration, whereas did not revert the antiedematogenic effect of ASA treatment. No significant change was observed when BOC-1 (200 ng/knee) was administered to mice that received zymosan but not ASA, or in animals treated with indomethacin (3 mg/kg, i.p.) instead. P values ≤ 0.05 were regarded as significant. All procedures were approved by the Committee for Animal Care and Use (CEUA-FIOCRUZ) under L0052-2002 license. **Conclusion** Taken together, these results point to a marked anti-inflammatory action of LXA<sub>4</sub> on knee joint inflammation. **Financial support** CNPq/FIOCRUZ

#### 04.031

Assessment of depressive behavior following the induction of chronic inflammation by complete Freund's adjuvant (CFA) in mice. Maciel IS<sup>1</sup>, Silva RBM<sup>1</sup>, Santos DS<sup>2</sup>, Calixto JB<sup>3</sup>, Morrone FB<sup>2</sup>, Campos MM<sup>4</sup> <sup>1</sup>PUCRS - Farmacologia, <sup>2</sup>PUCRS - Farmácia, <sup>3</sup>UFSC - Farmacologia, <sup>4</sup>PUCRS - Cirurgia-Odontologia

**Introduction:** Numerous studies have demonstrated a link between acute inflammation and behavioral alterations in animal models (Narita, et al., *J. Neur.* 31, 739, 2006). These studies indicate that induction of inflammation by bacterial lipopolysaccharide is associated with development of sickness behavior, characterized by anhedonia, decreased food intake and locomotion, social isolation and changes in circadian cycle (Dantzer, et al., *Nature Neuro Rev.* 9, 46, 2008). This work was aimed to characterize the depressive behavior in the mouse model of chronic inflammation induced by CFA. We have also evaluated the effects of the classical antidepressant drug imipramine in the responses induced by CFA. **Methods:** Male Swiss mice were used (8 per group, with 25 – 30 g). The experimental protocols were approved by the local Ethics Committee (07/03611, PUCRS). For the induction of chronic inflammation, the animals received an intraplantar injection of CFA (50 µl/paw). The control animals received 50 µl/paw of PBS (negative control). The animals were submitted to the following tests, 1 and 2 weeks after the administration of CFA: (i) *paw edema evaluation*: the increase in paw volume was measured in a plethysmometer (in µl; Ugo Basile, Comércio, Italy); (ii) *tail suspension test (TST)*: this test considers the immobility time (during a 6-min period of evaluation), as an index of depressive state (despair behavior). Separate groups of mice were treated with the classical antidepressant drug imipramine (10 mg/kg, i.p., 30 min prior to the tests). Statistical analysis was performed using analysis of variance (ANOVA) followed by the Tukey test. Values of  $P < 0.05$  were considered as indicative of significance (GraphPad Prism 4.02). **Results:** The intraplantar administration of CFA produced a marked and time-dependent edematogenic response ( $245 \pm 58$  µl and  $165 \pm 17$  µl, at 1 and 2 weeks after CFA, respectively). Of high interest, the inflammation evoked by CFA was accompanied by a marked increase of immobility time in TST test ( $59 \pm 6\%$  and  $155 \pm 17\%$ , at 1 and 2 weeks, respectively). The antidepressant drug imipramine (10 mg/kg, i.p., 30 min) was able to significantly prevent the depressive behavior induced by CFA ( $35 \pm 12\%$  and  $61 \pm 5\%$  of reduction, at 1 and 2 weeks, respectively), whereas it did not alter the paw edema formation ( $P > 0.05$ ). **Discussion:** The results obtained demonstrate for the first time that chronic inflammation induced by CFA is accompanied by depressive-like behavior, which is sensitive to the antidepressant imipramine. Additional studies are under development to further characterize the mechanisms involved in this response. **Financial support:** CAPES, CNPq and FAPERGS.

#### 04.032

Effects of the hydroalcoholic extract obtained from *Phyllanthus niruri* in the model of hemorrhagic cystitis induced by cyclophosphamide in mice. Boeira VT<sup>1</sup>, Santos Jr AA<sup>2</sup>, Martins JP<sup>3</sup>, Leal PC<sup>4</sup>, Calixto JB<sup>5</sup>, Morrone FB<sup>3</sup>, Campos MM<sup>6</sup> <sup>1</sup>PUCRS - Farmacologia, <sup>2</sup>INCT-PUCRS - Biologia Molecular e Funcional, <sup>3</sup>PUCRS - Farmácia, <sup>4</sup>QMC-CFM-UFSC, <sup>5</sup>UFSC - Farmacologia, <sup>6</sup>PUCRS - Cirurgia-Odontologia

**Introduction:** Hemorrhagic cystitis (HC) is a common side effect observed in patients under chemotherapy with cyclophosphamide (CYP). The urotoxic side effects of CYP are attributed to the metabolic compound acrolein, and can be partially prevented by the uroprotectant agent 2-mercaptoethene sulfate (Mesna) (Katz et al., J Cancer Res. Clin. Oncol., 121, 128, 1995). It has been demonstrated that *P. niruri* extract displays marked analgesic effects in several models of nociception (Martini et al., Neurochem. Res., 25, 211, 2000), and is able to protect the liver damage caused by oxidative stress in mice (Bhattacharjee et al., Food. Chem. Toxicol., 45, 817, 2007). The present study analyzed the effects of hydroalcoholic extract of *P. niruri* in a mouse model of cyclophosphamide-induced HC. **Methods:** Male Swiss mice were used (N= 6-8 per group; 25–30 g). All the experimental protocols are approved by the Local Ethics Committee (07/03611-PUCRS). Hemorrhagic cystitis was induced by a single administration of CYP (300 mg/kg, i.p.). Immediately after the i.p. injection of CYP, mice were housed in individual plastic cages to observe the spontaneous behavior for 4 h, for 2 min every half-hour. Three behavioral parameters were considered: (i) general activity (walking, rearing, climbing, grooming etc.); (ii) immobility time; and (iii) indicatives of visceral pain behavior ('crises'). In addition, the spontaneous behavior of mice was also scored according to the following scale: 0 = normal; 1 = piloerection; 2 = strong piloerection; 3 = laboured breathing; 4 = abdomen licking; and 5 = abdomen stretching and contractions (Olivar et al., Eur. J. Pain., 3, 141, 1999). In addition, we have performed the gross examination of bladders at 6 h, in order to determine the presence of edema and hemorrhage. The wet weight of bladders (g per 100 g of body weight) was also registered at this time-point (Gray et al., J. Urol., 136, 497, 1986). **Results:** The treatment with MESNA (80 mg/kg, i.p., 5 min before CYP administration and 160 mg/kg, p.o., 2 h after CYP, positive control drug) was capable of inhibiting the formation of hemorrhage ( $68 \pm 5$  %) and edema ( $78 \pm 11$  %), and also to diminish the behavioral score ( $85 \pm 7$  %), when compared to the control group (treated with CYP and saline). Interestingly, the treatment with the hydroalcoholic extract of *P. niruri* (30 mg/kg, p.o., 30 min before CYP administration and 2 h after CYP) was able to significantly inhibit the hemorrhage ( $44 \pm 13$  %) and edema ( $50 \pm 12$ %) formation. Moreover, the administration of *P. niruri* hydroalcoholic extract markedly reduced the behavioral score ( $81 \pm 7$ %), in comparison to the control group. Neither MESNA nor *P. niruri* hydroalcoholic extract displayed any behavioral effect *per se* ( $P > 0.05$ ). **Discussion:** The present results clearly indicate that hydroalcoholic extract of *P. niruri* might represent an important alternative to prevent the urotoxic effects following the chemotherapy with CYP. Additional studies are being conducted to verify the active compounds responsible for the protective effects of *P. niruri* extract. **Financial Support:** CNPq and PUCRS.



#### 04.033

Role of phosphatidylinositol-3 kinase in the inflammatory, nociceptive and pruritogenic responses induced by trypsin in mice. Pereira PJS<sup>1</sup>, Leal PC<sup>2</sup>, Calixto JB<sup>3</sup>, Morrone FB<sup>4</sup>, Campos MM<sup>5</sup> <sup>1</sup>PUCRS - Medicina e Ciências da Saúde, <sup>2</sup>QMC-CFM-UFSC, <sup>3</sup>UFSC - Farmacologia, <sup>4</sup>PUCRS - Farmácia, <sup>5</sup>PUCRS - Cirurgia-Odontologia

**Introduction:** It has been demonstrated that trypsin is able to evoke the classic signals of inflammation, including pain and pruritus, mainly by the activation of PAR-2 receptors and secondary production of several inflammatory mediators (Costa et al., *Br J Pharmacol.*, 154, 1094, 2008; Paszcuk et al., *Eur. J. Pharmacol.*, 581, 204, 2008). The present study evaluated the effects of AS605240, a selective PI3kg inhibitor, on the inflammatory, pruritogenic and nociceptive responses induced by trypsin in mice.

**Methods:** Male Swiss mice (8 per group, 25-30 g) were used. All the experimental protocols were approved by the Local Ethics Committee (07/03611-PUCRS). Animals were pretreated orally with the selective PI3kg inhibitor AS605240 (1 to 30 mg/kg), 30 min before trypsin injection. The control groups received saline by the same route. Oedema was induced by an intraplantar (i.pl.) injection of trypsin (30 µg/paw, 50 µl) in the right hindpaw. The left paw received the same volume of saline and it was used as the control. Oedema was determined with a plethysmometer, at different periods of time (0.5 to 6 h). Another parameter analyzed was the scratching behavior evoked by trypsin in the mouse dorsum (200 mg/site, 50 ml). Scratching was measured for 40 min, as the number of scratches with forepaws and hindpaws close to the injected site, and/or behind the ears. For the evaluation of spontaneous nociception, mice received an i.pl. injection of trypsin (300 mg/paw, 20 ml) into the right hindpaw, and the amount of time (in s) spent licking and/or biting the injected paw was recorded during 10 min.

**Results:** The intraplantar injection of trypsin (30 µg/paw) caused a marked and time-dependent paw oedema in mice (area under curve = 649 ± 26). AS605240 administered orally produced a significant and dose-dependent reduction of paw oedema induced by trypsin. The percentages of inhibition, calculated based on the area under the curve, were: 24 ± 7%, 46 ± 3%, 40 ± 7% and 42 ± 3%, for the doses of 1, 3, 10 and 30 mg/kg, respectively. In addition, the pretreatment with AS605240 produced a marked reduction of scratching behavior elicited by trypsin (200 µg/site) in the mouse dorsum. The inhibition percentages were 60 ± 8%, 57 ± 12% and 51 ± 8% for the doses of 1, 3 and 10 mg/kg, respectively. Finally, AS605240 promoted a significant reduction of spontaneous nociception induced by trypsin in the mouse paw, at the dose of 3 mg/kg (34 ± 9 % of inhibition). On the other hand, a higher dose of AS605240 (10 mg/kg) did not significantly alter this response. **Discussion:** The results of the present study suggest that PI3kg activation represents one of the mechanisms responsible for the oedematogenic, pruritogenic and nociceptive responses elicited by trypsin. Additional studies are in progress to better characterize the relevance of PI3kg in the effects of trypsin. **Financial support:** CAPES, CNPq, PUCRS.

#### 04.034

Evaluation of carbon nanotubes toxicity and immunogenicity *in vitro* using murine peritoneal macrophages. Cisalpino D<sup>1</sup>, Fagundes CT<sup>1</sup>, Souza DG<sup>2</sup>, Teixeira MM<sup>1</sup>  
<sup>1</sup>UFMG - Imunologia e Bioquímica, <sup>2</sup>UFMG - Microbiologia

**Introduction:** The development of nanomaterials for biomedical and biotechnological applications is an area of research that holds great promise for the near future and intense interest, and carbon-based nanostructures in particular, such as carbon based nanotubes (CNT's) are receiving an increasing level of attention. CNT's are presented in two major structural patterns: single wall carbon nanotubes (SWNT's) and multi wall carbon nanotubes (MWNT's). There is a very broad range of possible applications to CNT's in biomedical research and even clinical application, but before any medical applications can be developed, the fate and behavior of CNT's in mammalian systems must be explored. Our research group is assessing the immunogenic capabilities of different kinds of SWNT's and MWNT's by studying these materials effects on one of the most basic cell kinds in host immune response: the macrophage. **Methods:** Murine peritoneal macrophages were harvested and cultured in 96 well cell culture plates and incubated with decreasing concentrations of different types of SWNT's and MWNT's in two sets of experiments. In the first set we incubated the cells with concentrations of 5000, 500, 50 and 0.5µg of each type of SWNT's (standard, purified, carboxylated, L-Alanine conjugated and cutted carboxylated) and MWNT's (standard, purified and carboxylated). Also, LPS was used (50ng/ml) as a positive control for macrophage activation. After 24h of incubation with the CNT's and LPS, cell viability was assessed using the MTT viability assay. In the second set of experiments, TNF-α production was also assessed with enzyme linked immunosorbent assay (ELISA) using the cell culture supernatant. **Results and Discussion:** Our results clearly indicate a dose-dependent viability in the cultured cells. Higher doses of CNT's are more toxic to macrophages, and this toxicity decreases as the doses get lower. Also, some of the used types of CNT's are more toxic than others. TNF-α production was not detected in the CNT's test groups. Thus, we can conclude that lower doses of CNT's can be used safely for diverse *in vitro* applications (such as cell transfection) and that the CNT's apparently doesn't display any immunogenic activity. Further experiments will be conducted to evaluate other CNT's uses and cell response to them in varied conditions.

#### 04.035

Topical anti-inflammatory effects of statins and combination simvastatin + ezetimibe. Bracht L, Oliveira de Melo J, Magon TFS, Cuman RKN, Caparroz-Assef SM, Bersani-Amado CA UEM - Farmácia e Farmacologia

**Introduction:** The beneficial effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) in cardiovascular disease have generally been attributed to their cholesterol-lowering properties. However, an increasing number of *in vitro* and *in vivo* studies have indicated that statins have direct antiinflammatory effects that are not mediated by their hypocholesterolemic activity (TEIXEIRA et al., Eur J Pharmacol, v. 516, p. 282, 2005). Furthermore, previous studies have shown that, in addition to their favorable effects on lipid levels, combined therapy with ezetimibe + simvastatin significantly reduced C-reactive protein levels compared with statin monotherapy (PEARSON et al., Am J Cardiol, v. 99, p. 1706, 2007). In contrast, scarce evidence has shown whether such a combination has topical antiinflammatory properties. The aim of the present study was to investigate the topical antiinflammatory effects of simvastatin, atorvastatin, and a simvastatin + ezetimibe combination using croton oil ear edema in mice as the model of topical inflammation. **Methods:** All experiments were approved by the Animal Ethics Committee of Maringá University (062/2008). Simvastatin, atorvastatin, the simvastatin + ezetimibe combination, and dexamethasone (dissolved in 20 µL of 70% acetone) were topically applied simultaneously with croton oil (200 µg/ear, dissolved in 20 µL of 70% acetone) at the inner surface of each ear. Ear edema and myeloperoxidase activity, indicative of polymorphonuclear cell migration, were assessed 6 h after inflammatory stimuli. Ear edema was expressed as the increase in ear weight, and myeloperoxidase activity was expressed as augmentation in optical density at 460 nm. **Results:** Topical application of croton oil induced cutaneous inflammation at the ears of mice, which caused a significant increase in ear weight compared with the untreated ear. As a positive control, dexamethasone (0.1 mg/ear) significantly inhibited ear edema by 89%. Simvastatin also markedly reduced ear edema, an effect comparable to that of dexamethasone. The calculated inhibition was 71%, 83%, 70%, and 55% for simvastatin at 1.2, 0.6, 0.3, and 0.03 mg/ear, respectively. Similarly, atorvastatin (0.15 mg/ear) markedly inhibited ear edema by 78%. Treatment with the simvastatin + ezetimibe combination did not cause any additional reduction in ear weight compared with the same concentrations of simvastatin alone. All treatments produced remarkable inhibition of polymorphonuclear cell migration, reflected by a marked reduction of myeloperoxidase activity. For this parameter, the calculated inhibition was 97% for atorvastatin (0.15 mg/ear), 81% for simvastatin (0.03 mg/ear), and 86% for dexamethasone (0.1 mg/ear). **Discussion:** Altogether, these results consistently support the hypothesis that simvastatin and atorvastatin possess topical antiinflammatory properties and that combined simvastatin + ezetimibe therapy does not exert further beneficial effects in the croton oil ear edema model of topical inflammation in mice. The mechanisms underlying diminished leukocyte migration may be associated with downregulation of chemokines, such as MCP-1, RANTES, IL-6, and ICAM-1, as well as blockade of the induction of nitric oxide synthase (WEITZ-SCHMIDT, Trends Pharmacol Sci, v. 23, p. 482, 2002). **Financial Support:** CAPES/CNPq

#### 04.036

Mediators involved in the paw edema induced by batroxase isolated from *Bothrops atrox* snake venom. De Toni LGB<sup>1</sup>, Figueiredo MM<sup>2</sup>, Sartim MA<sup>1</sup>, Cintra ACO<sup>1</sup>, Franco, JJ<sup>1</sup>, Souza GEP<sup>2</sup>, Sampaio, SV<sup>1</sup> <sup>1</sup>FCFRP-USP - Análises Clínicas, Toxicológicas e Bromatológicas, <sup>2</sup>FCFRP-USP - Física e Química

**Introduction:** Ophidians accidents caused by *Bothrops* genus snakes are characterized by local and systemic reactions. Batroxase, a zinc-dependent metalloproteinase of PI class, is one of the bioactive components of these snakes venom and is responsible for the hemorrhagic, fibrinolytic and inflammatory effects of the venom<sup>1</sup>. The aim of this study was to evaluate the ability of batroxase from *Bothrops atrox* snake venom in inducing paw edema in rats and the inflammatory mediators involved in this response. **Methods:** The paw edema induced by intraplantar injection (ipl) of batroxase was evaluated in male Wistar rats (180-200g) by pletismometer (Ugo Basile, Italy). The values were expressed in milliliters (mL) of the difference between the left (batroxase) and right (saline) paws during 6 hours. Rats were pre-treated with celecoxib, a selective COX-2 inhibitor, (5 mg/kg, p.o.), dexamethasone (DEX), steroidal anti-inflammatory drug (0.5 and 2.0 mg/kg, s.c.), diphenhydramine (DIPH), H<sub>1</sub> receptor antagonist (5 and 10 mg/kg, s.c.), methysergide (MET), 5HT<sub>1</sub> and 5HT<sub>2</sub> receptors antagonist (5 and 10 mg/kg, s.c.) 1 hour before. Control animals received saline. The experimental protocols were submitted and approved by the Ethical Committee of University of São Paulo, Campus of Ribeirão Preto, SP, protocol number 09.1.255.53.0. **Results:** The ipl injection of batroxase (10, 20 and 40 µg/paw) elicited a dose and time-dependent paw edema. 20 and 40 µg/paw induced maximal edema formation. The dose of 20 µg was selected for the remaining experiments. The pre-treatment of animals with DEX 2mg/kg (2h: from 0.55±0.03 to 0.28±0.04), DIPH 10 mg/kg (2h: from 0.78±0.05 to 0.32±0.03) and MET (2h: from 0.82±0.03 to 0.59±0.03 and 0.50±0.05, 5 and 10 mg/kg, respectively) significantly reduced batroxase-induced paw edema while celecoxibe and dexamethasone 0.5 mg/kg do not alter this response. **Discussion:** Batroxase injected ipl into the rat paw causes dose-dependent edema. Prostaglandins seem do not be involved in this response. Histamine (H<sub>1</sub> receptors) and serotonin (5HT<sub>1</sub> and 5HT<sub>2</sub> receptors) seem to play a substantial role. Although deserving further investigation, the effectiveness of dexamethasone on batroxase-induced edema may be related to its ability to reduce mast cell degranulation and/or to reduce leukotrienes synthesis via phospholipase A<sub>2</sub> inhibition<sup>2,3</sup>. So, histamine and serotonin antagonists and dexamethasone may be useful to treat inflammatory signs after envenoming by *Bothrops atrox*. **Financial support:** CAPES/FAPESP/CNPq. [1]Matsui et al., *Biochim et Biophy Acta*, 1477: 146, 2000; [2] Zhou et al. *Allergy*, 63: 1177, 2008. [3] Barnes & Karin *New Engl. J. Med.* 336:1066; 1997

#### 04.037

Efeito do tratamento com o óleo essencial de *Zingiber officinale* Roscoe sobre a migração de leucócitos *in vivo*. Nogueira de Melo GA, Fonseca JP, Farinha TO, Bersani-Amado CA, Cuman RKN UEM - Farmácia e Farmacologia

**Introdução:** A espécie vegetal *Zingiber officinale* Roscoe é conhecida popularmente como gengibre. Os rizomas desta planta têm sido utilizados como especiaria e na medicina popular para o tratamento de diversas enfermidades, desde o desconforto gastrointestinal até processos infecciosos, existindo relatos na literatura sobre sua atividade anti-inflamatória. Neste trabalho foi investigado o efeito do tratamento com o óleo essencial de Gengibre (OEG) sobre a migração de leucócitos em vasos da microcirculação na fáscia espermática de ratos (Aprovação/CEAE nº 041/2008).

**Métodos:** Ratos machos da linhagem Wistar (220-240g) foram anestesiados com hidrato de cloral (500mg/kg; s.c.). A fáscia espermática interna foi exteriorizada, a microcirculação observada por um microscópio e as imagens foram captadas e gravadas através de software para captação de imagens e gravadas em microcomputador acoplado ao sistema. O número de leucócitos acumulados numa área de 2500cm<sup>2</sup> no tecido conjuntivo adjacente à vênula pós-capilar foi determinado 4 horas após a administração de carragenina (100µg) na bolsa escrotal. Os animais foram tratados com o OEG (100, 200 e 500mg/kg) ou indometacina (IND) (5mg/kg; *per os*) 30 minutos antes da injeção de carragenina. Os dados foram avaliados por Análise de Variância (Anova) seguida do teste de Tukey. **Resultados e Discussão:** Quatro horas após o estímulo inflamatório houve uma redução significativa no número de leucócitos migrados em animais tratados com OEG apenas nas doses de 200 e 500 mg/kg. (**Controle:** 14,9±0,8; **OEG<sub>100mg/kg</sub>:** 13,7±0,7; **OEG<sub>200mg/kg</sub>:** 10,0±0,9\*; **OEG<sub>500mg/kg</sub>:** 9,7±0,6\*; **IND:** 6,4±0,6\*\*; \*p<0,01/\*\*p<0,001). Os resultados preliminares obtidos *in vivo* demonstraram que o óleo essencial de Gengibre apresenta efeito sobre a migração de leucócitos durante a resposta inflamatória aguda inibindo a quimiotaxia.

**Apoio Financeiro:** CAPES/CNPq

#### 04.038

Prostaglandins in immune cell response in the histoplasmosis. Pereira PAT, Trindade BC, Secatto A, Santos DF, Nicolete R, Sorgi CA, Peres CM, Faccioli LH FCFRP- USP - Análises Clínicas, Toxicológicas e Bromatológicas

**Introduction:** Prostaglandins (PGs) participate as mediators of inflammation and immunomodulators of cytokines release during innate and adaptive immune response. In the histoplasmosis, serious pulmonary injuries occur due to the inhalation of conidial or mycelium fragments that reach the alveoli where they transform into the pathogenic yeast form. Previously, we showed an increase in leukotrienes synthesis in the lung of *Histoplasma capsulatum*-infected mice (*Infect. Immun.*, 72: 1637-1644, 2004), but nothing is known about the role of prostaglandins in this infection. **Objectives:** In this study, we investigated the role of prostaglandins in *H. capsulatum* infection in mice. **Methods and Results:** Mice were infected (i.t.) with  $5 \times 10^5$  (sublethal inoculum) or  $1 \times 10^6$  yeast/animal (lethal inoculum) and daily treated or not with celecoxib (1 or 5 mg/kg). Compared to sublethal *Hc*-infected mice, no changes in survival were observed in sublethal-infected mice that were treated with both doses of celecoxib. However, sublethal infected mice treated with celecoxib (1 mg/kg) presented reduction in (i) leukocyte counts in bronchoalveolar lavage fluid (BALF) at days 2, 14 and 28 postinfection (p.i.); (ii) lung colony forming unit (CFU) recovery at days 14 and 28 post infection, (iii) PGE<sub>2</sub> synthesis at day 14 p.i, and (iv) TNF- $\alpha$ , IL-1, IL-6, IL-8, IL-10, IL-4, IL-5 and IFN- $\gamma$  synthesis in the lung during infection. However, LTB<sub>4</sub> production increased at day 14 p.i. Also, administration of celecoxib (1 and 5 mg/kg) to lethal infected mice, reduced mortality from 100% to 20% and 58% respectively, and inhibited leukocyte accumulation in BALF (14 days p.i.) and CFU recovery from lung (28 days p.i.). **Conclusion:** These results suggest that PGs have a suppressive role in host defense during *Hc* infection **Financial Support:** FAPESP, FAEPA and CNPq.

#### 04.039

Anti-inflammatory effects of inosine in a murine model of ovalbumin-induced asthma. Lapa FR<sup>1</sup>, Ligeiro de Oliveira AP<sup>2</sup>, Golega B<sup>2</sup>, Tavares de Lima W<sup>2</sup>, Cabrini DA<sup>1</sup>, Santos ARS<sup>3</sup> <sup>1</sup>UFPR - Farmacologia, <sup>2</sup>ICB-USP – Farmacologia, <sup>3</sup>UFSC - Ciências Fisiológicas

**Introduction:** Inosine has been suggested exerts anti-inflammatory effects in a wide range of inflammatory conditions, including acute lung inflammation (Liaudet et al., *Ann. Surg.*, 235:568-78, 2002; Szabo et al., *Shock*, in press, 2009). The aim of this study was to evaluate the anti-inflammatory effects of inosine in a murine model of ovalbumin-induced asthma. **Methods:** Female Balb/c mice were sensitized with ovalbumin (OVA, 10 µg) (day 0) and boosted (day 7) subcutaneously with OVA (10 µg). At day 14 and 15, mice were challenged with aerosolized OVA (1% in PBS) and treated 30 min before the OVA challenges by intraperitoneal route with inosine (0.001-10 mg/kg). In addition, other group of animals was treated with A<sub>1</sub> (DPCPX) or A<sub>2A</sub> (ZM241385) adenosine receptor antagonist 30 min before the inosine treatment. On the day of experiments (24 h after the last OVA-challenge), the bronchoalveolar lavage (BAL) was obtained to perform the cell counting. Lungs fragments were obtained under sterile conditions and cultured-24 h (explant) for determination of cytokines. This study was approved by Ethics Committee of Federal University of Paraná (320) and University of São Paulo (58). **Results:** Inosine significantly reduced the OVA-induced lung inflammation, as indicated by decreased numbers of total leukocytes, macrophages, lymphocytes and eosinophils recovered in the BAL, when compared with the allergic control group, with ID<sub>50</sub> of 0.3 (0.05-0.35) mg/kg and inhibitions of 60 ± 2%, 49 ± 3%, 67 ± 6%, 97 ± 6% at dose of 10 mg/kg, i.p., respectively. Pre-treatment with ZM241385 reverted partially and totally the inhibitory effects of inosine (10 mg/kg, i.p.) on the total leukocyte cell migration and macrophage count in the BAL, respectively. In contrast, the treatment with DPCPX, an A<sub>1</sub> adenosine receptor antagonist, did not alter the inosine effects against both, total and differential leukocyte migration. In addition, the treatment with inosine (10 mg/kg, i.p.) reduced the levels of IL-4, IL-5 and IL-13 in OVA-challenged explants (61 ± 16%, 73 ± 8% and 80 ± 6%, respectively). The treatment with inosine reduced the levels of IL-5 and IL-13 in explants not challenged with inhibitions of 51 ± 1% and 52 ± 13%, respectively. The pretreatment with A<sub>1</sub> and A<sub>2A</sub> antagonists did not interfere with inosine effects in the explants cultures. **Discussion:** Inosine revealed an interesting anti-inflammatory effect on OVA induced lung inflammation, reducing the number of leukocytes in the BAL fluid. The effects observed with ZM241385 and DPCPX pre-treatment, suggests a partial involvement of A<sub>2A</sub> receptor in inosine effect on cell migration. Moreover, inosine effects might involve a possible inhibition on cytokine release, as observed on explants from OVA-challenged mice. These effects were probably not related with A<sub>1</sub> and A<sub>2A</sub> adenosine receptors and might suggest an inhibitory effect of inosine in Th2 lymphocyte response. **Financial supports:** REUNI, CAPES, CNPq and FAPESP

#### 04.040

Evidence of adenosine receptors involvement in the inosine anti-inflammatory effect in a mouse model of pleurisy. Lapa FR<sup>1</sup>, Cabrini DA<sup>1</sup>, Santos ARS<sup>2</sup> <sup>1</sup>UFPR - Farmacologia, <sup>2</sup>UFSC - Ciências Fisiológicas

**Introduction:** It is increasingly apparent that endogenous purines, including inosine, have been shown to exert immunomodulatory effects and presents a key role in regulation of inflammatory process. Thus, the aim of this study was to evaluate the inosine anti-inflammatory effects in the early phase (4h) of carrageenan (Cg) induced-pleurisy, as well as the participation of A<sub>1</sub> and A<sub>2A</sub> adenosine receptors. **Methods:** Mice (18-25 g) were challenged with a solution of Evans blue dye (25 mg/kg, 0.2 ml, i.v.) to evaluate the degree of exudation in the pleural space 30 min before the treatment with inosine (0.3-300 mg/kg, i.p.) or with A<sub>1</sub> (DPCPX) or A<sub>2A</sub> (ZM241385) adenosine receptor antagonists, i.p., 30 min prior to inosine. The pleurisy was induced by the intrapleural injection of carrageenan (Cg, 1%) 30 min after the treatments and 4 hours later, the exudates were collected to perform total and differential leukocyte counts, as well as the determination of Evans blue dye. This study was approved by Ethics Committee of the Universidade Federal do Paraná with protocol no. 320. **Results:** The inosine treatment (0.3-300 mg/kg, i.p., 30 min prior to Cg) produced a significant reduction in cell migration and pleural leakage that was not dose-related and was greatest at the dose of 10mg/kg, showing no increase of effect with the raise of dose. The inosine treatment did not affect the mononuclear migration but significantly reduced the pleural leakage, total leukocyte and neutrophil migration, with inhibitions 42 ± 11%, 48 ± 8%, 55 ± 11% at dose of 10 mg/kg, respectively. Inosine (10-300 mg/kg, i.p.) treatment reduced the inflammatory parameters with equal effectiveness, thus the smaller dose (10 mg/kg, i.p.) was chosen to study the involvement of adenosine receptors. In addition, the pre-treatment with A<sub>1</sub> (DPCPX) or A<sub>2A</sub> (ZM241385) receptor antagonists reverted the pleural leakage 77 ± 16 % and 83 ± 17 %; total leukocyte 68 ± 17 % and 70 ± 13 % and neutrophil migration 71 ± 15 % and 63 ± 9 %, respectively. **Discussion:** These results show that inosine presents an important anti-inflammatory effect in Cg-induced pleurisy, probably throughout the activation of A<sub>2A</sub> and A<sub>1</sub> adenosine receptors. Additional experiments will be carried out to evaluate the participation of cytokines, as well as other adenosine receptors in this inosine anti-inflammatory effect. **Grants:** REUNI, CAPES, CNPq and FAPESC



#### 04.041

Study of the anti-inflammatory effect the *Carapa guianensis* seed oil in mice. Gonçalves IB<sup>1</sup>, Figueiredo JG<sup>2</sup>, Bitencourt FS<sup>1</sup>, Cavalcante IJM<sup>4</sup>, Melo AT<sup>1</sup>, Sousa FA<sup>3</sup>, Gonçalves ACS<sup>4</sup>, Vasconcelos MAM<sup>5</sup>, Vale MR<sup>1</sup>, Alencar NMN de<sup>1</sup> <sup>1</sup>UFC - Fisiologia e Farmacologia, <sup>2</sup>UFC - Bioquímica e Biologia Molecular, <sup>3</sup>UFC - Farmacologia Bioquímica, <sup>4</sup>UFPA - Química, <sup>5</sup>Embrapa - Agroindústria

**Introduction:** *Carapa guianensis* Aublet (Meliaceae), popularly known as Andiroba, is a popular medicinal plant found in several countries. All parts of the tree, including its seed oil are used for medicinal purposes. Traditional Amazon Rainforest communities make a medicinal soap using *Carapa guianensis* seed oil for treatment of skin diseases, arthritis, rheumatism, ear infections. The present study investigated the anti-inflammatory effects of the *Carapa guianensis* seed oil. **Methods:** Animal handling and experimental protocols were registered on the Institutional Ethics Committee under number 59/2009. Induction of peritonitis by thioglycollate: mice (n=6) were injected i.p. with 1 ml of thioglycollate 3% (w/v) (Quershi, Eur. J. Immunol.141:2090, 1988). One hour before mice received orally 100, 200 and 400 mg/kg of the *Carapa guianensis* seed oil or vehicle (2% tween 80; 10 ml/kg; vehicle group). Controls group received the same volume of vehicle orally and saline i.p. (saline group). Six hours later, blood samples were withdraw from the retro-orbital plexus and centrifuged to obtain the serum. The serum level of nitric oxide (NO) was determined indirectly as the content of nitrite and nitrate (Feng, Circulation. 104: 700, 2001). Animals were killed and cells harvested by washing each peritoneal cavity with 3 ml saline (5 IU heparin/ml). Results were expressed as mean  $\pm$  S.E.M cells $\times 10^6$ /mL and NO<sub>2</sub>( $\mu$ M), compared using ANOVA – Bonferroni's test.  $p < 0.05$  was considered to be statistically significant. **Results and Discussion:** The pretreatment with *Carapa guianensis* seed oil decreased the neutrophil migration at doses of 200 (2113 $\pm$ 194.6) and 400 (1602 $\pm$ 104.1) mg/kg when compared with vehicle group (3286 $\pm$ 252.1). The level of serum NO was significantly increased in the vehicle group (4.73 $\pm$ 0.56) when compared with saline group (1.48 $\pm$ 0.28). Moreover, the mice pretreated with an oil only dose of 400 mg/kg (8.52 $\pm$ 0.22) showed an enhancement in the level of NO when compared with vehicle group. In conclusion, our suggest that *Carapa guianensis* seed oil presents anti-inflammatory activity with probable nitric oxide involvement. Further research would be of interest to explain the exact mechanism of this anti-inflammatory effect. **Acknowledgements:** Capes, Embrapa and CNPq.

#### 04.042

Effect of laser of low power, led and serum therapy in edematogenic effect caused by *Bothrops moojeni* snake venom (BmjV) and two isolated myotoxins. Nadur-Andrade N<sup>1</sup>, Barbosa AM<sup>1</sup>, Gogo JC<sup>2</sup>, Soares AM<sup>3</sup>, Zamuner SR<sup>2</sup> <sup>1</sup>UNIVAP - Pesquisa e Desenvolvimento, <sup>2</sup>UNIVAP - Fisiologia e Farmacologia, <sup>3</sup>FCFRP - Análises Clínicas Toxicológicas e Bromatológicas

**Introduction:** Envenomation caused by snakes of the genus *Bothrops* results in severe local effects in the victim characterized by edema formation, hemorrhage and myonecrosis, beyond serious systemic effect. Currently, the treatment used for ofidic accident is the serum therapy (ST). However, ST has been shown ineffective in the neutralization of local effect caused by the inoculation of the venom. In the present work, we evaluated the edematogenic reaction caused by (BmjV) or its isolated myotoxins (BmjTx I and BmjTx II). Also, we evaluate the ST and the effect of low power laser (LLP) and LED therapy as an alternative treatment in the neutralization of oedema formation. **Methodology:** Male Swiss mice (n=5), were used. Edema was measured by plethysmography after subplantar injection of BmjV (1 mg/paw), myotoxins (10 mg/paw) or saline (control) at 15, 30 min, 1, 3 and 6 h. The LLP was used in the wave length of 685 nm, power 30 mW, density of energy 2,2 J/cm<sup>2</sup>, area 0,2 cm<sup>2</sup>, 15" and the LEDs in the wave lengths 945 and 635 nm, power 110 and 120 mW, density of energy 4 J/cm<sup>2</sup>, area 1,2 cm<sup>2</sup>, time of 41" and 38" for the red LED (LED<sub>r</sub>) and infra-red ray (LED<sub>inf</sub>) respectively, with two applications, at 30 min and 3 h after the injection of the venom, the myotoxins or saline. The antiothropic serum (0.1 mL/5mg; i.v.) was applied immediately to the injection of the venom. **Ethics Committee:** A017/CEP/2008. **Results:** BmjV and myotoxins induced an edematogenic effect from 30 min to 6 h with maximum peak at 1 h, returning to normal values at 24 h. The ST was not efficient in neutralizing the edematogenic effect of the venom. However, the treatment with LLP reduced edema formation caused by the venom and myotoxins. The edema caused by the BmjV was reduced by 75, 78 and 74%, and 72, 74 and 72% for both myotoxins with LLP, LED<sub>inf</sub> e LED<sub>r</sub>, respectively, in the period of 1 up to 6 h after the injection of venom or myotoxins. **Discussion:** The current treatment used for bothropic accident is the serum therapy with bothropic antivenom, however this treatment does not revert the local effect induced by the venom of *Bothrops* genus<sup>(1)</sup>. Because of that, the search for alternatives to treat the local effect induced by *Bothrops* snakebite is essential. BmjV caused edema formation that was not neutralized by bothropic antivenom, with corroborates data from the literature<sup>(2)</sup>. The results demonstrated that the therapy with the LBP and LED infra-red ray and red had presented anti-inflammatory effect, in the used parameters. Similar result was observed in inflammatory model of paw edema induced by carragenina<sup>(3)</sup>, suggesting that the use of laser and LED therapy should be considered as a potentially therapeutic approach for the treatment of local effects of *Bothrops* species. <sup>1</sup>Picolo G, Chacur M, Gutiérrez JM, Teixeira CFP, Cury Y *Bra. J Med. Biol. Res.* 35 (10). P. 1221-1228, 2002; <sup>2</sup>Zamuner RS, Cruz-Hofling MA, Corrado AP, Hyslop S, Rodrigues-Simioni L *Toxicon*, v.44, n.3, p.259-272, 2004; <sup>3</sup>Honmura A, Ishii A, Yanase M, Obata J Haruki E *Lasers Surg Med.* 12: 441-449, 1992. **Financial support:** FAPESP, Fundação Valeparaibana de Ensino.

#### 04.043

Mechanisms involved in the failure of neutrophil chemotactic function in breast cancer patients treated with chemotherapy. Mendonça MAO<sup>1</sup>, Micheli DC<sup>1</sup>, Souto FO<sup>2</sup>, Alves-Filho JC<sup>2</sup>, Cunha FQ<sup>2</sup>, Murta EFC<sup>3</sup>, Tavares-Murta BM<sup>1</sup> <sup>1</sup>UFTM - Biological Sciences, <sup>2</sup>FMRP-USP - Pharmacology, <sup>3</sup>UFTM - Gynecology and Obstetric

**Introduction/Objective:** Cancer patients may present alterations in leukocyte functions. Chemotherapy may alter neutrophil function, even after recovery of bone marrow suppression, and favor an increase in the incidence and/or severity of infections. The aim of this study was to investigate mechanisms involved in the reduction of neutrophil migration after chemotherapy (CHT) in breast cancer patients.

**Methods and Results:** Thirty five breast cancer women at disease stage II to IV (Committee of the International Union against Cancer) and 17 healthy women (control group) were evaluated. The study protocol (number 519) was approved by the UFTM Committee on the Use of Human Subjects, and written informed consent was obtained from patients and volunteers. Peripheral venous blood was collected on diagnosis and 21 days after the 3<sup>rd</sup> or 4<sup>th</sup> cycles of anthracycline-based chemotherapy. Purified circulating neutrophils were assayed in a microchemotaxis chamber. Control neutrophils (n=5) incubated with sera of patients treated with CHT (n=8) displayed dose-dependent reduced migration in response to fMLP, LTB<sub>4</sub> and IL-8, compared to control neutrophils incubated with normal heterologous serum. The levels of nitric oxide (NO) metabolites (Griess reaction) and IL-6, IL-8, IL-10 and TNF- $\alpha$  (ELISA) were quantified in sera of 22 patients and 17 controls. Increased IL-8 concentrations (median; 25% - 75%) were found in patients (34.8; 0.0 – 100.5) than controls (0.0; 0.0 – 28.3) (p=0.02, Mann-Whitney), but cytokine levels did not significantly change after CHT. The NO production, at least in part produced by mononuclear cells, was significantly elevated after CHT (27.2; 18.3 – 45.6) compared to pre-treatment (18.4; 11.1 – 29.8) (p=0.01, Wilcoxon). The expression of the chemokine receptors CXCR1 and CXCR2 and CD11b (flow cytometry) and actin polymerization (fluorescence microscopy) were analysed in neutrophils obtained from 13 patients and 12 controls. The expression of both CXCRs was reduced in patients upon diagnosis, compared to controls, which was maintained after CHT. The actin polymerization (median, 25%-75% of mean fluorescence density) of activated neutrophils was significantly reduced in patients after CHT (8854, 4717 – 10817) compared to pre-treatment (9231, 7159 - 12120) (p=0.001, Wilcoxon). **Discussion/Conclusion:** Elevated systemic levels of IL-8 and the reduced expression of CXCRs may impair neutrophil migration in breast cancer patients. In patients treated with anthracycline-based chemotherapy, the production of circulating inhibitory mediators, such as NO, was associated with a reduction in the polymerization of actin filaments in neutrophils. **Financial support:** FAPEMIG, CNPq, CAPES, FINEP, FUNEPU.

#### 04.044

Role of PI3K- $\gamma$  pathway in the inflammatory response induced by dengue virus infection in mice. Valadão DF<sup>1</sup>, Costa VV<sup>2</sup>, Santos AG<sup>1</sup>, Morcatty TQ<sup>2</sup>, Amaral FA<sup>1</sup>, Fagundes CT<sup>2</sup>, Cisalpino D<sup>2</sup>, Silveira KD<sup>3</sup>, Lima CX<sup>4</sup>, Teixeira MM<sup>2</sup>, Souza DG<sup>1</sup> <sup>1</sup>UFMG - Microbiologia, <sup>2</sup>UFMG - Bioquímica e Imunologia, <sup>3</sup>UFMG - Fisiologia e Biofísica, <sup>4</sup>UFMG - Fisiologia e Farmacologia

**Introduction:** Dengue is one of the most important vector-borne viral diseases in the world. It's caused by four dengue related virus serotypes (DENV 1-4) and is transmitted by *Aedes* mosquito. Phosphoinositide 3-kinase (PI3K) is a family of enzymes that have important roles on signal transduction, regulation of cell activation, growth, differentiation, survival, migration and proliferation. **Objectives:** The aim of this study is to evaluate the role of PI3K- $\gamma$  pathway on inflammatory response induced by two different serotypes of dengue virus. **Methods:** This project was previously approved by CETEA/UFMG on number access 113/09. Wild type mice (C57BL/6) and knockout mice to PI3K- $\gamma$  (PI3K- $\gamma^{-/-}$ ) were infected with the adapted DENV-2 and DENV-3 serotypes by intraperitoneal route. After infection, lethality of animals was accompanied. We made the evaluation of disease signals (hematocrit, platelets and hepatic transaminases levels in serum), evaluation of inflammatory parameters (cytokines, chemokines, neutrophils (MPO) on spleen, liver and lungs. Tissue damage was verified by histological analyses in liver and lungs of both animals, and viral load in target organs was quantified by plaque assay. **Results and discussion:** PI3K- $\gamma^{-/-}$  mice exhibit a protective phenotype during DENV infection by the two serotypes. They showed only 40% (DENV-2) and 14% (DENV-3) of mortality in comparison of WT mice that had 100% and 80% of letality, respectively. The index of platelets in DENV-2 infected mice was 45% higher in PI3K- $\gamma^{-/-}$  mice in comparison with WT mice (Mean values: NI: 619.0, WT: 254.0 and PI3K- $\gamma^{-/-}$ : 533.0 platelets X10<sup>3</sup>/mL of blood) and in DENV-3 infected mice were 16% higher if compared with WT mice (Mean values: NI: 1175.0, WT: 645.0 and PI3K- $\gamma^{-/-}$ : 825.0 platelets X10<sup>3</sup>/mL of blood). Hematocrit levels in blood of DENV-2 infected PI3K- $\gamma^{-/-}$  mice were 14% lesser than WT mice (Mean values: NI: 41%, WT: 49% and PI3K- $\gamma^{-/-}$ : 44%) although, in DENV-3 infected mice this reduction was 31% (Mean values: NI: 34%, WT 48% and PI3K- $\gamma^{-/-}$ ), showing better clinical state of these animals. Hepatic transaminases (TGO) were 6X lower in PI3K- $\gamma^{-/-}$  mice (NI:30, WT: 344 and PI3K- $\gamma^{-/-}$  149 U/mL serum) and TGP were 20X lower (Mean values: NI: 50, WT: 1366 and PI3K- $\gamma^{-/-}$  361 U/mL serum) in comparison with WT mice in DENV-3 infected animals. Its can be correlated with less tissue damage showed in histological analyses in PI3K- $\gamma^{-/-}$  mice. TNF- $\alpha$  in DENV-2 infected mice were 8X lower in PI3K- $\gamma^{-/-}$  mice when compared with WT mice (Mean values: NI: 23 pg/mL, WT 412 pg/mL and PI3K- $\gamma^{-/-}$  207 pg/mL in spleen) and IL-6 levels were 33X lower in PI3K- $\gamma^{-/-}$  (Mean values: NI: 48 pg/mL, WT: 2929 pg/mL and PI3K- $\gamma^{-/-}$ : 1344 pg/mL). This results show the important participation of PI3K $\gamma$  pathway in DENV infection. **Financial Support:** INCT-CNPq, CAPES and FAPEMIG.

#### 04.045

Selective blockade of bradykinin-2 receptor activation reduces inflammatory response in an experimental model of articular acute gout. Morcatty TQ<sup>1</sup>, Costa VV<sup>1</sup>, Santos AG<sup>1</sup>, Valadão DF<sup>1</sup>, Amaral FA<sup>2</sup>, Coelho FM<sup>2</sup>, Tavares LD<sup>1</sup>, Teixeira MM<sup>2</sup>, Souza DG<sup>1</sup> <sup>1</sup>UFMG - Microbiologia, <sup>2</sup>UFMG - Bioquímica e Imunologia

**Introduction:** Gout arthritis is characterized by precipitation of monosodium uric acid crystals (MSUs) on several tissues, mainly on articular cavity, where can develop a local inflammation that can compromise the life quality of the patient. Basic mechanism of this disease is the intense neutrophil recruitment with the release of cytokines, chemokines, arachdonic acid metabolites, proteases and oxygen radicals. However, there are no efficient drugs against the symptoms of gout. Bradykinin (BK) is a vasoactive peptide that has been implicated in several inflammatory conditions. BK acts on two distinct receptors: B2 and B1 receptors. Here, we evaluated the role played by each BK receptor in an experimental model of articular acute gout. **Methods:** B2R deficient (B2R<sup>-/-</sup>), B1R-deficient (B1R<sup>-/-</sup>), B1R/B2R-deficient (B1R/B2R<sup>-/-</sup>), and their respective wild type (WT) control mice were used. UAC were prepared by precipitation of the crystals after the uric acid added in borate buffer solution (pH 8.5). For the experiments, a dose of 100µg of UAC was diluted (borate buffer plus uric acid – pH 8.5) and a volume of 10µL was injected in the tibio-femoral joint. Hypernociception was measure by a digital analgesimetro (Insight mod. EFF-301). Sample of periarticular tissue were removed for neutrophil quantification (MPO). A joint lavage (BSA 3%; 10µL) was been to evaluate the cell infiltration on articular space, which was performed total cell (Neubauer clamber) and differential count (Cytospin3 - Shandon). All procedures have been approved by local ethics committee - CETEA/UFMG (protocol 165/08). **Results:** Using C57/BL6 mice, we made a temporal curve (3, 6, 9, 12, and 24 hours after UAC injection) to evaluate the best time according to inflammatory parameters analyzed. The time chosen was 6 and 15 hours after the MSU injection, where there were an increase in hypernociception, elevated MPO levels and neutrophils in knee cavity and periarticular tissue compared to vehicle mice. BR2<sup>-/-</sup> but not, BR1<sup>-/-</sup> and BR1/BR2<sup>-/-</sup> mice, showed important reduction in neutrophil infiltration in knee cavity and MPO levels in periarticular tissue after the MSU injection compared to WT ones. These results were accompanied to a reduction of the intensity of hypernociception in BR2<sup>-/-</sup> animals. **Discussion:** Our results present an interesting animal model of acute gout due to a direct injection of MSU on articular joint as well as the measurement of the hypernociception in these mice. BR2<sup>-/-</sup>mice showed lesser inflammatory conditions compared to WT littermates, suggesting an important mechanism of how gout disease can promote the compromising of articular homeostasis, and showing an important role o bradykinin in this model. Financial support: CNPq/ CAPES/ FAPEMIG.

#### 04.046

Efeito do extrato bruto e das frações da *Cayaponia podantha* sobre a resposta inflamatória tópica. Oliveira de Melo J, Arruda LLM, Bracht L, Truiti MCT, Caparroz-Assef SM, Cuman RKN, Bersani-Amado CA UEM - Farmácia e Farmacologia

**Introdução:** Várias espécies do gênero *Cayaponia* tem sido utilizadas na medicina tradicional com finalidade analgésica, antimicrobiana, antiofídica, diurética, depurativa e no tratamento de doenças cutâneas. No presente estudo foi investigado o efeito do extrato bruto e das frações da *Cayaponia podantha* (Cp) sobre a resposta inflamatória tópica utilizando o teste de edema de orelha induzido por óleo de cróton (Oc). Também foi avaliada a atividade da enzima mieloperoxidase (MPO), um marcador de influxo celular. **Métodos:** Foram utilizados camundongos Swiss (25-30 g). O edema de orelha foi induzido pela aplicação tópica de Oc (200 µg) na face interna das orelhas de camundongos. A seguir foram aplicados topicamente 20 µl do extrato bruto da Cp nas doses de 1,25; 2,5 e 5,0 mg, e 20 µl das frações hexânica (FH), acetato (FA) e metanólica (FM), na dose de 5,0 mg, na orelha esquerda. Na orelha direita foi aplicado 20 µl do veículo. Após 6 h os animais foram submetidos à eutanásia, as orelhas seccionadas e pesadas em balança analítica. Posteriormente, as secções das orelhas foram empregadas para a determinação da atividade da MPO. O protocolo para esses experimentos foi aprovado pelo Comitê de Conduta Ética no uso de Animal em Experimentação da UEM (024/2006). Os dados foram avaliados estatisticamente usando Anova seguida do teste de Tukey. **Resultados:** A aplicação do Oc induziu uma reação inflamatória intensa na orelha de camundongos e um aumento significativo na atividade da MPO. O extrato bruto e as frações da Cp inibiram significativamente a intensidade do edema (Cp<sub>2,5</sub>= 48% P<0,01; Cp<sub>5,0</sub>=64%; FH<sub>5,0</sub>=81% e FA<sub>5,0</sub>=67% P<0,001) e a atividade da MPO (Cp<sub>2,5</sub>= 95%; Cp<sub>5,0</sub>=99%; FH<sub>5,0</sub>=90% e FA<sub>5,0</sub>=89% P<0,001). A aplicação tópica do extrato bruto de Cp na dose de 1,25 mg e da FM na dose de 5,0 mg, não alterou o desenvolvimento do edema de orelha e a atividade da MPO. **Discussão:** Estes resultados mostraram que o extrato bruto e as frações da Cp apresentam efeito Anti-inflamatório tópico quando aplicados em camundongos. Entretanto, é importante ressaltar que as frações FH e FA, apresentaram maior eficácia indicando que os constituintes do extrato, responsáveis por tal atividade, possuem estruturas químicas com característica apolar. **Apoio Financeiro:** CAPES/CNPq

#### 04.047

Aprotinin treatment inhibits eosinophil recruitment induced by ovalbumin in immunized mice. Braga AD, Miranda JP, Ferreira GM, Klein A ICB-UFMG - Farmacologia

**Introduction:** Aprotinin is a potent *in vitro* and *in vivo* proteinase inhibitor known to inhibit trypsin and kallikrein. Here, we evaluated the effects of systemic or local administration of aprotinin on the eosinophil recruitment induced in response to allergen challenge in sensitized mice, and their role on eotaxin-1/CCL11 or leukotriene (LT)<sub>4</sub>-induced eosinophil recruitment. **Methods:** BALB/c mice were immunized with ovalbumin (OVA) in aluminum hydroxide, and fifteen days after the first immunization challenged through the intra pleural (i.pl.) injection of OVA 15 minutes after the aprotinin i.pl. injection or 1 h after subcutaneous (s.c.) administration of aprotinin. Eosinophil migration was assessed after 48 h. (UFMG Animal Ethics Committee Protocol number 079/08). **Results:** Both local aprotinin injection (0.01 mg.kg<sup>-1</sup>) or systemic aprotinin injection (1mg.kg<sup>-1</sup>) inhibited the eosinophil recruitment induced by OVA (i.pl.: PBS+PBS, 0.2 ± 0.08; PBS + OVA, 0.8 ± 0.2; \*Aprotinin + OVA, 0.04 ± 0.01.10<sup>5</sup> eosinophils/cavity, \*p<0.01 ANOVA followed by Tukey test; s.c: PBS + PBS, 0.7 ± 0.1; PBS + OVA, 11.3 ± 2.0; \*Aprotinin + OVA, 3.0 ± 0.5. 10<sup>5</sup> eosinophils/cavity, \*p<0.01 ANOVA followed by Tukey test). Pretreatment with aprotinin s.c. also inhibited eosinophil recruitment induced by eotaxin/CCL11 (PBS, 0.3 ± 0.2; Eotaxin, 2.5 ± 0.3; \*Aprotinin + Eotaxin, 0.6 ± 0.1. 10<sup>5</sup> eosinophils/cavity, \*p<0.01 ANOVA followed by Tukey test), but did not inhibit the eosinophil migration induced by LTB<sub>4</sub> (PBS, 2.5 ± 0.7; LTB<sub>4</sub>, 9.5 ± 1.7; Aprotinin + LTB<sub>4</sub>, 10.4 ± 2.1. 10<sup>5</sup> eosinophils/cavity). **Discussion:** The results are consistent to show the ability of aprotinin to inhibit eosinophil migration into mouse pleura cavity, and proteinases involvement modulating the eosinophil recruitment induced by eotaxin-1/CCL11, a C-C chemokine known to activate eosinophils *in vitro* and induce their migration *in vivo*. Moreover aprotinin does not appear to act via the inhibition of LTB<sub>4</sub> action. Taken together our findings suggest that eotaxin-1/CCL11 effects on eosinophil migration could be modulated by proteinases such as trypsin or kallikreins. **Financial support:** FAPEMIG.

#### 04.048

Study of the inflammatory reaction induced by *Bothrops jararacussu* snake venom (BjsuV) and two isolated myotoxins. Guimarães-Souza L<sup>1</sup>, Barbosa AM<sup>1</sup>, Gogo JC<sup>2</sup>, Soares AM<sup>3</sup>, Zamuner SR<sup>1</sup> <sup>1</sup>UNIVAP - Inflammation, <sup>2</sup>UNIVAP - Physiology, <sup>3</sup>FCFRP-USP Clinical Analysis, Toxicology and Bromatology

**Introduction:** Venoms from snakes of genus *Bothrops* cause pronounced local inflammatory reaction in the victims, which are characterized by oedema formation and leukocyte influx. In the present work, the ability of BjsuV and two toxins isolated from this venom, BthTX I and II, to cause oedema formation and leukocyte influx were investigated. Also, we use the Laser therapy of low power (LBP) and LED as an alternative treatment in the neutralization of oedema formation and leukocyte influx, caused by the venom and myotoxins. **Methodology:** Groups of male Swiss mice (n=5) were used. The edema was measured by plethysmography after subplantar injection of BjsuV (2.5 mg/paw) or BthTX I or BthTX II (10 mg/paw) or saline (control). The leukocyte influx was evaluated 6 h after venom or myotoxins injection in the peritoneal cavity, after injection of 5 µg of the venom, 40 and 20 µg of BthTX I and II, respectively. The LBP was used in the wave length of 685 nm, power 30 mW, density of energy 2,2 J/cm<sup>2</sup>, area 0,2 cm<sup>2</sup>, 15" and the LEDs in the wave lengths 945 and 635 nm, power 110 and 120 mW, density of energy 4 J/cm<sup>2</sup>, area 1,2 cm<sup>2</sup>, time of 41" and 38" for the red LED (LEDr) and infra-red ray (LEDinf) respectively, being two applications, at 30 min and 3 h after the injection of the venom, toxins or saline. **Ethics Committee: A028/CEP/2007. Result:** The venom of BjsuV and myotoxins were able to cause a significant edema formation between 30 min and 6 h with a peak at 1 h. The LBP and the LEDs, in the used parameters, had reduced the oedema formation. The edema caused by the BjsuV, at 1 h peak, was reduced by 39, 41 and 46%, by 49, 54 and 67% for BthTX I and by 51, 61 and 58% for BthTX II with LBP, LEDinf e LEDv, respectively. BjsuV caused a significant increase of leukocytes influx at 6 h, as well as toxins. The treatment with the laser and LEDs caused a reduction of Polymorphonuclears cells (PMN), in the order to 88, 90 and 84% after the injection of BjsuV; 54, 83 and 70% after BthTX I and by 55, 78 48% after the injection of BthTX II, with LBP, LEDinf e LEDv, respectively. **Discussion:** These results indicated that the BjsuV and myotoxins induced oedema formation and leukocyte influx in the local of their injection, these data are in accordance with Olivo et al., (2007)<sup>(1)</sup> and Zuliani (2005)<sup>(2)</sup>. The therapy with the LBP and LEDs had presented anti-inflammatory effect, in the used parameters, and these results are in accordance with others from the literature<sup>(3; 4)</sup>, suggesting that the use of laser and LED therapy should be considered as a potentially therapeutic approach for the treatment of local effects of *Bothrops* species. <sup>(1)</sup>Olivo, R.A. *Toxicon* 49, 670, 2007; <sup>(2)</sup>Zuliani, J.P. *Toxicon*, 45, 335, 2005.; <sup>(3)</sup>HONMURA, A. *Lasers Surg Med.* 12: 441, 1992.; <sup>(4)</sup>Barbosa, A.M. *Toxicon*, 51, 1236, 2008. Gratefulness to the financial support: FAPESP, Fundação Valeparaibana DE Ensino



#### 04.049

Anti-inflammatory potential of the extracts and fractions of *Baccharis trimera*. Freitas GM, Vigliano MV, Sabino KCC, Coelho MGP UERJ - Bioquímica

**Introduction:** *Baccharis trimera* (family Asteraceae) is a plant known as “carqueja”, which is found in the South America and has been used to treat gastrointestinal and liver diseases, angine, diabetes and inflammatory processes. **Material and Methods:** The plant was collected and identified in the Herbarium Bradeanum (HB 86447). The extracts were prepared by immersing 30 g of dry material in distilled water (EABt) or ethanol 80% (EEBt) for 24 h. The fractions of EEBt were obtained by a liquid-liquid partition of the extract made with hexane, dichloromethane and ethyl acetate. The anti-inflammatory activity was analyzed in two models. In the air pouch model, male SW mice received injections of sterile air in the back on days 0 and 3, to form a cavity; on the day 6, animals were treated with ASA 100 mg/kg and at different doses of the extracts and fractions by i.p. route, followed by injection of carrageenan 1% in the cavity 1 h after treatment; after 4 h, the animals were sacrificed and the exudate collected and the total and differential cell determined. The tissues were removed and examined macroscopically by observing the occurrence of vasodilatation, as well as the presence of fibrin. In the model of vascular permeability, male SW mice were treated with indomethacin 10 mg/kg and the doses of 0.3 and 3.0 mg/kg of extracts, followed by injection of the Evans blue (i.v.) 1 h after treatment; then, the animals received an injection of acetic acid 0.7% (i.p.) and 30 min later were sacrificed, the peritoneal cavity washed with saline 0.9% and the absorbance read at 630 nm. All animal experiments were approved by the ethics committee of IBRAG-UERJ (protocol 05/2009). **Results:** The results for the vascular permeability model were: control ( $0.352 \pm 0.049$ ); Indomethacin ( $0.21 \pm 0.058$ ); EABt 0.3 mg/kg ( $0.158 \pm 0.010$ ); EABt 3 mg/kg ( $0.135 \pm 0.026$ ); EEBt 0.3 mg/kg ( $0.135 \pm 0.026$ ); EEBt 3 mg/kg ( $0.158 \pm 0.060$ ). In the air pouch model, received the following rates of inhibition of cell migration: ASA (46.8%), EABt (28.3%), EEBt (48.5%), Fr DCM 30 mg/kg (88.0 %), Fr DCM 300 mg/kg (68.5%), Fr HEX 30 mg/kg (69.7%) and Fr HEX 300 mg/kg (79.8%). Regarding the differential count, the extracts and fractions affected only the migration of neutrophils. All results represent the mean $\pm$ SD and analysed by ANOVA followed by Tukey post-test. **Discussion:** The analysis of these results demonstrate that the extracts of *Baccharis trimera* inhibit the increased vascular permeability, and block the migration of cells, particularly neutrophils, to the inflammatory site. The fractions of ethanolic extract were also able to inhibit cell migration. The analysis of tissues showed severe reduction in vasodilatation as well as fibrin deposition in animals treated with the extracts and fractions of this plant. These activities may be related to inhibition of synthesis of inflammatory mediators that induce vasodilatation, for example, nitric oxide and prostaglandins, as well as chemotactic factors. **Financial support:** CNPq, FAPERJ e UERJ.

#### 04.050

PI3K $\gamma$  is crucial for inflammatory reaction caused by graft *versus* host disease. Castor MGM<sup>1</sup>, Rezende B<sup>2</sup>, Bernardes, PTT<sup>3</sup>, Silva AFC<sup>3</sup>, Resende BC<sup>2</sup>, Vieira AT<sup>2</sup>, Arantes RME<sup>4</sup>, Silva TA<sup>5</sup>, Teixeira MM<sup>2</sup>, Pinho V<sup>3</sup> <sup>1</sup>UFMG - Fisiologia e Farmacologia, <sup>2</sup>ICB-UFMG – Bioquímica e Imunologia, <sup>3</sup>ICB-UFMG - Morfologia, <sup>4</sup>UFMG - Patologia Geral, <sup>5</sup>FMRP-USP - Farmacologia

**Introduction:** Allogeneic hematopoietic stem cell transplantation (HSCT) is an evolving technology in cancer therapeutics. One of the most important complications associated with this increasingly common treatment is acute graft-versus-host disease (GVHD), the immunologic attack of transplanted donor T lymphocytes against foreign host tissues. Mononuclear phagocytes and other leukocytes are thought to be responsible for both initiation of graft-versus-host reaction and for the subsequent injury to host tissues after complex interactions with cytokines and chemokines. Chemotactic factors bind to seven-transmembrane-domain receptors and activate heterotrimeric G-proteins. Downstream of these proteins a complex interrelated signaling network is activated and may result in the activation of PI<sub>3</sub>K isoforms mainly PI<sub>3</sub>K $\gamma$ . This study proposed to verify the role of PI<sub>3</sub>K $\gamma$  to development of GVHD. **Methods:** To GVHD induction the recipients mice, B6D2F1, were exposed to sublethal dose of gamma radiation (4 Gy). Two days after the transplant, the recipients mice received intravenously  $3 \times 10^7$  splenocytes of parental mice, C57BL/6J (WT) or C57BL/6J PI3k $\gamma$ <sup>-/-</sup>. This study was approved by ethics committee of UFMG (CETEA) number 024/09. After transplant, there was monitoring to evaluation of disease clinical signs and inflammatory reaction. **Results and discussion:** Our data demonstrated that absence of PI<sub>3</sub>K $\gamma$  in donor cells prevented mortality and diminished weight loss. Also decrease the occurrence and severity of disease clinical and histopathological signs. Additionally, decreased the concentration of pro-inflammatory cytokines and chemokines including TNF- $\alpha$ , IFN- $\gamma$ , CCL2, CCL3 and CCL5 in small intestine. Also, the recruitment of CD8<sup>+</sup>, CD4<sup>+</sup>, CD11c<sup>+</sup> cells and macrophages to the small intestine was decrease. In liver, we showed a decrease of CCL3 level and a reduction of CD8<sup>+</sup> cells and macrophages. Mechanistically, we observed an inhibition of leukocytes rolling and adhesion in intestine venules when used a PI<sub>3</sub>K $\gamma$  selective inhibitor and also when the donor cells are deficient to gene of PI<sub>3</sub>K $\gamma$ . Despite decreased GVHD, the absence of PI<sub>3</sub>K $\gamma$  in donor cells still dealt normally with injection of the mastocytoma cell line P815 remains the graft versus tumor response. This study suggests that the inhibition of PI<sub>3</sub>K $\gamma$  in donor cells may be an interesting therapeutic strategy to control inflammatory response associated to GVHD without affecting the ability of the graft to deal with leukemia cells. Financial support: CAPES, CNPq e Fapemig.

#### 04.051

*Lonomia obliqua* venom-induced cytoskeletal alterations and increased expression of inflammatory molecules in endothelial cell. Rodrigues GS<sup>1</sup>, Nascimento-Silva V<sup>1</sup>, Moraes JA de<sup>1</sup>, Guimarães JA<sup>2</sup>, Barja Fidalgo TC<sup>1</sup> <sup>1</sup>UERJ - Farmacologia, <sup>2</sup>UFRGS - Centro Biotecnologia

**Introduction:** The contact with the caterpillar *Lonomia obliqua* causes numerous accidents, especially in the southern region of Brazil, where it is considered a public health problem. The *Lonomia obliqua* venom causes disseminated intravascular coagulation and a consumptive coagulopathy, that can lead to a hemorrhagic syndrome. The venom also causes hemorrhage, but through increased fibrinolysis. *In vivo* and *in vitro* studies have shown that this venom contains several toxins with procoagulant, anticoagulant and antithrombotic activities. These toxins are also able to induce activation of the endothelium. In this work, we aim to define the effects of *L. obliqua* venom on endothelial cell (EC) activation, evaluating the changes in the cytoskeleton dynamics and the expression of pro-inflammatory molecules. **Materials and Methods:** *L. obliqua* caterpillar bristle extract (LOCBE) was obtained as described (Bohrer et al., *Toxicon* 2007 49:663-9). Cell viability was assessed by Trypan blue exclusion. EC were incubated in the absence or in the presence of the LOCBE (1-30 µg/mL) for different periods of time. Protein levels were detected by Western blot analysis. F-actin was stained with rhodamine phalloidin. **Results:** The treatment of EC with LOCBE did not affect cell viability. However, LOCBE was also able to induce actin cytoskeleton alterations as well as focal adhesion kinase (FAK) phosphorylation and its subsequent association to actin. Importantly, EC treated with LOCBE showed increased expression of COX-2, NOS-2 and HO-1. The signaling pathways involved in such effects are under investigation. **Discussion:** Initially the venomous proteins act enzymatically on the victim's plasma components generating effects such as intense coagulation, fibrinolysis and some degree of inflammation. In a second moment, venom components would stimulate a cellular response through up-regulation of several genes, causing activation and migration of inflammatory cells. In this work we show that LOCBE exerts a direct effect on endothelial cells, increasing the expression of molecules which are crucial to the onset of inflammatory responses. These findings enhance our understanding of Ionomism and may contribute to the development of more effective treatments in order to control the symptoms associated to the contact with this caterpillar. **Financial Support:** FAPERJ, CNPq, SR-2/UERJ

#### 04.052

PAF participates of the inflammatory reaction caused by graft versus host disease (GVHD). Rezende B<sup>1</sup>, Castor MGM<sup>2</sup>, Bernardes PTT<sup>3</sup>, Silva AFC<sup>4</sup>, Vieira AT<sup>4</sup>, Vilela MC<sup>5</sup>, Resende BC<sup>4</sup>, Teixeira MM<sup>4</sup>, Pinho V<sup>4</sup> <sup>1</sup>ICB-UFMG - Bioquímica e Imunologia e Morfologia, <sup>2</sup>UFMG - Fisiologia e Farmacologia, <sup>3</sup>ICB-UFMG - Morfologia, <sup>4</sup>UFMG - Bioquímica e Imunologia, <sup>5</sup>ICB-UFMG

**Introduction:** Acute GVHD occurs when transplanted donor-derived T cells recognize and react to histo-incompatible recipient antigens and cells. It is a rapidly progressive syndrome characterized by profound wasting, immunosuppression and target organs injury by donor cells, including the intestines, liver, spleen, skin and lung. Platelet Activation Factor is a lipidic inflammatory mediators that participate of cell recruitment to injury areas. This study proposes to verify the effects of donor cells PAF absence in acute GVHD. **Methods:** For induction of GVHD the recipient mice, B6D2F1, were exposed to a sublethal dose (4 Gy) of gama radiation. Two days after, they received intravenously  $3 \times 10^7$  splenocytes of parental mice, C57BL/6J (WT) or C57BL/6J PAF<sup>-/-</sup>. After transplant, there was monitoring for evaluation of clinical disease and inflammatory reaction. **Results and discussion:** Absence of PAF in donor cells (PAF<sup>-/-</sup>) prevented mortality, reduced the occurrence and severity of clinical signs of disease. It also reduced bacterial translocation to peritoneal cavity, blood and liver and the levels of pro-inflammatory cytokines and chemokines (TNF-alpha, IFN-gamma, CCL2) in the small intestine of GVHD mice. A diminution in the rolling and adhesion of leukocytes in the mesenteric venules was also observed in GVHD mice treated with a PAF specific blocker (PCA4248). Furthermore, we observed that graft versus leukemia (GVL) effect did not modify by PAF absence in donor cells. Graft versus leukemia is the graft effect against tumor, eliminate the remains tumor. This way PAF participates of GVHD inflammatory responses and without to interfere in the action of donor cells against tumor cells (GVL) may be an interesting therapeutic application among the management strategies of this disease.

#### 04.053

Sexual dimorphism on cytokines expression in the temporomandibular joint: the role of gonadal hormones. Torres-Chavez KE<sup>1</sup>, Fischer L<sup>2</sup>, Teixeira JM<sup>1</sup>, Parada CA<sup>3</sup>, Tambeli CH<sup>1</sup> <sup>1</sup>FOP-UNICAMP - Physiology, <sup>2</sup>UFPR - Physiology, <sup>3</sup>IB-UNICAMP - Physiology and Biophysics

**Introduction:** Temporomandibular joint (TMJ) pain conditions are more prevalent and severe in women than in men and are generally characterized by a local inflammatory reaction of the articular tissue. Although therapeutic approaches have been developed with the purpose of inhibit pro-inflammatory cytokines action, little studies have been focused on the role of gonadal hormones on the cytokines expression. The aim of this study was to investigate whether gonadal hormones affect formalin-induced cytokines expression in the rat TMJ. **Methods:** The study was approved (N<sup>o</sup>905-1) by the Committee on Animal Research of the University of Campinas. Male and female wistar rats were used. Formalin (1.5%) or its vehicle (0.9% NaCl) was injected in TMJ and the expression of TNF- $\alpha$ , IL1- $\beta$ , IL-6 and CINC-1 was measured, using an immunoassay ELISA kit, in intact and gonadectomized animals receiving or not hormone administration. Data were analyzed by ANOVA and Tukey post hoc test ( $p \leq 0.05$ ) and the results are expressed as mean  $\pm$  SEM. **Results:** The expression of TNF- $\alpha$ , IL-1 $\beta$  and CINC-1 was significantly greater in males (477 $\pm$ 41; 1687 $\pm$ 138; 250 $\pm$ 28, respectively) than in proestrus (359 $\pm$ 18; 1083 $\pm$ 87; 118 $\pm$ 9, respectively) or diestrus (319 $\pm$ 12; 834 $\pm$ 47; 121 $\pm$ 7, respectively) females, while the expression of IL-6 was significantly greater in diestrus (4649 $\pm$ 345) and proestrus (4238 $\pm$ 546) females than in males (972 $\pm$ 102). The expression of TNF- $\alpha$ , IL-1 $\beta$  and CINC-1 was similar between sham-operated proestrus (370 $\pm$ 16; 1048 $\pm$ 110; 149 $\pm$ 18, respectively), sham-operated diestrus (347 $\pm$ 30; 750 $\pm$ 115, respectively) and ovariectomized (OVX) females (364 $\pm$ 25; 1233 $\pm$ 137; 153 $\pm$ 18, respectively) and between OVX receiving vehicle (358 $\pm$ 19; 1335 $\pm$ 151; 188 $\pm$ 12, respectively), estradiol (344 $\pm$ 25; 1034 $\pm$ 60; 108 $\pm$ 3, respectively) or progesterone (348 $\pm$ 19; 1095 $\pm$ 100; 99 $\pm$ 4, respectively) administration. The expression of IL-6 was significantly greater in sham-operated proestrus (5751 $\pm$ 1036) and diestrus (5077 $\pm$ 1148) than in OVX females (888 $\pm$ 61) and it was significantly greater in OVX females receiving estradiol (3259 $\pm$ 208) or progesterone (3231 $\pm$ 330) administration than in those receiving vehicle (1019 $\pm$ 69). The expression of TNF- $\alpha$ , IL-1 $\beta$  and CINC-1 was significantly greater in sham-operated (437 $\pm$ 41; 1749 $\pm$ 112; 338 $\pm$ 40; respectively) than in ORX males (296 $\pm$ 16; 927 $\pm$ 128; 110 $\pm$ 9; respectively) while the expression of IL-6 was similar between sham-operated (999 $\pm$ 130) and ORX (696 $\pm$ 60) males. **Discussion:** This study demonstrated that the release of TNF- $\alpha$ , IL-1  $\beta$ , CINC-1 and IL-6 during TMJ inflammation is modulated by gonadal hormones. Testosterone appears to increase the release of TNF- $\alpha$ , IL-1  $\beta$  and CINC-1, while ovarian hormones increase the release IL-6. The clinical approaches developed with the purpose of inhibiting the action of pro-inflammatory cytokines are starting to supplant traditional immunosuppressive therapies and further studies should confirm the importance of taking into account the hormonal status of the patients before determining the more effective therapeutic agent and its dose. **Financial Support:** CAPES and FAPESP, Brazil.

#### 04.054

Obesity enhances the eosinophil trafficking from bone marrow to lung parenchyma in obese mice. Calixto MC<sup>1</sup>, Lintomen L<sup>1</sup>, Schenka A<sup>2</sup>, Saad MJA<sup>3</sup>, Antunes E<sup>1</sup>  
<sup>1</sup>UNICAMP - Farmacologia, <sup>2</sup>FCM-Unicamp - Patologia, <sup>3</sup>UNICAMP - Clínica Médica

**Introduction:** Increases in eosinophil (EO) numbers in the tissues, blood, and bone marrow (BM) are a hallmark of asthma and, in general, elevated numbers correlate with disease severity. Epidemiological data indicate that obesity increases the prevalence and incidence of allergic asthma (Camargo et al., 1999). Increased fat mass, particularly with central obesity, leads to production of cytokines and chemokines, such as IL-6, TNF- $\alpha$  and eotaxin (Vasudevan et al, 2006). Studies have shown that genetically obese mice exhibit innate airway hyperresponsiveness (Shore et al., 2003; Shore & Johnston, 2006), but little attention has been given to the allergic pulmonary EO recruitment in obese animals. Since selective accumulation of eosinophils into the airways has become a central concept of the asthma pathology, this study was designed to evaluate the time-course eosinophil trafficking from bone marrow to peripheral blood, and recruitment into the airways in obese allergic mice, along with measurements of levels of Th1/Th2 cytokines, adipocytokines and eotaxin in OVA-sensitized C57BL/6 obese mice have been carried out in this study. **Methods and Results:** Male C57bl6/J mice (initial weigh 14.5 $\pm$ 0.9 g) received a high fat diet for 10 weeks. On the eighth week, animals were sensitized with a s.c. injection of OVA (100  $\mu$ g dissolved in Al(OH)<sub>3</sub>). Two weeks thereafter, mice were intranasally challenged with OVA (10  $\mu$ g), after which eosinophil counts in bronchoalveolar lavage fluid (BALF) and bone marrow were evaluated. The experimental protocols were approved (n<sup>o</sup>1496-1) by the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation (COBEA). High-fat diet mice exhibited a significant increase in body weight and epididymal fat, as well as increased total serum cholesterol levels compared with non-obese groups. Intranasal challenge with OVA in sensitized mice largely increased the EO counts in BAL at 48 h and 72 h post-OVA challenge (0.67 $\pm$ 0.06 and 0.12 $\pm$ 0.03 $\times$ 10<sup>6</sup>/BAL, respectively). Eosinophils were nearly absent in the non-sensitized mice. The sensitized obese mice showed a delayed EO emigration to BAL, peaking at 72 h post-OVA challenge (0.2 $\pm$ 0.04 $\times$ 10<sup>6</sup>/BAL). In addition, the morphological analysis showed that lung parenchyma of sensitized obese mice presented a markedly higher EO infiltration at both 48 h and 72 h post-OVA challenge when compared with non-obese mice. In BM, a significant increase in counts of both mature and immature EO was also found in sensitized obese (1.73 $\pm$ 0.32 $\times$ 10<sup>6</sup>/ml and 0.49 $\pm$ 0.07  $\times$ 10<sup>6</sup>/ml, respectively) compared with sensitized non-obese mice (0.28 $\pm$ 0.07 $\times$ 10<sup>6</sup>/ml and 0.15 $\pm$ 0.05 $\times$ 10<sup>6</sup>/ml, respectively). The levels of TNF- $\alpha$ , IL-6, IL-10, IL-5 and eotaxin significantly increased in BAL of sensitized obese mice, peaking at 72 h-post OVA challenge. **Conclusion:** We have established an experimental model in C57bl6/J obese mice that clearly show a potentiation of EO influx in response to OVA challenge. In obese mice, EO are likely to be retained in the lung parenchyma exerting their effector functions in promoting the pathogenesis of airways diseases. **References:** Camargo, C.A. et al. *Arch Intern Med.* 1999, 159(21):2582-8. Vasudevan, A.R. et al. *J Clin Endocrinol Metab*, 2006, 91(1):256–261. Shore, S.A. et al.. *J Appl Physiol.* 2003, 95(3):938-45. Shore, S.A, Johnston R.A.. *Pharmacol. Ther.*, 2006, 110(1):83-102. **Financial support:** CAPES

#### 04.055

Estrogen and androgen receptors mediate the effect of estradiol and testosterone on formalin-induced temporomandibular joint inflammation. Torres-Chavez KE<sup>1</sup>, Fischer L<sup>2</sup>, Tambeli CH<sup>1</sup> <sup>1</sup>FOP-UNICAMP - Physiology, <sup>2</sup>UFPR - Physiology

**Introduction:** Pain in the temporomandibular joint (TMJ) region is a major symptom associated with temporomandibular disorders, which are generally characterized by a local inflammatory reaction of the articular tissue. We have previously demonstrated that formalin-induced TMJ nociception and inflammation, are decreased during high ovarian hormone levels of rat the estrous cycle, as well as by estradiol or testosterone administration in gonadectomized rats. Taken together, these findings suggest that the attenuation of TMJ inflammation is a potential mechanism by which estradiol and testosterone attenuate TMJ nociception. The aim of this study was to investigate if the reduction of plasma extravasation (PE) and mieloperoxidase (MPO) levels induced by estradiol is blocked by the estrogen receptor antagonist (ICI 182780) and if that induced by testosterone is blocked by the androgen receptor antagonist (flutamide).

**Methods:** The study was approved (N<sup>o</sup>905-1) by the Committee on Animal Research of the University of Campinas. Orquidectomized (ORX) male and ovariectomized (OVX) female wistar rats were used. Formalin (1.5%) or its vehicle (0.9% NaCl) was injected in TMJ. The intensity of PE was determined by measuring the concentration of Evan's blue dye extravasated in the TMJ tissue and the intensity of leukocyte migration was determined by measuring MPO activity in the TMJ tissue. Data were analyzed by ANOVA and Tukey post hoc test ( $p \leq 0.05$ ) and the results are expressed as mean  $\pm$  SEM. **Results:** Formalin-induced TMJ PE and MPO were significantly lower in OVX females receiving estradiol ( $25.3 \pm 2.3$ ;  $1.43 \pm 0.1$ , respectively) than in those receiving vehicle ( $53.35 \pm 4.12$ ;  $2.73 \pm 0.47$ ; respectively) administration. Formalin-induced TMJ PE and MPO were significantly lower in ORX males receiving testosterone ( $47.44 \pm 2.7$ ;  $1.20 \pm 0.2$ ; respectively) or estradiol ( $49.83 \pm 2.7$ ;  $1.11 \pm 0.1$ ; respectively) than in those receiving vehicle ( $65.91 \pm 3.27$ ;  $3.61 \pm 0.64$ ; respectively) administration. The decrease in PE and MPO induced by estradiol was blocked by ICI 182780 ( $64.80 \pm 8.1$ ;  $2.09 \pm 0.1$ ; respectively) in females and in males ( $71.96 \pm 5.8$ ;  $2.54 \pm 0.33$ ; respectively), while that induced by testosterone was blocked by flutamide ( $73.00 \pm 4.7$ ;  $3.91 \pm 0.2$ ; respectively) but not by ICI 182780 ( $49.74 \pm 4.2$ ;  $0.99 \pm 0.1$ ; respectively).

**Discussion:** This study demonstrated that the anti-inflammatory effect of estradiol is mediated by estrogen receptors and that it is not sex specific, since estradiol also reduced TMJ inflammation in males. Although testosterone might be aromatized to estradiol, which in fact, mediates many of the effects attributed to androgens in the males, the anti-inflammatory effect of testosterone is mediate exclusively by androgen receptors. Further studies are necessary to evaluate the potential therapeutic interest in developing drugs that potentiate or mimic the anti-inflammatory effect of estradiol and testosterone, such as estrogen or androgen receptor ligands, devoid of conventional estrogenic or androgenic activity. **Financial Support:** CAPES and FAPESP, Brazil.

#### 04.056

Effects of pulmonary exposure to staphylococcal type B enterotoxin in a murine allergic model. Squebola Cola, DM, Souza IA, Gomes G, Mello GC, Antunes E UNICAMP - Farmacologia

**Introduction:** *Staphylococcus aureus* is a gram-positive bacterium often found in the normal microflora of skin, upper respiratory and gastrointestinal tracts in humans. This bacterium produces and secretes the so-called staphylococcal enterotoxins (SEs), which are a family of structurally related heat-stable 23 to 29 kDa proteins. We have previously shown that exposure of rat airways to staphylococcal enterotoxin type A and B (SEA and SEB) evokes a large influx of neutrophils in bronchoalveolar lavage fluid (BAL) by mechanisms involving over-expression of cytokine-induced neutrophil chemoattractant (CINC-2), iNOS and COX-2, as well as enhanced production of TNF- $\alpha$  and IL-6 (DeSouza et. al, 2005, 2006). Clinical evidences have shown an association between bacterial organisms and pathogenesis and/or exacerbation of bronchial asthma, supporting the hypothesis that prior exposure to staphylococcal enterotoxin (SEs) exacerbates the allergic diseases. Therefore, this study aimed to investigate the effect of prior exposure to SEB on ovalbumin (OVA)-induced allergic pulmonary inflammation in BALB/c mice. **Methods:** This study was approved by the Ethical Committee for animal studies of UNICAMP (Protocol n° 1657-2). Balb/c mice were immunized with a s.c injection of OVA (100  $\mu$ g) dissolved in Al (OH)<sub>3</sub>. Two weeks later, mice were intranasally challenged with OVA (10  $\mu$ g/50 $\mu$ l PBS), after which eosinophil counts in bronchoalveolar lavage fluid (BALF) and bone marrow were evaluated. Mice were intranasally exposed with either to SEB (1  $\mu$ g) or sterile PBS (control group) 12 h prior to OVA challenge. **Results:** No differences in eosinophil number in BAL fluid of OVA-challenged mice were found between control and SEB groups. In bone marrow, however, SEB exposure caused an enhancement of immature and mature forms of eosinophils in OVA-challenge mice ( $2,58 \pm 0,94$  and  $2,25 \pm 0,63 \times 10^6$  cells/ml respectively;  $P < 0.05$ ) compared with PBS group ( $1,23 \pm 0,27$  and  $0,55 \pm 0,09 \times 10^6$  cells/ml, respectively). **Conclusions:** Our findings show that airways exposure to SEB increases the number of mature and immature eosinophils in bone marrow. The mechanism involved in this process is under current investigation. **References:** DeSouza IA et.al. *Br. J. Pharmacol.* 146(6): 781-91.2005. DeSouza IA; et al. *Toxicol. Appl Pharmacol.* 217 (1): 107-13. 2006. **Support:** FAPESP



#### 04.057

*In vivo* participation of nitric oxide in hyperproliferative epidermal phenomena in mice. Mendes DAGB<sup>1</sup>, Silva CD<sup>1</sup>, Prudente AS<sup>1</sup>, Assreuy J<sup>2</sup>, Otuki MF<sup>3</sup>, Cabrini DA<sup>1</sup> <sup>1</sup>UFPR - Farmacologia, <sup>2</sup>UFSC - Farmacologia, <sup>3</sup>UEPG - Ciências Farmacêuticas

**Introduction:** In the skin, nitric oxide (NO) has been implicated in numerous homeostatic functions, including keratinocyte proliferation, angiogenesis and wound healing. However, it also mediates pathological conditions, such as immune-mediated skin diseases. Thus, the aim of this study was to investigate the role of NO upon the hyperproliferation of epidermal keratinocytes by analyzing the effects of NOS inhibitors and a NO donor in an animal model of chronic skin inflammation caused by croton oil.

**Methods:** The chronic inflammatory process was induced by multiple application of croton oil (0.4 mg/ear) for 9 days on alternate days. Mice were topically treated with the N<sup>G</sup>-nitro-L-arginine-methyl ester (L-NAME, 10 µmol/ear), Aminoguanidine (AG, 10 µmol/ear), Sodium nitroprusside (SNP, 2.5 µmol/ear), 7-Nitroindazole (7-NI, 1 µmol/ear) or Dexamethasone (0.1 mg/ear), starting on the 5<sup>th</sup> day of experiment. Edema was measured daily as the increase in ear thickness, and on the last day 6 mm sample of ear tissue were collected, weighed and analysed using the following parameters: histology, immunohistochemistry (Proliferating Cell Nuclear Antigen), myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) enzymatic activity (n=5). Procedures have been approved by our Institutional Ethics Committee under the number 299/UFPR. **Results:** Multiple applications of croton oil induced formation of ear edema that started at 24 h after the first dose and increased within days of treatment. Application of dexamethasone or L-NAME reduced the edema during all treatment period when compared to the control group. However, the NO donor SNP caused a significant increase in the croton oil induced-ear edema. L-NAME, 7-NI and dexamethasone reduced ear weight by 16.9 ± 4.8, 18.3 ± 9.1 and 43.8 ± 2.0% respectively, while in groups treated with AG and SNP, ear weight increase by 24.9 ± 5.7 and 22.0 ± 9.2%, respectively, when compared to control group. Histological analysis showed that L-NAME, AG and 7-NI were able to prevent the increase in epidermis width by 28.2 ± 5.3, 20.7 ± 5.3 and 20.5 ± 3.4%, respectively, as well as dexamethasone (53.5 ± 3.5%), while SNP application increased it by 35.6 ± 5.7%. In the PCNA staining, multiple applications of croton oil promoted an increase in the number of proliferating cells in ear skin of control group (87.1 ± 6.8 cells/field). Application of L-NAME, AG and dexamethasone reduced (57.3 ± 3.9, 73.3 ± 2.7 and 37.0 ± 1.8 cells/field, respectively), while SNP raised (100.8 ± 6.7 cell/field) the number of PCNA positive cells. In the AG and SNP-treated ears, the MPO activity was further increased in 52.8 ± 23.9% and 74.4 ± 47.9% respectively, while in dexamethasone group it was reduced by 47.0 ± 3.4%, in comparison to control value. Ears treated with 7-NI or dexamethasone showed a decrease in 14.6 ± 6.1 and 34.4 ± 7.2%, respectively, of NAG activity in comparison to control results. However, topical application AG augmented in 16.3 ± 6.3% NAG activity induced by repeated skin treatment with croton oil. **Discussion:** Therefore, in the skin NO produced by iNOS is involved in the control of hyperproliferation of keratinocytes in the epidermis, with the contribution of nNOS. Besides, in the animal model of cutaneous chronic inflammation by croton oil, NO is involved in the exudation and leukocytes migration, with participation of all three NOS isoforms. **Support:** CNPq; CNPq/CAPES (J.A.)

#### 04.058

15D-PGJ2 treatment following acute *Trypanosoma cruzi* infection controls immune response. Rodrigues WF<sup>1</sup>, Miguel CB<sup>1</sup>, Napimoga MH<sup>2</sup>, Chica JEL<sup>3</sup> <sup>1</sup>UNIUBE - Biopatologia e Biologia Molecular, <sup>2</sup>UNIUBE - Biologia Celular e Molecular, <sup>3</sup>UFTM - Biologia Celular

**Introduction:** In the acute phase of *Trypanosoma cruzi* infection is associated with a strong inflammatory reaction in the heart. The acute infiltration of immune cells in *T. cruzi*-associated myocarditis is induced by a Th1-biased immune response. 15d-PGJ2 belongs to a new class of anti-inflammatory compounds with possible important clinical applications. Thus, we evaluated the effects of 15d-PGJ2 administration in the acute phase of experimental Chagas' disease. **Methods:** All experimental procedures were approved by the Ethical Committee for Animal Research of the University of Uberaba (#055/2009). Three days after infection of C57BL/6 mice with Colombian strain of *T. cruzi*, animals were treated during 7 days with 15d-PGJ2 (1mg/kg; daily). Histological procedures (H&E staining) were done using the heart muscle for analyze the inflammatory infiltrate (leukocyte subpopulations). A separate set of slides were immunohistochemically stained to count the number of parasite nests in the muscles. Serum levels measurement of cytokines were carried out using ELISA assay. **Results:** The results showed that the 15d-PGJ2 reduced the inflammatory infiltrate, specially the lymphocytes and neutrophils ( $p < 0.05$ ) however a slight increase in monocyte numbers subpopulation in the heart tissue was found. On the other hand, a decreased volume density of amastigotes nests in cardiac muscle was found ( $4,75 \pm 0,91$  in infected animals versus  $1,52 \pm 1,67$  in *T. cruzi*-treated animals). Also, *T. cruzi*-infected animals treated with 15d-PGJ2 displayed a statistical increase in IL-10 levels ( $256,49 \pm 49,0$  in infected animals versus  $375,17 \pm 42,0$  in *T. cruzi*-treated animals), but IFN- $\gamma$  levels were unchanged. **Discussion/Conclusion:** Taken together we demonstrated that 15d-PGJ2 is able to control the immune response and decreased the volume density of amastigote nests in the acute phase of infection. Financial Support: FUNEPU/UFTM; PAPE/UNIUBE

#### 04.059

Anti-inflammatory properties of antithrombin purified from *Bothrops jararaca* snake plasma in mice: preliminary results. Morais-Zani, K, Nunes FPB, Tanaka-Azevedo AM -  
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**Introduction:** Antithrombin is an important inhibitor of several coagulation serine proteases, including factors X<sub>a</sub>, IX<sub>a</sub>, XI<sub>a</sub> and thrombin. Besides, a large number of recent studies have shown that human antithrombin has anti-inflammatory actions, which are independent of its effects on coagulation. **Objective:** The aim of this work was to investigate the effects of *B. jararaca* antithrombin (BjAT) on cell migration induced by carrageenan (cg) in mice. **Methods:** Antithrombin was purified from *B. jararaca* plasma by HiTrap Heparin HP column. The BjAT (20 µg/100 µL i.v.) or saline (100 µL) was administered 1 hour before intraperitoneal injection of cg (300 µg/200 µL) or saline (sal) (200 µL) in male Swiss mice (18-22 g). After 4 hours of cg injection or sal, cell migration to the peritoneal cavity was evaluated. The count of total peritoneal cells was determined in Neubauer's hemocytometer and differential counts were performed in smears stained with panchromatic stain. A total of 100 cells were counted by optical microscopy. **Results:** Pre-treatment with BjAT diminished cg-induced cell-influx into the peritoneal cavity, when compared with the group was pretreated with sal (sal+cg). The decrease in cell migration in animals pretreated with BjAT was 41% (sal+cg: 4.66 ± 0.56, BjAT+cg: 2.74 ± 0.31; p<0.05). A significant decrease of 82% was observed for polymorphonuclear cells in animals pretreated with BjAT (sal+cg: 3.50 ± 0.81, BjAT+cg: 0.60 ± 0.09; p<0.05). **Conclusion:** The results demonstrated that BjAT significantly inhibited migration of polymorphonuclear cells to peritoneal cavity. Thus we could suggest that BjAT presents anti-inflammatory properties.

#### 04.060

The fundamental role of IFN- $\gamma$  and nitric oxide in the inflammatory response induced by dengue virus infection in mice. Costa VV<sup>1</sup>, Fagundes CT<sup>2</sup>, Valadão DF<sup>1</sup>, Silveira KD<sup>3</sup>, Morcatty TQ<sup>1</sup>, Santos AG<sup>1</sup>, Lima CX<sup>4</sup>, Prospero T<sup>1</sup>, Silva TA<sup>5</sup>, Amaral FA<sup>1</sup>, Teixeira MM<sup>2</sup>, Souza DG<sup>1</sup> <sup>1</sup>UFMG - Microbiologia, <sup>2</sup>UFMG - Bioquímica e Imunologia, <sup>3</sup>UFMG - Fisiologia e Biofísica, <sup>4</sup>UFMG - Fisiologia e Farmacologia, <sup>5</sup>UFMG - Patologia, Clínica e Cirurgia Odontológicas

**Introduction:** Dengue is one of the most important vector-borne viral diseases in the world that affects humans and constitutes a serious world health problem. It's caused by four dengue related virus serotypes (DENV 1-4) and is transmitted by *Aedes* mosquito. Although knowledge about the disease pathogenesis is incipient, an important role is attributed to cytokines in host response to infection. Among them, we highlight IFN- $\gamma$ , a cytokine with important functions in immunity to infectious agents.

**Objectives:** The aim of this study was to evaluate the role played by IFN- $\gamma$  and by one of its effectors molecules, nitric oxide, during response to dengue virus infection.

**Methods:** This project was previously approved by CETEA/UFMG on access number 113/09. Wild type mice (C57BL/6), deficient mice to IFN- $\gamma$  (IFN- $\gamma^{-/-}$ ) and iNOS (iNOS $^{-/-}$ ) were infected with 10PFU/100 $\mu$ L of the adapted DENV-3 diluted on PBS by the intraperitoneal route. After infection, lethality of animals was accompanied for 14 days. We made the evaluation of hematological signals: hematocrit, platelets, plasmatic albumin and hepatic transaminases levels in serum (specific Kits Bioclin/Quibasa), evaluation of inflammatory parameters (cytokines and chemokines by specific ELISA and neutrophils by the evaluation of MPO activity on spleen, liver and lungs). Hypernociception was measured by a digital analgesimetro (Insight mod. EFF-301). Tissue damage was verified by histological analyses in liver and lungs of both animals. Expression of iNOS was evaluated by Immunohistochemical detection of iNOS on liver of WT and IFN- $\gamma^{-/-}$  animals. *Production of NO was evaluated in culture of DCs by Griess method* and viral load in target organs was quantified by plaque assay in permissive cells (LLC MK-2).

**Results:** WT mice were able to produce IFN- $\gamma^{-/-}$  after infection with DENV-3. Furthermore, IFN- $\gamma^{-/-}$  mice presented significantly higher lethality after infection when compared with WT control mice. This reduced survival rate was associated by more severe disease manifestation when compared to control littermates, assessed by increase in thrombocytopenia, haemoconcentration and hypernociception, besides elevated systemic levels of IL-6 and CXCL-1 in spleen, lungs and serum. In addition, there was elevated concentrations of hepatic transaminases that correlated with higher tissue damage in IFN- $\gamma^{-/-}$  when compared with WT controls. Tissue damage in lungs of IFN- $\gamma^{-/-}$  was evident. This enhanced susceptibility to infection was characterized by loss in control of viral replication after infection, what we deduced to be associated with reduced ability of NO-production by IFN- $\gamma^{-/-}$  mice. This hypothesis was confirmed by *Immunohistochemical detection of iNOS on liver of WT and no production in IFN- $\gamma^{-/-}$* , suggesting that IFN- $\gamma$  stimulus is fundamental for the production of NO in dengue virus infection. *In vitro* DCs were able to produce NO when stimulated together with DENV-3 and IFN- $\gamma$  but, not when infected with DENV-3 alone, corroborating one more time our hypothesis. The same worse phenotype response was observed in iNOS $^{-/-}$  mice in all evaluated parameters.

**Conclusion:** Here, we conclude that IFN- $\gamma$ -induced NO production is a fundamental pathway during response to DENV-3 infection. In absence of these two molecules, there is reduced ability of viral replication control by host, resulting in a more severe disease manifestation. Strategies capable to enhance production of these two molecules may result in benefits during the control of primary infection by dengue virus.

**Financial Support:** CNPq, CAPES and FAPEMIG.

#### 04.061

Major role for endothelins in sustaining symptoms in mouse models of inflammatory bowel disease. Claudino RF, Leite DFP, Bento AF, Chichorro JG, Marcon R, Calixto JB, Rae GA UFSC - Farmacologia

**Introduction:** Evidence suggests that endothelin (ET) levels are elevated in the plasma, but reduced in colonic mucosa of patients with inflammatory bowel disease (IBD), and that blockade of their receptors might ameliorate some inflammatory signs associated with IBD. This study attempts to clarify the roles of ETs and mechanisms associated to ETA/ETB receptors in mouse models of colitis. **Methods:** Colitis was induced by intracolonic administration of TNBS (1.5 mg in 0.1 mL of 50% ethanol) to anesthetized male Balb/c mice (20-25 g). Starting 24 h after colitis induction, mice were treated with vehicle, Atrasentan (**Atra**, ETA receptor antagonist, 1-10 mg/kg once daily, i.v.), A-192621 (ETB receptor antagonist, 20 mg/kg once daily, i.v.) or dexamethasone (**Dex**, 1 mg/kg twice daily, s.c.). Mice were monitored for body weight loss and overall mortality up to 72 h. Surviving mice were killed at 72 h after TNBS for evaluation of the following parameters in colonic samples: microscopic and macroscopic damage, colon weight, MPO activity and cytokine levels (MIP-2, KC, IL-1 $\beta$ , IL-10 and IL-13). Levels of mRNA for ET-1 and ET-2, ET<sub>A</sub> and ET<sub>B</sub> receptors were assessed at 24, 48 and 72 h after TNBS by Real Time RT-PCR. Expression of adhesion molecules E-selectin and  $\beta_2$ -integrin was also assessed at 72 h, by immunostaining. The influence of Atra (10 mg/kg, once daily, i.v.) on some parameters in colitis induced by ingestion of dextran sodium sulfate (DSS, 3%) solution was also assessed. All protocols were previously approved by UFSC's Ethics Committee on Animal use (No. PP00062). **Results:** At 72 h after TNBS, mice displayed high mortality and survivors (only 35%) showed signs of severe illness characterized by bloody diarrhea and profound weight loss. Atra or Dex treatment enhanced survival rate (73 and 90%, respectively). Mice treated with Atra (but not A-192621 or Dex) rapidly recovered from body weight loss. Macroscopic and microscopic damage and MPO activity were increased ~15-fold in colon tissue of TNBS-treated mice relative to controls, and Atra and Dex significantly reduced all 3 parameters. TNBS-induced colitis increased colonic IL-1 $\beta$ , MIP-2 and KC levels at 72 h by 6.5, 2.4 and 3.7-fold, respectively. Such increases were abolished by Atra or Dex. Samples from TNBS-treated mice also showed 1.8- and 2.4-fold reductions in IL-10 and IL-13 levels, respectively. Dex restored levels of both anti-inflammatory cytokines, but Atra only restored IL-10. Both treatments inhibited the marked increase in colonic E-selectin and  $\beta_2$ -integrin immunostaining (5.7- and 3.7-fold, respectively) induced by TNBS. Real time RT-PCR detected a decrease in ET-1 mRNA at 24 h, but not at 48 and 72 h after TNBS. ET-2 mRNA was decreased at 24 and 72 h, but increased at 48 h. mRNA for both ET<sub>A</sub> and ET<sub>B</sub> receptors was increased at all 3 time points after colitis induction. Atra also effectively reduced body weight loss and several inflammatory parameters in colonic samples (i.e. disease activity index, colon length and macroscopic damage) taken from of DSS-treated mice. Altogether, these results show that colonic injury and inflammatory parameters seen in two mouse IBD models are highly responsive to reversal by treatment with a selective ET<sub>A</sub> receptor antagonist. **Financial Support:** CAPES, CNPq, PRONEX, FAPESC.

#### 04.062

PI3K (phosphoinositide 3-kinases) – promising targets for the treatment of gouty arthritis. Tavares LD<sup>1</sup>, Costa VV<sup>2</sup>, Amaral FA<sup>2</sup>, Fagundes CT<sup>2</sup>, Teixeira MM<sup>2</sup>, Souza DG<sup>2</sup> <sup>1</sup>UFMG - Fisiologia e Farmacologia, <sup>2</sup>UFMG - Bioquímica e Imunologia

**Introduction:** Hyperuricemia may result in the deposition of monosodium urate monohydrate (MSU) crystals in joints and soft tissues. When shed from deposits or precipitated de novo acute inflammation may result. The pathogenic mechanisms are still not fully characterized. For most patients, therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids for acute episodes and prevention of recurrence with agents that lower the serum uric acid levels are highly effective. However, there remain many patients with chronic polyarticular gout for whom these therapies are not sufficient because of ineffectiveness, toxicity, or comorbidities. MSU crystals induce a variety of inflammatory cytokines and chemokines including tumor necrosis factor (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, CXCL1 (IL-8 in humans and KC in mice). Phosphoinositide 3-kinases (PI3K) have long been considered promising drug targets for the treatment of inflammatory and autoimmune disorders as well as cancer and cardiovascular diseases. The isoform p110 $\gamma$  of class I phosphoinositide 3-kinase (PI3K) plays a major role in leukocyte chemotaxis. Consequently, emphasis has been placed on developing PI3K  $\gamma$  inhibitors to treat disease states that result from inappropriate tissue accumulation of leukocytes, such as gouty arthritis. Thus, the aim of this study is to evaluate the role of PI3K- $\gamma$  pathway in the pathogenesis of gouty arthritis model and to evaluate possible targets for the treatment of this disease.

**Methods:** Wild type male C57/BL6 and wild type (WT) and PI3K knock-out (PI3K<sup>-/-</sup>) mice were used. Uric acid crystals (UAC) were prepared by precipitation of the crystals after the uric acid added in borate buffer solution (pH 8.5). For the experiments, a dose of 100 $\mu$ g of UAC was diluted (borate buffer plus uric acid – pH 8.5) and a volume of 10 $\mu$ L was injected in the tibio-femoral joint. Hypernociception was measured by a digital analgesimeter (Insight mod. EFF-301). Samples of periarticular tissue were removed for cytokines and chemokines (ELISA) analysis and neutrophil quantification (MPO). A joint lavage (BSA 3%; 10 $\mu$ L) was used to evaluate the cell infiltration in articular space, which was performed by total cell count (Neubauer chamber) and differential count (Cytospin3 - Shandon). **Results:** Using C57/BL6 mice, we made a temporal curve (3, 6, 9, 12, and 24 hours after UAC injection) to evaluate the best time according to inflammatory parameters analyzed. The time chosen was 6 hours after the UAC injection, where there was an increase in hypernociception, elevated MPO levels and neutrophils compared to vehicle mice. Using the PI3K<sup>-/-</sup> mice, we observed a reduction in the number of articular cell infiltration after the UAC injection compared to WT ones. These results were accompanied by a reduction of the MPO values and to low levels of the cytokine IL-1 $\beta$  and the chemokine MIP-2. **Discussion:** We show that PI3p110 $\gamma$ (<sup>-/-</sup>) mice are largely protected in mouse models of gouty arthritis; this protection correlates with defective neutrophil migration, reduction of hypernociception and suppression of expression of chemokine MIP-2 and in cytokines such as IL-1 $\beta$  and TNF- $\alpha$ , further validating PI3K $\gamma$  as a therapeutic target in this model. Financial Supports: CNPq and CAPES

#### 04.063

Modulation of integrins on eosinophils from bone marrow and peripheral blood after allergen challenge in mice: role of nitric oxide. Pelaquini EH<sup>1</sup>, Fernandes LGR<sup>2</sup>, Toledo AP<sup>1</sup>, Tamashiro WMSC<sup>2</sup>, Ferreira HHA<sup>1</sup> <sup>1</sup>USF - Inflamação, <sup>2</sup>UNICAMP - Imunologia e Microbiologia

**Introduction:** Mobilization of eosinophil (EOs) from the bone marrow (BM) to peripheral blood (PB) and their trafficking to lungs are features of allergic inflammation. This process is mediated by VLA-4 (CD49d/CD29), LFA-1(CD11a/CD18) and Mac-1 (CD11b/CD18) integrins. Our previous study suggested that inhibition of NO synthesis could delay efflux of eosinophils from BM (Ferreira et al., *Bioch. Pharmacol.* 68:631, 2004). **Objective:** Now we investigated whether NO modulates the expression of EO integrins in BM and PB of allergic mice. **Materials and Methods:** All experiments were approved by the AEC/USF (protocol 002.11.08). BALB/c mice previously sensitized with ovalbumin (OVA) were injected with the iNOS selective inhibitor, 1400W, or non-selective inhibitor, L-NAME, 2h before and 4 and 12h after OVA-challenge. The control group received only saline. BM and PB Eos, collected at 24 and 48 hours after OVA-challenge, were stained with FITC-conjugated anti-CCR3, PE-conjugated anti-CD49d, APC-conjugated anti-CD11b and PeCy7-conjugated anti-CD11a. Flow cytometric analysis of the cells with high content of granules/CCR3+ were performed with a FACS-Aria. **Results:** Analysis of EOs from BM or PB of controls showed that antigen challenge had no effect on LFA-1 expression at any time studied. A reduction in Mac-1 expression (~40%) was observed in EOs from both compartments but in different periods; at 24h on BM EOs and later, at 48h, in PB EOs. The expression of VLA-4 was not modified on BM EO at any time examined. Nevertheless, a reduction of 81% in VLA-4 expression was observed in PB EOs at 48h after antigen challenge in control mice. Treatment of mice with NO inhibitors showed that 1400W, but not L-NAME, resulted in an increase in LFA-1 and Mac-1 expressions (40 and 85% increases, respectively) on BM EOs at 24h, as compared to control mice. At this time, treatments had no effect on VLA-4 expression of BM EOs. However, in the PB compartment, 1400W or L-NAME dropped in ~40% and ~76%, respectively, the expression of Mac-1 and VLA-4 on EOs at 24h. Interestingly, at 48h, both treatments increased CD11a expression on PB EOs (42% and 247% elevations for 1400W and L-NAME, respectively). Conversely, effects on VLA-4 expression differed: 1400W increased expression by 64% whereas a reduction of 74% was seen on PB EOs from L-NAME-treated mice. **Conclusions:** These results suggest that, in sensitized control mice, downregulation of EO integrin expression can reduce cell adherence to BM and PB components, facilitating cell egress to circulation and to lungs at distinct times after OVA-challenge. Mac-1 might play an important role in BM EOs mobilization at 24h, whereas both Mac-1 and VLA-4 were involved in EO migration from PB to lungs at 48h after OVA challenge. Increased Mac-1 expression on BM EOs from 1400W-treated mice at 24h, as well as increased Mac-1 and VLA-4 expressions on PB EOs induced by 1400W and L-NAME, could produce a reduced efflux of both EOs from BM and PB and an impaired influx of these cells to lungs. The opposing effects of L-NAME on Mac-1 and VLA-4 need to be elucidated. In conclusion, we suggest that NO interferes with EO migration to lungs by modulating integrin expression on BM and PB cells. **Financial support:** FAPESP and CNPq.

#### 04.064

The role of insulin on signaling pathways and mediator in LPS – induced acute lung injury in diabetic rats. Ribeiro LF Jr<sup>1</sup>, Martins JO<sup>1</sup>, Ferracini M<sup>1</sup>, Anger DB<sup>1</sup>, Martins DO<sup>2</sup>, Sannomiya P<sup>3</sup>, Jancar S<sup>1</sup> <sup>1</sup>ICB-USP - Imunologia, <sup>2</sup>ICB - Fisiologia e Biofísica, <sup>3</sup>InCor-HC-FMUSP - Experimentação

**Introduction:** It has long been recognized that diabetic patients are more susceptible to infections and that their inflammatory response is impaired. Reversal of the impaired responses is attained by treatment of the animals with insulin. There is evidence that insulin through direct or indirect effects regulates the inflammatory response. The lipopolysaccharide (LPS) is known to induce systemic inflammation, which affects several organs among them the lung, and in consequence acute lung injury (ALI) may develop. Binding of LPS to toll-like receptor 4 (TLR-4) triggers a complex sequence of events leading to increased expression of specific genes through nuclear factor (NF)- $\kappa$ B and release of a plethora of mediators which are involved in inflammatory response. We showed before that insulin modulates the development of LPS-induced ALI in diabetic rats. We found that in ALI caused by intratracheal instillation of LPS the neutrophil infiltration and concentration of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-10 in the airways are significantly reduced in diabetic rats compared to non-diabetic controls. The present study was designed to investigate signaling pathways and mediators in the course of LPS-induced acute lung inflammation in alloxan-induced diabetic rats and the effect of insulin treatment. **Methods:** Diabetic male Wistar rats (alloxan, 42 mg/kg, i.v., 10 days) and control rats received intratracheal instillation of LPS (750 mg/0.4 mL) or saline. Some diabetic rats were given neutral protamine Hagedorn (NPH) insulin (4 IU, s.c.) 2 hours before LPS. After 6 hours, bronchoalveolar lavage (BAL) was performed for mediators release and lung tissue was homogenized for analysis of LPS-induced signaling pathways. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by The Ethical Committee for Animal Research of the Biomedical Sciences Institute, University of São Paulo (licence n° 139, page 64, book 2). **Results:** Relative to control rats, diabetic rats exhibited a significant reduction in LPS-induced phosphorylation of ERK (64%), p38 (70%), Akt (67%), PKC- $\alpha$  (57%) and PKC- $\delta$  (65%) and in the expression of inducible nitric oxide synthase (32%) and cyclooxygenase-2 (67%) in the lung homogenates. The BAL fluid concentration of nitric oxide (47%) and IL-6 (49%) were also reduced in diabetic rats whereas the cytokine-induced neutrophil chemoattractant (CINC)-2 levels was increased 23% and CINC-1 was not different from control animals. Treatment of diabetic rats with insulin completely or partially restored all these parameters. In conclusion, data presented show that insulin regulates MAPK, PI3K, PKC pathways, the expression of the inducible enzymes, COX-2 and iNOS, and the levels of IL-6 and CINC-2 in LPS-induced lung inflammation in diabetic rats. **Discussion:** These results suggest that insulin could modulate cellular signal transduction factors, restoring completely or partially the inflammatory response of diabetic rats during LPS-induced acute lung injury. This research was supported by FAPESP and CNPq, Brazil.



#### 04.065

Anti-inflammatory evaluation of *Coronopus didymus* in the pleurisy and paw oedema models in mice. Souza MM<sup>1</sup>, Teimosan, T. B.<sup>1</sup>, Mora TC<sup>2</sup>, Biavatti MW<sup>3</sup>, Bürger C<sup>1</sup>, Claudino VD<sup>4</sup>, Darmarco ED<sup>5</sup>, Frode TS<sup>6</sup> <sup>1</sup>CCS-UNIVALI, <sup>2</sup>NIQFar-UNIVALI, <sup>3</sup>UFSC - Farmácia, <sup>4</sup>UNIVALI - Ciências Farmacêuticas, <sup>5</sup>FURB - Farmácia, <sup>6</sup>UFSC - Análises Clínicas

**Introduction-** The use of *Coronopus didymus* (CD) or “mastrunço” in traditional medicine in Brazil for infusion, decoction or feeding against inflammatory and pain process is a common practice in folk medicine in several countries but mainly in Brazil..

**Methods:** the purpose of this study was to investigate the anti-inflammatory effect of hydroalcoholic extract obtained from the leaves of *Coronopus didymus* in the mouse model of pleurisy and paw oedema in rats. The animals (rats, 250-300g / mice, 25-30g) were obtained from the *Biotério Central* UNIVALI and the experimental protocols were submitted to the ethics committee CEP-UNIVALI and approved (CEP-407/07). Both process being induced by various flogistic agents (carrageenan/Cg; bradykinin /BDK; histamine HIS, substance P/SP; dextran/ DEX; PGE<sub>2</sub> ), and to observe their effects on leukocyte migration, myeloperoxidase (MPO), and adenosine-deaminase (ADA) activities and nitric oxide (NO) levels. **Results and Discussion** CD (200-600mg/kg) administered by the oral route (v.o.) inhibited the leukocyte (60.0±1.42%) neutrophils (82.75±1.29%), MPO (42.30±4.23%), and ADA (57.89± 1.94% ) activities as well as NO levels (64.28±2.15%) in Cg induced pleurisy. CD also inhibited total and differential leukocytes (neutrophils) pleurisy induced by BDK (75.15 ± 2.11%/ 46.24 ± 2.02%) HIS (51.29 ± 0.91%/ 42.42 ± 2.05%) or SP (80.44 ± 1.45%/64.56 ± 3.21%). In addition, CD was effective in reducing paw oedema induced by Cg (72.79±1.13%), SP(68.26 ± 0.78%), DEX (61.00 ± 0.98% ) BDK (66.66.±0.77% and PGE<sub>2</sub> (53.346.±1.18). **Conclusion:** Together the results demonstrate the anti-inflammatory effect of CD partially validating the popular use, in addition several mechanisms, including the inhibition of (BDK, HIS, SP, NO and PGE<sub>2</sub>) release and/or action, appear to account for the *Coronopus didymus* effect. **Financial support:** CNPq - FAPESC

#### 04.066

Avaliação da participação do óxido nítrico na função pulmonar e hiperreatividade das vias aéreas de camundongos silicóticos. Dias DF, Ciambarella BT, Ferreira TPT, Arantes ACS de, Silva PMR, Martins MA FIOCRUZ - Fisiologia e Farmacodinâmica

**Introdução:** A silicose é uma doença pulmonar crônica causada pela exposição prolongada a partículas de sílica, caracterizando-se por intensa fibrose e comprometimento da função respiratória. Uma ampla gama de mediadores inflamatórios participa de forma conjunta neste processo, com destaque para o óxido nítrico (NO). Este estudo teve como objetivo investigar o envolvimento do óxido nítrico no comprometimento da função respiratória na silicose, com ênfase sobre a hiperreatividade das vias aéreas. **Métodos:** Camundongos Swiss-Webster, selvagens C57 Bl6 (iNOS<sup>+/+</sup>) e nocautes para a enzima NO sintase induzida (iNOS<sup>-/-</sup>) foram anestesiados e instilados com sílica (10 mg), por via intranasal, sendo as análises realizadas 7 e 28 dias após. Como parâmetros constaram: i) mecânica respiratória (resistência e elastância) avaliada por pletismografia de corpo inteiro invasiva (Buxco), ii) nível de óxido nítrico no lavado broncoalveolar quantificado pela técnica de Griess e iii) alterações morfológicas avaliadas por técnicas histológicas clássicas. O tratamento com doador de óxido nítrico DETANonoato (0.0,25 µmoles/kg) foi realizado uma vez ao dia durante 5 dias alternados. Todos os procedimentos utilizados foram aprovados pelo Comitê de Ética de Uso de Animais (CEUA) da FIOCRUZ (Protocolo 0213-4). **Resultados:** Verificamos que, em ambos os tempos de análise, os animais silicóticos apresentaram níveis basais de resistência e elastância superiores aos dos controles, assim como uma resposta broncoconstrictora exacerbada frente à aerolização com o agente colinérgico metacolina. Níveis de óxido nítrico aumentados foram detectados no lavado broncoalveolar de camundongos silicóticos, tanto em 7 como em 28 dias pós-sílica. A estimulação intranasal de camundongos normais com o composto doador de óxido nítrico DETANonoato foi capaz de induzir uma quadro de hiperreatividade das vias aéreas, atestado pela reposta aumentada tanto de resistência como de elastância. Em paralelo, notamos que o parênquima pulmonar dos animais iNOS<sup>-/-</sup> não apresentou alterações morfológicas em comparação ao dos iNOS<sup>+/+</sup>, enquanto que na condição da silicose, os camundongos iNOS<sup>-/-</sup> apresentaram uma marcada redução do processo inflamatório e fibrótico, em 7 e 28 dias, respectivamente. A análise morfométria indicou uma redução significativa da área de granuloma nos animais iNOS<sup>-/-</sup> instilados com sílica quando comparados aos iNOS<sup>+/+</sup>. **Conclusão:** Nossos resultados mostram que os camundongos silicóticos apresentaram resposta de hiperreatividade das vias aéreas à estimulação com metacolina, em clara associação com a geração de óxido nítrico, em ambas as fases (aguda e crônica) da doença. Os camundongos nocautes para iNOS apresentaram resposta de hiperreatividade abolida em relação aos selvagens, assim como marcada redução dos componentes inflamatório e fibrótico. Isto é indicativo de que o NO parece desempenhar um papel relevante no quadro de alteração da função pulmonar na silicose, e que este parece estar associado ao comprometimento do parênquima pulmonar. **Apoio Financeiro:** PAPES 4/FIOCRUZ, CNPq e FAPERJ.

#### 04.067

Reduced expression of cxc receptors in neutrophils of patients with uterine cervical neoplasia. Fernandes Jr PC<sup>1</sup>, Micheli DC<sup>2</sup>, Mendonça MAO<sup>2</sup>, Murta EFC<sup>1</sup>, Tavares-Murta BM<sup>2</sup> <sup>1</sup>UFTM - Gynecology and Obstetrics, <sup>2</sup>UFTM - Biological Sciences

**Introduction:** Neutrophil migration is a key event in the inflammatory response, mainly mediated by CXC chemokines. Neutrophils may play a role in the host response against uterine cervical cancer, since a defective neutrophil migration was associated to invasive stages. Nevertheless, in women at pre-invasive stages submitted to surgery, increased neutrophil migration towards chemoattractants was observed compared to pre-treatment, suggesting that even at non-invasive stage patients may present alterations in the capacity of inflammatory response (Fernandes Jr *et al*, *Int J Gynecol Cancer*, 17: 1068, 2007). **Objective:** To evaluate the expression of CXCR1 and CXCR2 receptors in circulating neutrophils of patients with uterine cervical neoplasia at different disease stages. **Methods and Results:** This study enrolled sixteen women with uterine cervical neoplasia without prior treatment, divided in groups: 1) cervical intraepithelial neoplasia, at pre-invasive stage (CIN group, n=8) and, 2) invasive cervical cancer (INV group, n=8). Controls were 15 healthy women without cytological alterations at Papanicolau exam. The study protocol (number 933) was approved by the UFTM Committee on the Use of Human Subjects. The expression of the chemokine receptors CXCR1 and CXCR2 was quantified in peripheral blood neutrophils by flow cytometry (20.000 events) and results were expressed (means  $\pm$  SD) by 3 quantitative parameters: fluorescence intensity, absolute and percentage number of neutrophils per group. The expression of both CXCRs was reduced in patients compared to controls, as indicated by at least one quantitative parameter. CXCR1 expression was diminished in CIN group than controls, respectively, as seen by percentage values ( $94,9 \pm 4,6\%$  vs.  $98,2 \pm 2,6\%$  neutrophils,  $p < 0,05$ ), but not by absolute number of neutrophils ( $7640,8 \pm 2624,1$  vs.  $9130,7 \pm 3536,8$ ) or fluorescence intensity ( $508,9 \pm 267,6$  vs.  $660,4 \pm 215,4$ ). CXCR1 expression was reduced in INV group compared to controls, as indicated by percentage values ( $96,2 \pm 3,1\%$  vs.  $98,2 \pm 2,6\%$  neutrophils,  $p = 0,093$ ) and fluorescence intensity ( $599,9 \pm 393,5$  vs.  $660,4 \pm 215,4$ ,  $p = 0,087$ ). The expression of CXCR2 was reduced in CIN group than controls, as indicated by percentage values ( $63,2 \pm 32,9\%$  vs.  $95 \pm 4\%$  neutrophils,  $p < 0,05$ ) and absolute number of neutrophils ( $5591,1 \pm 3614,2$  vs.  $9145,8 \pm 3518,8$ ;  $p < 0,05$ ), but not by fluorescence intensity ( $116,1 \pm 74,3$  vs.  $96,7 \pm 31,9$ ). In INV group, the expression of CXCR2 was reduced compared to controls, respectively, as indicated by percentage values ( $80,5 \pm 24,7\%$  vs.  $95 \pm 4,0\%$  neutrophils,  $p < 0,05$ ), but not by absolute number of neutrophils ( $7720,5 \pm 3269,8$  vs.  $9145,8 \pm 3518,8$ ) or fluorescence intensity ( $96,1 \pm 68,1$  vs.  $96,7 \pm 31,9$ ). **Discussion/Conclusion:** Patients with uterine cervical neoplasia have reduced expression of CXCR1 and CXCR2 in circulating neutrophils, which may account for inhibition of neutrophil migration. The reduced expression of CXC receptors was detected even at pre-invasive stages, which may contribute to an early impairment in the host immune response. **Financial Support:** FAPEMIG, CNPq, FINEP.

#### 04.068

Effects of organic selenium compound - *Selemax*® in experimental models of rheumatoid arthritis. Arruda MCC<sup>1</sup>, Vieira AT<sup>1</sup>, Martins FS<sup>1</sup>, Silveira KD<sup>2</sup>, Fagundes CT<sup>2</sup>, Silva TA<sup>3</sup>, Nicoli JR<sup>4</sup>, Teixeira MM<sup>1</sup> <sup>1</sup>UFMG - Bioquímica e Imunologia, <sup>2</sup>UFMG - Fisiologia e Biofísica, <sup>3</sup>FMRP-USP - Farmacologia, <sup>4</sup>UFMG - Microbiologia

**Introduction:** Rheumatoid arthritis (RA) is a chronic and auto-immune inflammatory disease that mainly targets the synovial membrane, cartilage and bone. It affects 1% of the population and is associated with significant morbidity and increased mortality. Selenium is an essential trace element with antioxidant properties able to modulate the anti-inflammatory and immune responses. *Selemax*® (Biorigin, Lençóis Paulista, SP, Brazil) is an inactive yeast enriched with organic selenium. The aim of the present study was to investigate the effects of *Selemax* administration in a model of antigen induced-arthritis and adjuvant-induced arthritis (AIA). **Material and methods:** Male C57Bl6j mice were immunized subcutaneously with methylated bovine serum albumin (mBSA) on day 0. Fourteen days later, murine AIA was induced by intraarticular (IA) injection into the knee (stifle) joint. For control, the same volume of PBS was injected into the joint of immunized mice. Animals were killed 24 hours after antigen challenge for cytokines measurement, myeloperoxidase (MPO) activity quantification and histological analysis. The treatment with different doses of *Selemax*® 0.1%, 1% and 10% (mixed with animals' food) was initiated on the 7<sup>o</sup> day after the immunization. In the adjuvant-induced arthritis model, female Rotzman rats received a single dose with *Mycobacterium butyricum* (400µg) in water- oil into dorsal root of the tail. In the 10<sup>o</sup> day, when arthritis was fully developed, treatment with dose choice *Selemax*® (1,0% ) was initiated until the end of experiment. The rats were sacrificed on day 16 for the same assessment of inflammatory parameters above. All experiments received prior approval from the UFMG ethics committee (CETEA certificate 166/06). **Results and discussion:** In the arthritis model, neutrophils role is well established. In antigen-induced arthritis model and in the adjuvant-induced arthritis, the treatment of mice with all doses of *Selemax*® decreased significantly the recruitment of neutrophil in tissue as assessed by assaying MPO activity ( $P < 0.001$  all treated groups compared with vehicle). The concentration of pro-inflammatory cytokines, such as, TNF- $\alpha$  (vehicle  $192.7 \pm 24.13$  N=4; treated  $70.07 \pm 34.36$  N=5 ), IL-1 $\beta$  (vehicle  $961.0 \pm 192.4$  N=5; treated  $224.8 \pm 52.16$  N=5) and CXCL1 (vehicle  $371.6 \pm 71.62$  N=5; treated  $80.51 \pm 29.78$  N=5) was significantly decreased in the *Selemax*-treated group in the mice model. In adjuvant- model, it was observed a decrease of paw oedema (about 32%) and a reduction of hypernociception ( $P < 0.01$ ). This reduction may be correlated, partially, with the inhibition of the pro-inflammatory cells influx. This study demonstrates a possible anti-inflammatory activity of *Selemax*® as observed in both animals models of RA. This compound may represent a new therapeutic adjunct through diet supplementation of RA's patients. **Financial Support:** Biorigin, CNPq, Fapemig.

#### 04.069

Cysteinyl leukotriene receptors expressed on membranes of human eosinophil granules mediate secretion directly from within eosinophil granules. Neves JS<sup>1</sup>, Radke A<sup>2</sup>, Weller PF<sup>2</sup> <sup>1</sup>UFRJ/Harvard Medical School - Medicine, <sup>2</sup>Harvard Medical School - Medicine

**Background:** Cysteinyl leukotrienes (cys-LTs) and their receptors (CysLTR) have clear roles in pathophysiological conditions such as asthma and other allergic diseases. Eosinophils are known to contain an abundance of preformed proteins stored within their cytoplasmic granules and to express both CysLT<sub>1</sub>R and CysLT<sub>2</sub>R. We previously demonstrated that eosinophil granules express cytokine receptors on their membranes and can function, upon extrusion from eosinophils, as independent secretory organelles releasing granule constituents in response to activating chemokines and cytokines (Neves et al., PNAS, 105:18478, 2008). **Objectives:** We evaluated the expression of CysLTRs on eosinophil granule membranes and their functional roles in eliciting protein secretion from within eosinophil granules. **Methods:** We studied secretory responses of human eosinophil granules isolated by subcellular fractionation. Granules were stimulated with cys-LTs and eosinophil cationic protein (ECP) and cytokines were measured in supernatants by ELISA and a cytokine multiplex assay, respectively. Receptor expression on granule membranes and eosinophils was evaluated by flow cytometry and western blot. **Results:** The ligand-binding amino-terminal domains for both CysLT<sub>1</sub>R and CysLT<sub>2</sub>R were expressed on granule surface membranes. After cys-LT stimulation, granules secreted ECP, but not cytokines. Granule ECP secretion in response to LTC<sub>4</sub>, LTD<sub>4</sub> and even LTE<sub>4</sub> was inhibited by montelukast, suggesting that there are other montelukast-inhibitable mechanisms, besides CysLT<sub>1</sub>R, mediating Cys-LT-elicited ECP secretion from granules. Cys-LTs, especially LTE<sub>4</sub> can act as agonists at P2Y<sub>12</sub> receptor (P2Y<sub>12</sub>R) (Nonaka et al, BBRC, 337:281, 2005). MRS 2395, a P2Y<sub>12</sub>R antagonist, dose-dependently inhibited ECP release induced by cys-LTs, suggesting a role for this purinergic receptor in cys-LT-mediated granule secretion. In confirmation, we demonstrated P2Y<sub>12</sub>R membrane expression on eosinophils and isolated granules. **Conclusion:** Our findings extend the recognition that cell-free eosinophil granules are secretion competent organelles. Granules respond to cys-LTs, including extracellularly formed LTE<sub>4</sub>, via membrane-expressed receptors that elicit granule protein secretion. Thus, secretion from eosinophil granules can be initiated by receptor-mediated mechanisms elicited by intracellular and extracellular Cys-LTs. Supported by NIH grants AI020241, AI022571, AI051645 and an unrestricted educational grant from Merck.

#### 04.070

15D-PGJ2 decreases eosinophilia following allergen challenge. Alves CF<sup>1</sup>, Correia TRMF<sup>2</sup>, Bonacini Cheraim A<sup>3</sup>, Napimoga MH<sup>4</sup> <sup>1</sup>UNIUBE - Biologia Molecular, <sup>2</sup>UNIUBE - Biopatologia e Biologia Molecular, <sup>3</sup>UFF - Patologia Experimental, <sup>4</sup>UNIUBE - Biologia Celular e Molecular

**Introduction:** Subcutaneous heat-coagulated egg white implants (EWI) induce chronic, intense local eosinophilia in mice, followed by asthma-like responses to ovalbumin challenge. It is now established that 15d-PGJ2 negatively regulates cellular functions through its intracellular targets such as peroxisome proliferator-activated receptor-g (PPAR-g). The objective of the present study was to evaluate the use of 15d-PGJ2 in the EWI model and determine the 15d-PGJ2 effect. **Methods:** All experimental procedures were approved by the Ethical Committee for Animal Research of the University of Uberaba (#049/2009). EWI animals were challenged by i.p. administration with ovalbumin (10 ug/mice) followed by subcutaneous injection of 15d-PGJ2 (1 mg/kg) every 12 hours for 48hs. After that period, the leukocytes were harvested from peritoneal cavity and eosinophils were counted after eosinophil peroxidase staining (EPO). **Results:** Treatment of EWI carriers with 15d-PGJ2 decrease the eosinophil migration to the peritoneal cavity as well decrease the eosinophilopoiesis in the bone marrow of EWI animals. **Discussion/Conclusion:** We demonstrated that 15d-PGJ2 decreases eosinophil recruitment. These observations suggest that the use of pharmacological 15d-PGJ2 may be a novel therapeutic modality for the treatment of allergic diseases by negatively regulating the eosinophil functions. Financial Support: PAPE/FAPEMIG 2009/006

#### 04.071

Estudos adicionais sobre a propriedade anti-inflamatória do óleo essencial da resina de *Protium heptaphyllum* March. (Almécega) em roedores. Alves AAR.<sup>1</sup>, Amaral MPM<sup>2</sup>, Oliveira NNPM<sup>1</sup>, Carvalho AA<sup>3</sup>, Chaves MH<sup>3</sup>, Oliveira FA<sup>1</sup> <sup>1</sup>UFPI - Plantas Medicinais, <sup>2</sup>UFPI - Bioquímica e Farmacologia, <sup>3</sup>CCN-UFPI - Química

**Introdução:** A espécie *Protium heptaphyllum* March., conhecida popularmente como almécega, encontrada na Amazônia e em outras regiões do País, produz uma resina bastante utilizada na medicina popular como cicatrizante e anti-inflamatório. Em trabalhos anteriores, o óleo essencial obtido da resina de *Protium heptaphyllum* (OEP) demonstrou atividade anti-edematogênica ao inibir, significativamente, os edemas induzidos por carragenina, dextrana e histamina em ratos e camundongos. O objetivo deste trabalho é ampliar os estudos com o OEP avaliando sua atividade em modelos animais de inflamação crônica. **Metodologia:** Licença de Autorização do C.E.P/UFPI 02/08. Camundongos Swiss machos foram previamente tratados (v.o.) com veículo, OEP (50, 100 e 200 mg/kg) ou indometacina (10 mg/kg) 1 h antes da administração intraplantar (pata traseira direita) de zymosan (50 µL, 2%). O edema formado foi avaliado por pletismometria (mL) 1, 2, 3, 4, 24 e 48 h após a injeção do agente inflamatório. Em outro experimento, camundongos Swiss (20-30g) foram divididos em grupos ( $n=8$ /grupo). O dorso dos animais foi depilado, feita uma incisão onde foram implantados, subcutaneamente, 2 “pellets” de algodão esterilizados (10 mg/cada), um de cada lado do dorso. Os animais foram tratados (v.o.) com veículo (10 mL/kg), OEP ou ibuprofeno (300 mg/kg), uma vez ao dia, por 7 dias consecutivos. Ao final do oitavo dia, os animais foram sacrificados, os “pellets” de algodão retirados e determinado os pesos úmido e seco (mg). **Resultados:** Zymosan promoveu intenso edema com efeito máximo na 3<sup>a</sup> h ( $0,52 \pm 0,02$ ) de seu desenvolvimento, mantendo-se elevado até a 48<sup>a</sup> h. O pré-tratamento dos animais com o OEP (100 e 200 mg/kg) inibiu significativamente ( $p<0,05$ ) o edema elicitado por zymosan nos tempos de 1 (41; 42%), 2 (37; 36%), 3 (33; 38%), 4 (30; 34%), 24 (22; 31%) e 48 h (31; 43%), respectivamente. Da mesma forma, indometacina (10 mg/kg), foi capaz de reduzir o edema em todos os tempos observados 1 (34%), 2 (51%), 3 (35%), 4 (52%), 24 (36%) e 48 h (44%). **Discussão:** Os resultados obtidos fornecem evidências adicionais que o OEP possui uma ou mais substâncias com propriedades anti-inflamatórias ao reduzir o edema induzido por zymosan e a formação de tecido granulomatoso induzido por pellets de algodão, inibindo a fase proliferativa do processo inflamatório. Esses efeitos podem ser devido à inibição de mediadores inflamatórios, migração celular e acúmulo de colágeno nos sítios da inflamação. Conclui-se, portanto, que o óleo essencial da resina de *Protium heptaphyllum* possui propriedade anti-inflamatória modulando a fase crônica do processo inflamatório. Apoio Financeiro: CNPq/CAPES/UFPI

#### 04.072

Exacerbation of pulmonary allergic inflammation after rat airways exposition to a kunitz-type inhibitor. Ruiz KF<sup>5</sup>, Mello GC<sup>1</sup>, Souza IA<sup>1</sup>, Macedo MLR<sup>2</sup>, Squebola Cola, DM<sup>1</sup>, Antunes E<sup>1</sup> <sup>1</sup>FCM-UNICAMP - Farmacologia, <sup>2</sup>UNICAMP – Bioquímica

**Introduction:** DMTI-II is a Kunitz-type inhibitor isolated from *Dimorphandra mollis* seeds that causes eosinophil influx into the rat peritoneal cavity at early time after injection (4 h). Our preliminary study has shown that the intranasal instillation of DMTI-II prior to allergen (ovalbumin; OVA) challenge exacerbates the eosinophil influx in bronchoalveolar lavage (BAL) fluid. The present study aimed to investigate whether DMTI-II administered after OVA challenge also exacerbates the allergic eosinophil influx into the BAL fluid. **Methods:** This study was approved by the Ethical Committee for animal studies of UNICAMP (Protocol n° 1454-1). Male Wistar rats were sensitized by subcutaneous injection of OVA. Fourteen days later, sensitized rats were challenged with OVA (1000 µg) or instilled with PBS (200 µL). DMTI-II (10 µg) or sterile PBS buffer (control group) was instilled 4 h after OVA challenge; BAL fluid was collected at 24 h post-OVA challenge. **Results:** The eosinophil counts in BAL fluid from DMTI-II (10 µg)-exposed rats were significantly enhanced ( $0.8 \pm 0.4 \times 10^6/\text{BAL}$ ) when compared with control group ( $0.3 \pm 0.07 \times 10^6/\text{BAL}$ ;  $p < 0.05$ ;  $n = 5$ ). No significant differences were found for the mononuclear cells and neutrophils. **Conclusions:** The airways post-exposure to DMTI-II exacerbates the allergic pulmonary eosinophil influx. This capacity of DMTI-II to recruit eosinophils is likely to reflect the allergen properties of proteinase inhibitors belonging to the Kunitz family. **Support:** FAPESP



#### 04.073

Nitric oxide controls pecam-1 expression on endothelium by a mechanism dependent on IL-10 secretion. Hebeda CB<sup>1</sup>, Tamura EK<sup>2</sup>, Markus RP<sup>2</sup>, Farsky S<sup>1</sup> <sup>1</sup>FCF-USP - Análises Clínicas e Toxicológicas, <sup>2</sup>IB-USP – Fisiologia

**Introduction:** The dual role of nitric oxide (NO) on leukocyte migration has been extensively investigated. We have shown that *in vivo* chronic blockade of NO synthesis, elicited by L-NAME treatment (20mg/kg/day; 14 days; oral route), impairs leukocyte-endothelium interactions in non-inflammatory conditions dependent, partially, on reduced L-selectin and PECAM-1 expression on leukocytes and endothelium, respectively (Farsky *et al.*, *Inflamm. Res.* 53 (442); Hebeda *et al.*, *Biochem. Biophys. Res. Commun.* 377(694), 2008). Additionally, L-NAME treatment also inhibits PECAM-1 expression on endothelial cells stimulated by LPS, indicating that NO acts as a pro-inflammatory mediator on LPS-induced neutrophil recruitment. Here we investigated the role of L-NAME treatment on neutrophil migration into LPS-inflamed peritoneum and on the expression of adhesion molecules and cytokine secretion. **Methods:** Male Wistar rats (180 ± 30g) were treated with L-NAME (20mg/kg/day; 14 days; oral route) whereas control animals received only water. One set of control and L-NAME-treated animals was inflamed with LPS (5mg/kg; i.p.) on the last day of the treatment. Four hours later, peritoneal migrated leukocytes were quantified in a Neubauer chamber and differential counts were realized in staining smears; cremaster muscle was collected to quantify synthesis and expression of PECAM-1 using RT-PCR assay and immunohistochemistry, respectively; blood was collected to quantify cytokine secretion by ELISA. Circulating lymphocytes (Percoll<sup>®</sup> gradient) were obtained from another set of animals, treated only with water or L-NAME, and were stimulated *in vitro* with LPS (5mg/mL) to quantify supernatant cytokine secretion and NO production by Griess reaction. Primary culture of endothelial cells was obtained from cremaster muscle of non-treated rats according to Chen *et al.*, *Microvasc. Res.* 50(119), 1995 and modified by Lotufo *et al.*, *Eur. Journ. Pharmacol.* 534(258), 2006. Supernatant of previous lymphocyte culture obtained from control and L-NAME treated rats were incubated with endothelial cells in the presence or absence of LPS (1mg/mL; 4 hours). After that, PECAM-1 expression was quantified on endothelial cells using FITC-labeled monoclonal antibody by confocal microscopy. The experiments were conducted according to the Ethics Committee in Animal Experiments n.53/2008 – Number Protocol – 60. **Results:** L-NAME-treated rats presented reduced numbers of polymorphonuclear (40% vs. control) and mononuclear leukocytes (45% vs. control) in inflamed peritoneum; reduced expression of PECAM-1 on endothelial cells (control=146±1.15; L-NAME=138±0.93) and increased serum levels of IL-10 (control=47.40±0.62; L-NAME=55.27±0.71). Lymphocytes of L-NAME-treated rats secreted higher levels of IL-10 (control=278.4±13.10; L-NAME=726.3±3.24). Primary cultured endothelial cells incubated with supernatant of L-NAME-lymphocytes presented lower PECAM-1 expression after LPS incubation (control=511.4±68.16; L-NAME=112.1±19.88). **Discussion:** Here we demonstrated that NO modulates leukocyte migration into the LPS-inflamed peritoneum. This effect may involve a control of NO on IL-10 secretion by lymphocytes, which exert an inhibitory effect on PECAM-1 expression on endothelial cells. This is a new pathway of the role of NO on leukocyte-endothelium interaction. Financial support: FAPESP 05/60329-0 and Capes.

#### 04.074

*In vivo* hydroquinone (hq) exposure impairs functional activity of peritoneal cells. Hebeda CB<sup>1</sup>, Macedo SMD<sup>1</sup>, Cavalcanti DM<sup>1</sup>, Ferreira Jr JMC<sup>3</sup>, Sousa MGT<sup>1</sup>, Almeida SR<sup>1</sup> <sup>1</sup>FCF-USP - Análises Clínicas e Toxicológicas, <sup>2</sup>Instituto Butantan - Imunoquímica

**Introduction:** HQ is a phenolic compound found in cigarette smoking, medicines and foods. It is also obtained from endogenous metabolism of benzene. We have shown that rats exposed to HQ (5 or 10 mg/kg; ip) present impaired leukocyte migration to the lung during allergic inflammation. Here we investigated the role of HQ exposure on functional activities of peritoneal cells related to acquired inflammatory response.

**Methods:** Male Wistar rats were exposed to HQ (5 or 10 mg/kg), ip; once a day, 8 doses with an interval of 2 days every 5 doses. Control animals received vehicle (saline:etanol; 1:10). Twenty four hours after later exposures, resident peritoneal cells were collected and stimulated *in vitro* with ovalbumine (OVA) to assess CD40, CD80, CD86, CD18 and ICAM-1 membrane expression by flow cytometer; to investigate concentrations of IFN-gamma, IL-10 and IL-4 in the supernatant by ELISA; and to quantify killing activity of *Candida albicans* using optical microscopy. The experiments were conducted according to the Ethics Committee in Animal Experiments n.53/2008 – Number Protocol – 65. **Results:** HQ did not modify expressions of CD80, CD40, CD86, CD18 and ICAM-1 on peritoneal cells; did not alter concentrations of IL-4 and IL-10, but reduced concentrations of IFN-gamma (37% vs. control); and reduced killing activity (331% vs. control). Incubation of cells with recombinant IFN-gamma reversed the decreased killing activity. **Discussion:** Altogether, the obtained results suggest that *in vivo* exposure to HQ reduces peritoneal cell functions related to acquired inflammatory response, by a mechanism dependent on reduced IFN-gamma secretion. Financial Support: CAPES; FAPESP 03/04013-8.

#### 04.075

Circulating mediators are involved in the impairment of neutrophil migration in patients with uterine cervical neoplasia. Micheli DC<sup>1</sup>, Fernandes Jr PC<sup>2</sup>, Murta EFC<sup>2</sup>, Tavares-Murta BM<sup>1</sup> <sup>1</sup>UFTM - Biological Sciences, <sup>2</sup>UFTM - Gynecology and Obstetrics

**Introduction:** Uterine cervical cancer is the third most common cancer in women worldwide. We have demonstrated reduced function of circulating neutrophils in invasive cancer, compared to patients at early disease stage. The surgical removal of tumor in patients at pre-invasive stage increased neutrophil migration, suggesting that the production of soluble mediators by tumor cells altered the function of circulating leucocytes. **Objective:** To investigate the involvement of circulating mediators in the reduced capacity of neutrophil migration in patients with uterine cervical neoplasia at different stages. **Methods/Results:** Patients with uterine cervical neoplasia were grouped as: 1) cervical intraepithelial neoplasia, at pre-invasive stage (CIN group) and, 2) invasive cancer (INV group). Healthy women served as controls. The study protocol (number 445 and 933) was approved by the UFTM Committee on the Use of Human Subjects. Circulating purified neutrophils from controls (n=15) were treated with heterologous normal serum (50%, n=5) or sera (0,5; 5 and 50%) obtained from CIN (n=5) or INV (n=5) groups, and were assayed in a micro-chemotaxis chamber. Control neutrophils treated with sera of both patient groups showed dose-dependent reduced migration in response to fMLP, LTB<sub>4</sub> and IL-8. The migration of control neutrophils was not altered by normal sera but was completely inhibited by sera from patient groups towards all chemoattractants ( $p < 0,05$ ). Since circulating cytokines and nitric oxide (NO) inhibit neutrophil migration in different experimental models, the levels of TNF- $\alpha$ , IL-6, IL-8 and IL-10 (ELISA) and nitrite (Griess reaction) were assessed in serum and/or supernatants of cultured neutrophils or mononuclear cells. The serum concentrations of IL-6 and IL-8 (median; 25-75%, pg/ml) were elevated in patients (n=40) (IL-6: 5,6; 3,6-12,8; IL-8: 11,3; 3,6-16,0) than controls (n=13) (IL-6: 1,7; 1,3-3,5; IL-8: 2,4; 0,7-8,3). The levels of IL-6 were increased in both CIN (n=18,  $p < 0,05$ ) and INV (n=22,  $p < 0,001$ ) patients than controls, but higher levels were detected in INV group (7,2; 4,5-14,3) compared to CIN group (3,8; 2,5-8,4). The IL-8 concentrations were significantly increased in INV group (12,8; 6,4-17,7) compared to controls, but not in CIN patients (8,4; 2,6-15,6). The serum levels of TNF- $\alpha$  and IL-10 did not differ between groups. Nevertheless, the production of TNF- $\alpha$  and IL-10 in the neutrophil supernatants was higher ( $p < 0,01$  and  $p < 0,05$ , respectively) in patients (n=11) than controls (n=7), accounted for by INV group, while the production of nitrite (mM, mean  $\pm$  SD) by mononuclear cells was significantly elevated by both CIN (n=6, 10,9  $\pm$  0,9) and INV (n=5, 11,3  $\pm$  2,0) groups compared to control cells (2,6  $\pm$  0,8) ( $p < 0,001$ ). **Discussion/Conclusion:** Patients with uterine cervical neoplasia at invasive stages produce diverse systemic mediators with inhibitory effect on neutrophil function. Most important, even at non-invasive stage, patients are able to produce circulating inhibitory mediators such as IL-6 and NO, suggesting impairment of the inflammatory response at very early stages of the disease. **Financial Support:** FAPEMIG, CNPq, FUNEPU

#### 04.076

Histological changes induced by extracts of *Pterodon polygalaeflorus* in model of acute inflammation. Vicente LS, Silva GP, Freitas GM, Marques PR, Coelho MGP UERJ - Bioquímica

**Introduction:** The genus *Pterodon* is included in the family Fabaceae and is composed of five species widely distributed in Brazil. Extracts prepared from the fruits of these plants are used in folk medicine as anti-rheumatic, anti-inflammatory and analgesic preparations. Previous studies showed antinociceptive activity (Coelho, J Ethnopharmacol 98:109,2005), anti-inflammatory (Silva, J Pharm Pharmacol 55:135,2004), anti-arthritic (Sabino, Phytother Res 13: 613, 1999) for extracts of *Pterodon pubescens*. The objective was to evaluate anti-inflammatory activity of hexane extract of *Pterodon polygalaeflorus* (EHxPpg) in acute inflammation induced by carrageenan in the air pouch model. **Material and Methods:** In the back of the SW male mice (30-40 g, 4 to 6 months) was injected 5 ml (s.c.) of sterile air and after 3 days, plus 3 ml of sterile air to keep the air pouch. Six days after, each group (n = 5) was treated orally with 1mg/kg of EHxPpg, ASA (100 mg/kg) or vehicle (ethanol 15% with 1.25% Tween-20). After 1 h, health animals received injection of saline and the other carrageenan 1% into the pouch. After 4 h, the cavity was washed with NaCl 0.9% EDTA 2 mM (1 ml), the volume of exudate was measured and the total number of cells determined. For histological evaluation, the tissues from the air pouch were removed, fixed in buffered formalin 10% (pH 7.4) for 7 days, processed, stained with HE and observed under light microscopy. All animal experiments were approved by the ethics committee of IBRAG-UERJ protocol 05/2009. **Results and Discussion:** Vascular changes such as vasodilation and consequent increase in vascular permeability are the first events observed in the inflammatory process. The increase in vessel size can increase the flow of blood and leukocytes that migrate to the site of stimulation. In this study, this effect can be observed mainly in animals in the vehicle group in which there was an area of intense vascularization and an intense deposition of layers of fibrin, due to the presence of fibroblasts at the site. Tissues of animals treated with EHxPpg showed obvious reduction of histopathological features of inflammation, such as reduction in the vascularization, in the size of the vessels and the quantity of cells, when compared to controls. Furthermore, there was some decrease in the area of fibrin deposition in this group. **Conclusion:** The EHxPpg inhibited the acute inflammation induced by carrageenan in the model of air pouch. **Financial support:** CNPq, FAPERJ e UERJ.

#### 04.077

Short-term induction of thrombocytopenia delays periodontal healing in rats with periodontal disease: participation of endostatin and vascular endothelial growth factor. Herrera BS<sup>1</sup>, Coimbra LS<sup>2</sup>, Figueiredo MN<sup>2</sup>, Spolidorio DMP<sup>2</sup>, Muscará MN<sup>3</sup>, Spolidório LC<sup>1</sup> <sup>1</sup>FOAr-UNESP - Patologia, <sup>2</sup>FOAr-UNESP - Fisiologia e Patologia, <sup>3</sup>USP - Farmacologia

**Introduction:** Platelets contain factors, including vascular endothelial growth factor (VEGF) and endostatin that can modulate the healing process. We evaluated the effects of severe thrombocytopenia on periodontal healing in rats and determined the contribution of VEGF and endostatin to the healing process. **Methods:** The Experimental protocols were approved by the State University of Sao Paulo's Animal Care and Use Committee (number 06/2006). Briefly, rats were distributed into three test groups and two control groups. Cotton ligatures were placed at the gingival margin level of the lower first molar in the test groups. Sham-operated rats and rats in one of the periodontitis groups were killed 15 days later. Rats in the remaining two periodontitis groups had the ligatures removed in order to study the spontaneous recovery from the periodontal disease 15 days later, and these rats were treated with rabbit ant platelet serum, in order to induce thrombocytopenia, or normal rabbit serum. An additional group without ligatures received antiplatelet serum in the same period. **Results:** After ligature removal, rats treated with normal rabbit serum showed reduced myeloperoxidase activity, decreased alveolar bone loss and increased numbers of blood vessels. Thrombocytopenia caused a delay in alveolar bone regeneration, a decrease in the number of vessels and a modest decrease in myeloperoxidase activity. In the rats with periodontitis, serum endostatin concentrations were slightly decreased and serum VEGF remained unchanged compared with sham-operated animals. After ligature removal, a significant VEGF increase and endostatin decrease were observed in the rats treated with normal rabbit serum. Thrombocytopenia led to a dramatic fall in both VEGF and endostatin concentrations. **Discussion:** Thrombocytopenia leads to a delay of periodontal healing in the situation of experimental periodontitis, which might be mediated in part by a decrease in the serum concentration of VEGF and endostatin derived from the platelets. However, other factors derived from the platelets may also have contributed to a delay of periodontal healing in the rats with thrombocytopenia. **Financial support:** FAPESP

#### 04.078

Regulatory effect of annexin-1 on the acute phase of silicosis in mice. Trentin PG<sup>1</sup>, Santos TPO<sup>1</sup>, Ferreira TPT<sup>1</sup>, Arantes ACS de<sup>1</sup>, Pires ALA<sup>1</sup>, Flower RJ<sup>2</sup>, Perretti M<sup>2</sup>, Martins MA<sup>1</sup>, Silva PMR<sup>1</sup> <sup>1</sup>FIOCRUZ - Fisiologia e Farmacodinâmica, <sup>2</sup>William Harvey - Biochemical Pharmacology

**Introduction:** Silicosis is a chronic occupational disease caused by inhalation of free crystalline silica particles and is characterized by an intense inflammatory response followed by fibrosis and granuloma formation. There are several endogenous mediators able to regulate negatively inflammatory responses, in order to ensure the control of such processes. Glucocorticoids are considered as important agents based on their anti-inflammatory activity, which has been shown to be, at least partially, dependent on the generation of an intermediate protein named annexin-1. In this study we investigated the regulatory role of annexin-1 in the acute phase of the experimental model of silicosis in mice. **Methods:** Anesthetized Balbc (ANX1<sup>+/+</sup>) and annexin-1 knockout mice (ANX1<sup>-/-</sup>) were intranasally instilled with silica particles (10 mg) or saline, and analyses performed 7 days post-silica. Lung function (resistance and elastance) and airways hyperreactivity to the aerosolization of methacholine (3 - 27 mg/mL) were evaluated by whole body invasive plethimography (Buxco System). Morphological alterations were analyzed by classical histological techniques including staining with Hematoxylin-Eosin and Picrus-Sirius for granuloma formation and collagen deposition, respectively. Cytokines and chemokines were quantified by ELISA. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (license 0213-4). **Results:** ANX1<sup>+/+</sup> silicotic mice showed increased levels of basal lung resistance and elastance as well as airways hyperreactivity to methacholine. Marked inflammatory and fibrotic responses including leukocyte infiltration, collagen deposition and granuloma formation were also noted in the ANX1<sup>+/+</sup> silicotic mice. Higher amounts of chemokine (KC, MIP-2 and MCP-1) and cytokine (TNF $\alpha$ , TGF $\beta$  and INF $\gamma$ ) generation were detected in silicotic animals. The ANX1<sup>-/-</sup> control mice showed normal basal levels of lung resistance and elastance and a clear exacerbation of the response to methacholine provocation. In the case of ANX1<sup>-/-</sup> silicotic mice, an exacerbation of lung elastance but not resistance was detected after methacholine provocation. The percentage of granuloma area was similar in the lungs of ANX1<sup>-/-</sup> and ANX1<sup>+/+</sup> animals, though the former exhibited a more marked inflammatory infiltrate and collagen deposition compared to the later. Generation of inflammatory and fibrotic chemokines (KC and MCP-1) and cytokines (TNF $\alpha$  and INF $\gamma$ ) was also increased in the lung tissue of ANX1<sup>-/-</sup> as compared to ANX1<sup>+/+</sup> mice. **Conclusion:** Altogether our findings show the annexin-1 seems to play a relevant regulatory role in the control of inflammation and fibrotic response in experimental silicosis in mice. In addition, they indicate that treatment with the entire protein or its derivatives may constitute a potential pharmacological tool for the treatment of chronic fibrotic lung diseases such as silicosis. **Financial support:** FIOCRUZ, CNPq, FAPERJ (Brazil).

#### 04.079

Effect of thalidomide on lung inflammation caused by silica particles in mice. Ciambarella BT, Santos TPO, Azevedo, RB, Arantes ACS de, Trentin PG, Dias DF, Ferreira TPT, Jurgilas PB, Cordeiro RSB, Martins MA, Silva PMR IOC-FIOCRUZ - Fisiologia e Farmacodinâmica

**Introduction:** The inhalation of silica particles leads to development of silicosis, an occupational disease characterized by leukocyte infiltration, collagen deposition and granuloma formation. TNF- $\alpha$  is a cytokine with proinflammatory and profibrotic properties which has been involved in several inflammatory lung diseases. Thalidomide is a compound effective against some chronic lung diseases based on its property to inhibit TNF- $\alpha$  generation. Thus, in this study we investigated the potential effect of thalidomide on the experimental model of silicosis in mice. **Methods:** Swiss-Webster mice were instilled with silica (10 mg/50  $\mu$ l) and control mice received the same volume of saline. Thalidomide was (25 and 100mg/kg, p.o.) administered curatively, once a day during 7 consecutive days, starting 21 days post-silica. All analyzes were made 28 days after silica provocation and included: i) lung function (resistance and elastance) and airways hyperreactivity to methacholine (3 – 27 mg/ml) evaluated by whole body invasive plethymography (Buxco System); ii) morphological alterations were analyzed by classical histological techniques using H&E staining and by morphometry and iii) collagen content in the lung tissue was measured by Sircol technique. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (license 0213-4). **Results:** We noted that silicotic mice showed an increase in the basal levels of lung resistance and elastance, in parallel with airways hyperreactivity to aerosolization with the bronchoconstrictor agent methacholine. Higher levels of chemokines (KC) and cytokines (INF- $\gamma$  and TGF- $\beta$ ) were also noted in the lung tissue of silicotic mice in comparison to those of control animals. Curative administration of silicotic mice with thalidomide led to suppression of the increased basal levels of lung resistance and elastance as well as of airways hyperreactivity to methacholine aerosolization. Thalidomide also significantly reduced lung tissue alterations such as collagen deposition and granuloma formation. The generation of KC, INF- $\gamma$  and TGF- $\beta$  was also sensitive to treatment with thalidomide. **Conclusion:** Our findings show that the failure of lung function as well as inflammatory and fibrotic components associated with silicosis in mice were clearly sensitive to treatment with thalidomide. They also indicate TNF- $\alpha$  seems to be an important pharmacological target of the disease and that thalidomide can be considered as a potential therapeutic approach when considering fibrotic lung diseases such as silicosis. **Financial support:** FIOCRUZ, CNPq, FAPERJ.

#### 04.080

*Geissospermum vellosii* (Pau-pereira): antinocicepção induzida pelo extrato e fração com atividade anticolinesterásica. Costa TWR<sup>1</sup>, Louback LS<sup>2</sup>, Miranda ALP<sup>2</sup>, Lima JA<sup>3</sup>, Pinto AC<sup>3</sup> <sup>1</sup>UNISUAM - Enfermagem, <sup>2</sup>LASSBio-FF-UFRJ - Fármacos, <sup>3</sup>UFRJ - Química

**Introdução:** A Doença de Alzheimer é caracterizada pela deposição de emaranhados neurofibrilares e acúmulo do peptídeo  $\beta$ -Amilóide nas membranas neuronais, promovendo decaimento da concentração de acetilcolina nas sinapses do sistema nervoso central. Associado a este processo ocorre neuroinflamação, devido à ativação das células da Glia. A atividade anticolinesterásica e reversão da amnésia induzida por escopolamina de uma fração de *Geissospermum vellosii* (Pau Pereira), rica em alcalóides indólicos, cuja geissospermina é o majoritário, foi estudada por este grupo de pesquisa (Lima, JA; *Pharmacol. Biochem. Behav.*; 42; 508; 2009). Relatos da literatura mostram inibidores da acetilcolinesterase com atividade antinociceptiva (Yoon MH; *Acta Anaesthesiol. Scand.*; 47; 1079; 2003). O objetivo deste trabalho foi avaliar a atividade antinociceptiva do extrato etanólico (EE) e da fração do Pau Pereira. **Métodos:** A atividade antinociceptiva foi avaliada em camundongos através do teste de nocicepção induzida por formalina (2,5%; 20  $\mu$ L i.pl.), um ensaio bifásico, com uma fase aguda inicial (0-5 min; dor neurogênica) seguida por uma fase tônica prolongada (15-30 min; dor inflamatória). O tempo de lambida/mordida foi cronometrado e considerado indicativo de dor; e através do teste de contorção abdominal induzida por ácido acético (0,1N; 10  $\mu$ L/g de animal; i.p.). As contorções abdominais foram contadas no tempo de 10-30 min após o estímulo doloroso. No teste de formalina o EE foi empregado na dose de 30 mg/kg e a fração 10, 30 e 60 mg/kg, administrados por via i.p. e no teste de contorção EE e fração a 30 mg/kg por via oral. O tratamento foi feito 1h antes do estímulo nociceptivo. O uso de animais nestes experimentos foi aprovado pelo comitê de ética da UFRJ e está em processo de liberação do número de licença. **Resultados e Discussão:** No teste da formalina, o EE e a fração inibiram a segunda fase inflamatória, mas não a fase neurogênica, com porcentagens de inibição de 82,8%\* para o EE (30 mg/kg) e de 21,5\*; 84,9\* e 100%\* para as doses de 10, 30 e 60 mg/kg da fração, respectivamente. Na contorção abdominal o EE e a fração reduziram a nocicepção em 35,8%\* e 48,5%\*, respectivamente (n=8-10 animais, \*p<0,05). Estes dados demonstram atividade antinociceptiva para o EE e fração do Pau-pereira. É apontado o envolvimento de receptores colinérgicos nicotínicos ( $\alpha 7$ ;  $\alpha 4\beta 2$  e  $\alpha 3\beta 2$ ) e muscarínicos (M3) em processos antinociceptivos. Embora o isolamento dos alcalóides e o mecanismo de antinocicepção ainda precisem ser desvendados, o fato da fração rica em alcalóides apresentar atividade anticolinesterásica nos dá indicativos de que a antinocicepção pode ser devido ao aumento da concentração de acetilcolina. Além disso, a segunda fase da formalina e a contorção abdominal, onde houve atividade do EE e fração, são caracterizadas pela relevante participação de componentes inflamatórios, o que indica uma possível atividade anti-inflamatória para o pau pereira. A busca por substâncias com atividade dual, anticolinesterásica e anti-inflamatória pode ser útil no retardo da progressão da doença de Alzheimer. **Apoio Financeiro:** CAPES, CNPq, FAPERJ.



#### 04.081

*N*-acetylcysteine prevents baker yeast-induced inflammation and fever. Saraiva ALL<sup>1</sup>, Ferreira APO<sup>1</sup>, Pasin JSM<sup>1</sup>, Schirmer VR<sup>1</sup>, Ratzlaff V<sup>1</sup>, Ferreira J<sup>2</sup>, Mello CF<sup>1</sup> <sup>1</sup>UFSM - Fisiologia e Farmacologia, <sup>2</sup>UFSM - Química

**Introduction:** In infectious fever, the activation of immune cells triggers the transcription of innate immune proteins, including cytokines which induce cyclooxygenase-2 and consequent prostaglandin E<sub>2</sub> production in the anterior hypothalamus, ensuing fever. Recent studies have demonstrated that N-acetylcysteine (NAC), a precursor of glutathione, protects against several inflammatory diseases. However, it is poorly known whether it interferes with fever, an inflammation-dependent processes. Therefore, we investigated the effects of NAC on fever and inflammatory response induced by intraperitoneal administration of baker yeast. **Methods:** Wistar rats (26-28 days of age) were used. The rectal temperature (T<sub>R</sub>) was measured by the insertion of a thermistor probe into the rectum of animal. To determine whether NAC prevented baker yeast induced-fever and peritoneal inflammation, after measuring the basal rectal temperature (T<sub>Rb</sub>) at 08:00 a.m., the animals were injected with vehicle (pyrogen-free 0.9% NaCl, 5 ml, s.c.) or NAC (500 mg/kg, s.c.). One hour later, they received baker yeast (135 mg/kg, i.p., 10 ml/kg) or vehicle (pyrogen-free 0.9% NaCl, i.p., 10 ml/kg) and T<sub>R</sub> was recorded every hour for 6 hours. Protein extravasation and leukocyte number were evaluated in peritoneal lavage 4 hours after yeast injection; cytokine content was evaluated in peritoneal lavage and hypothalamus. The centrally-mediated antipyretic and peritoneal anti-migratory effect of NAC was accessed by injection of vehicle 100 µL/site or NAC 50 µg/100 µL/site, i.t., 2 hours after yeast injection. T<sub>R</sub> was recorded every hour for 6 h after yeast injection. Experiments were approved by the Committee on the Use and Care of Laboratory Animals of our University (Process number: 23081.013228/2008-78). Cytokine levels were measured using a commercially available ELISA Kit from R&D System and the leukocyte count was performed in Neubauer chamber. **Results:** Systemic administration of NAC prevented fever induced by baker yeast [F(5,185) = 2.7; p<0.05]. In addition, NAC decreased leukocyte migration [F (1,19)= 10.83; p < 0.01], plasma protein extravasation [ F(1,14)= 9.05; p<0.01] and decreased tumor necrosis factor (TNF)-α [F(1,21)= 4.39; p < 0.05], interleukin (IL)-1β [F (1,21) = 5.68; p < 0.05] release induced by baker yeast in peritoneal lavage and IL-1b release in hypothalamus [F(1,15)=11.71; p<0.01]. The central administration of NAC (50 µg, i.t. also prevented baker yeast-induced fever [F(1,18)= 12.13; p < 0.01], but did not alter leukocyte migration to peritoneal cavity. **Discussion:** These data constitute circumstantial evidence that NAC is a antipyretic compound and that hypothalamic IL-1b, but not TNF-α, are involved in baker yeast-induced fever. It is also remarkable that NAC presented an anti-inflammatory effect on yeast-induced peritonitis. Other important finding is that the central administration of NAC reduced yeast-induced fever, with no impact on peritoneal leukocyte migration, suggesting that increase in central SH availability may be more relevant for the antipyretic effect of NAC than its peripheral anti-inflammatory action. Therefore we suggest that NAC antipyretic effect is due to inhibition IL-1β production in hypothalamus. **Finances support:** CNPq, CAPES and FAPERGS.

#### 04.082

Derivados tiazolidinônicos 2,4-dissubstituídos (LPSF/GQ-138 e LPSF/GQ-140) como possíveis candidatos a fármacos anti-inflamatórios. Lins TUL, Araújo LCC, Oliveira TB, Pitta IR, Lima MCA, Galdino SL, Silva TG UFPE - Antibióticos

**Introdução:** Por possuir vários sítios de substituição, o anel 4-tiazolidinona nos leva a um grande número de análogos estruturais que apresentam diversas atividades biológicas como, por exemplo, atividade anti-inflamatória (Liesen et al, 2008). As tiazolidinonas têm sido alvo de estudos a fim de se obter novas moléculas potencialmente bioativas que melhorem a eficácia farmacológica e minimizem os efeitos colaterais do tratamento convencional dos processos inflamatórios. Sendo assim, este trabalho teve por objetivo avaliar a atividade anti-inflamatória de dois novos derivados tiazolidinônicos 2,4 dissubstituídos: LPSF/GQ-138 e LPSF/GQ-140.

**Métodos:** Foram utilizados camundongos albinos Swiss (*Mus musculus*), machos, com 60 dias de vida e peso variando de  $20 \pm 5$  gramas. Os experimentos foram executados segundo as diretrizes do Comitê de Ética para Experimentação Animal da UFPE sob número de registro 23076.006242/2009-75. Para a avaliação da atividade anti-inflamatória, os animais receberam como tratamentos: solução de NaCl 0,9% (0,1mL/10g v.o.; controle negativo), indometacina (28mMol/kg v.o.; controle positivo) e os derivados tiazolidinônicos LPSF/GQ-138 e LPSF/GQ-140 (10mMol/kg v.o.). Após 1h os animais foram submetidos à injeção de 0,25mL de carragenina (1%) na cavidade peritoneal com o objetivo de induzir a resposta inflamatória. Decorridas 4h, os animais foram eutanasiados em câmara de CO<sub>2</sub> e submetidos à cirurgia para abertura do abdome, e a cavidade peritoneal foi lavada com 2 mL de solução de NaCl 0,9% com EDTA e o líquido coletado com seringa 3mL estéril (GUPTA et al., 2005). O exsudato obtido foi processado em analisador hematológico Micros 60, onde foram contados os leucócitos polimorfonucleares (PMNL) presentes. **Resultados:** Os derivados tiazolidinônicos LPSF/GQ-138 e LPSF/GQ-140 testados na dose de 10mMol/kg exibiram significativa inibição da migração leucocitária (71,0% e 65,5%, respectivamente). Os resultados da contagem de PMNL foram para o derivado LPSF/GQ-138 ( $3,5 \times 10^3 \pm 0,3$  células/mm<sup>3</sup>), para o derivado LPSF/GQ-140 ( $3,6 \times 10^3 \pm 1,1$  células/mm<sup>3</sup>) e para o controle negativo ( $12,1 \times 10^3 \pm 3,8$  células/mm<sup>3</sup>). O valor da inibição percentual da migração leucocitária para o grupo controle positivo indometacina foi 54,30% apresentando contagem de PMNL de  $5,52 \times 10^3 \pm 1,2$  células/mm<sup>3</sup>. Os dados representam a média do número de células  $\pm$  desvio padrão.

**Discussão/Conclusão:** Os dois compostos testados apresentaram atividade anti-inflamatória bastante promissora, reduzindo significativamente a migração celular. Observou-se que os percentuais de inibição do LPSF/GQ-138 (71,0%) e do LPSF/GQ-140 (65,5%) foram superiores ao da droga padrão indometacina (54,30%). Entretanto, é necessária a continuidade dos estudos, testando outras doses para se observar a relação dose-resposta. **Citação Bibliográfica:** Gupta M et al. *J Ethnopharmacol*, 98, 267, 2005. Liesen, AP. et al *Química Nova*, 31, 369, 2008. **Apoio Financeiro:** CNPQ/FACEPE

#### 04.083

Avaliação da inibição da migração celular pelo derivado tiazolidinônico 3,5-dissubstituído LPSF/GQ-15. Silva TG, Pereira DTM, Lins TUL, Barbosa KPS, Galdino SL, Lima MCA, Pitta IR UFPE - Antibióticos

**Introdução:** Os derivados tiazolidínicos, grupo de moléculas estruturalmente relacionadas caracterizados pelo anel tiazolidínico, são extensamente estudados devido às suas diferentes atividades biológicas: antimicrobiana, antineoplásica, antidiabética e inibidora da aldose redutase. Estudos recentes têm demonstrado o potencial dos derivados tiazolidínicos em inibir a resposta inflamatória, caracterizada pela liberação de diferentes mediadores endógenos pró-inflamatórios (prostaglandinas e leucotrienos), a partir da conversão do ácido araquidônico pelas enzimas ciclooxigenase e lipoxigenase. As tiazolidinonas também têm ação no receptor nuclear PPAR $\gamma$ . Neste trabalho, apresentaremos a avaliação da atividade anti-inflamatória do derivado tiazolidinônico- 3,5-dissubstituído LPSF/GQ-15. **Métodos:** A atividade anti-inflamatória foi avaliada com base na inibição de leucócitos polimorfonucleares (Teste "Air-Pouch") induzida pelo agente flogístico carragenina (cg) 1%. Foram utilizados camundongos de ambos os sexos albinos Swiss (*Mus musculus*). Os experimentos foram executados segundo as diretrizes aprovadas pela Comissão de Ética para Experimentos com Animais/UFPE (Protocolo N°016269/2006-23). Inicialmente, foram produzidos bolsões de ar por injeção 2,5 mL de ar estéril na área dorsal do animal (dia - 0). Decorridos 72 horas, mais 2,5 mL de ar estéril foi injetado na cavidade a fim de manter o espaço (dia - 3). Decorrido um novo intervalo de 72 horas (dia-6), os animais receberam por via oral, o derivado tiazolidinônico 3,5-dissubstituído LPSF/GQ-15, nas doses de 0,03, 0,3 e 3,0 mg/kg, o controle e o padrão (piroxicam) na dose de 3,0 mg/kg. Os compostos testados e o padrão piroxicam foram previamente dissolvidos na solução de tween 80/água destilada (2:98 v/v) considerada como o veículo. Uma hora após a administração do derivado, os animais receberam uma injeção de 1 mL de solução de carragenina 1%, diretamente na cavidade previamente formada pelas injeções de ar estéril (bolsão), com o objetivo de induzir a resposta inflamatória. Decorridas 6 horas após a aplicação do estímulo, os animais foram sacrificados por deslocamento cervical e as bolsas lavadas com 3 mL de solução salina com EDTA como líquido de arraste. O exsudato foi diluído em solução de Turk, e a contagem dos leucócitos polimorfonucleares (PMNL) foi feita em câmara de Neubauer. **Resultados:** Os resultados da contagem de PMNL/mL ( $\times 10^6$ ) nas doses testadas foram: 3 mg/kg ( $10,72 \pm 1,3$ ); 0,3 mg/kg ( $18,97 \pm 0,03$ ); 0.03 mg/kg ( $23,05 \pm 1,67$ ) ; Piroxicam 3mg/kg ( $15,06 \pm 1,91$ ); Controle com carragenina ( $49,18 \pm 1,91$ ). Os valores apresentados são significativos para o intervalo de confiança de 95% (ANOVA, teste de Bonferroni). **Discussão/Conclusão:** A atividade anti-inflamatória avaliada através do *air pouch*, demonstrou que o derivado tiazolidinônico-3,5-dissubstituído (LPSF/GQ-15) apresentou atividade anti-inflamatória significativa em relação ao controle em todas as doses testadas, observando um efeito dose-dependente. Portanto, o LPSF/GQ-15 é um candidato a protótipo de fármaco anti inflamatório. **Apoio Financeiro:** CNPq

#### 04.084

Effect of n-acetylcysteine on platelet adhesion of LPS-treated rats. Casarin AL, Pires MEL, Cardelli NJA, Antunes E, Marcondes S UNICAMP - Farmacologia

**Introduction:** Severe sepsis and septic shock are life threatening complications of infections and the most common cause of death in intensive care units. Lipopolysaccharide (LPS) is a component of the outer membrane of Gram negative bacteria and has a pivotal role in inducing Gram negative sepsis. Septic shock triggers the production of various substances including reactive oxygen species (ROS). Platelets are believed to take part in the pathophysiology of sepsis, but data are still conflicting. Therefore, in the present work we decided to study the effects of intraperitoneal injection of N-acetylcysteine (NAC), a potent antioxidant, on platelet adhesion 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 1368-1). Male Wistar rats (250-320 g) were injected i.p. with saline or LPS (from *E. coli*, 1 mg/kg). At 2 and 6h thereafter, blood was collected in ACD-C (9:1 v/v). In a second experimental group, N-acetylcysteine was injected i.p. (150 mg/kg) 30 min after LPS or saline injection. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min and the platelets were washed using citrated buffer (pH 6.0). Washed platelet adhesion was evaluated using fibrinogen-coated 96-well microtiter plates. Platelets were maintained in the plate for 30 min. After that, the plate was washed and adherent platelets were incubated with the acid phosphatase substrate for 1h. The plate was read by a microplate reader set at 405nm. **Results:** Treatment of rats with LPS for 2 and 6 h significantly increased spontaneous platelet adhesion to fibrinogen-coated plates when compared to the control rats (increase of 40 and 60%, respectively). In contrast, in thrombin-stimulated platelets LPS reduced platelet adhesion at 6h ( $50\pm 11\%$  and  $11\pm 3\%$  of adhesion in absence and presence of LPS, respectively). At 2 h, LPS did not present any effect on stimulated-platelet adhesion. In NAC-treated rats, the spontaneous and thrombin-stimulated platelet adhesion were not modified when compared to control rats. Treatment of rats with both LPS and NAC reverted the stimulatory effect of LPS on spontaneous adhesion. In addition, NAC significantly reduced thrombin-stimulated platelet adhesion in LPS-treated rats at 2h (LPS:  $51\pm 7\%$  and LPS + NAC:  $18\pm 9\%$ ,  $p < 0.05$ ). In LPS-treated rats at 6 h, treatment with NAC had no effect on stimulated-platelet adhesion ( $n=3$ ). **Discussion:** Our results showed that NAC influences platelet adhesion of LPS-treated rats by mechanisms involving inhibition of ROS generation and maintenance of antioxidant systems. **Supported by:** CAPES

#### 04.085

Avaliação da atividade antioxidante no efeito gastroprotetor da fração hexânica de *Zanthoxylum rhoifolium* Lam. em modelos de úlcera por etanol. Passos FFB<sup>1</sup>, Fernandes HB<sup>1</sup>, Piauilino CA<sup>1</sup>, Carvalho KIM<sup>1</sup>, Machado, FDF<sup>1</sup>, Chaves, MH<sup>2</sup>, Oliveira RCM<sup>1</sup>, Oliveira FA<sup>1</sup> <sup>1</sup>NPPM-CCS-UFPI -, <sup>2</sup>UFPI - NPPM/CCS, <sup>3</sup>UFPI - NPPM, <sup>2</sup>UFPI - Química

**Introdução:** *Zanthoxylum rhoifolium* Lam (Rutaceae), popularmente conhecida como mamica-de-cadela, é uma árvore originária do Brasil, com distribuição na mata pluvial e atlântica. Na medicina popular, suas partes são utilizadas como estomáquico e no tratamento das dispepsias. O objetivo desse trabalho foi dar continuidade a estudos já realizados, avaliando os efeitos e os mecanismos de ação envolvidos na atividade protetora gástrica da fração hexânica de *Z. rhoifolium* (FHZR). **Métodos:** Licença de Autorização do C.E.P. /UFPI 003/2008. Foram utilizados camundongos fêmeas (25-30 g, n=8), as quais foram tratadas v.o. com veículo (Tween 80 3%, 10 mL/kg), FHZR (125, 250 e 500 mg/kg) ou N-acetilcisteína (NAC- 750 mg/kg, i.p.). Após 1h dos tratamentos, os grupos receberam (v.o.) etanol<sub>abs</sub> (0,2 mL/animal). Decorridos 30 minutos da administração do etanol, os animais foram sacrificados, os estômagos retirados e abertos pela grande curvatura, lavados com salina 0,9% gelada e analisados por planimetria (mm<sup>2</sup>) e os dados expressos em termos de porcentagem. A atividade antioxidante da FHZR, para mensurar a absorvância para GSH foi avaliada através da metodologia descrita por Sedlak e Lindsay (1968) e a atividade enzimática da catalase (CAT) foi analisada pelo método de Beers e Sinzer (1952). Em ambos os métodos a porção glandular dos estômagos foi retirada. No primeiro método os estômagos foram homogeneizados com uma quantidade de solução de EDTA (0,02M) correspondente ao peso do tecido numa proporção de (1:1) e absorvância foi lida a 412nm dentro de 5 minutos da adição do DTNB contra um homogenato branco. A quantidade de GSH foi expressa em absorvância a 412 nm. Na avaliação da CAT, os estômagos foram homogeneizados em solução tampão fosfato de potássio (pH 7,4) e a absorvância lida a 240nm dentro de 6 minutos após a adição de uma solução reagente de peróxido de hidrogênio. O valor da absorvância foi medida para uma curva padrão de CAT e expressa em mmol/minuto/ 100mg de tecido. **Resultados e Discussão:** De acordo com os resultados obtidos, a FHZR apresenta efeito gastroprotetor no modelo de úlcera induzida por etanol<sub>abs</sub> nas doses de 125, 250 e 500 mg/kg exibindo proteção significativa (p<0,05) (47,96%, 36,09% e 76,6%, respectivamente) quando comparado ao controle normal. NAC, droga padrão, protegeu em 71,9% (p<0,05). Na leitura da absorvância para o GSH, os grupos tratados com NAC (750 mg/kg) e com FHZR (500 mg/kg), foram capazes de aumentar de forma significativa (23,65% e 14,51%, respectivamente, p<0,05), a absorvância quando comparados ao controle normal. Da mesma forma, no ensaio da atividade da CAT, os grupos tratados com NAC (750 mg/kg) e com FHZR (500 mg/kg), foram capazes de aumentar de forma significativa (p<0,05) (130,46 e 136,67%, respectivamente), os níveis de CAT quando comparados ao controle normal. GSH e CAT são agentes importantes na proteção da mucosa gástrica nas lesões induzidas por etanol, onde o aumento do dano é acompanhado por uma diminuição nos níveis desses compostos na mucosa gástrica. Os resultados indicam um forte envolvimento de GSH e de catalase no efeito gastroprotetor dessa fração. **Apoio:** PROF/CAPES/UFPI.

#### 04.086

Avaliação da atividade anti-inflamatória dos derivados tiazolidinônicos 3,5-dissubstituídos no modelo de peritonite induzida por carragenina. Malta DJN, Paula MJD, Souza MA, Oliveira CF, Galvão CM, Lima MCA, Galdino SL, Pitta IR, Silva TG UFPE - Antibióticos

**Introdução:** Na busca de um novo candidato a agente terapêutico podemos destacar moléculas contendo o anel 4-tiazolidinona. Compostos que apresentam esse anel heterocíclico em sua estrutura, possuem grande interesse científico, pois diversos estudos descritos na literatura demonstram seu amplo espectro de atividades biológicas dos seus diferentes derivados. Vários estudos demonstram que receptor ativador de proliferação de peroxissomo gama (PPAR $\gamma$ ), desempenha um papel importante nos processos inflamatórios. Um dos ligantes sintéticos do PPAR são as tiazolidinonas (troglitazona, ciglitazona, pioglitazona, rosiglitazona, troglitazona), os fármacos Anti-inflamatórios não-esteroidais (indometacina, fenoprofeno, ácido flufenâmico) e outros. Baseado nesses fatos, três derivados tiazolidinônicos 3,5-dissubstituídos (LSPF/GQ-115, LSPF/GQ-125 e LSPF/GQ-192) foram avaliados no modelo de peritonite induzido por carragenina. **Métodos:** Foram utilizados camundongos Swiss (*Mus musculus*) machos, pesando entre 25 a 30 g, com idade média de 60 dias, oriundos do Biotério do Departamento de Antibióticos/UFPE. Os experimentos foram executados segundo as diretrizes aprovadas pela Comissão de Ética para Experimentos com Animais/UFPE (Processo N°016098/2006-13). Para a indução da peritonite em camundongos, os animais foram divididos em grupos (n=6) e submetidos à injeção de 0,25mL de solução de carragenina (1%) na cavidade peritoneal. Ao grupo controle foi administrado soro fisiológico (0,9%) e ao grupo tratado os compostos da série 5-benzilideno-3-benzil-tiazolidina-2,4-diona, LSPF/GQ-115, LSPF/GQ-125 e LSPF/GQ-192, na dose de 3 mg/kg, 1 hora antes da indução da peritonite. Decorridas 4 horas, os animais foram sacrificados por deslocamento cervical e imediatamente submetidos à cirurgia para abertura do abdome, em seguida a cavidade peritoneal foi lavada com 2 mL de solução salina com EDTA e o exsudato coletado. A contagem global de células foi feita câmara de Neubauer. **Resultados:** A avaliação da atividade anti-inflamatória dos derivados tiazolidinônicos 3,5-dissubstituídos, no modelo de peritonite induzida por carragenina, apresentou os seguintes resultados para contagem de PMNL / mL ( $\times 10^5$ ): LSPF/GQ-115 ( $29,40 \pm 4,05$ ); LSPF/GQ-125 ( $25,21 \pm 3,02$ ); e LSPF/GQ-192 ( $10,33 \pm 4,83$ ), Grupo controle salina ( $94,5 \pm 4,64$ ). O percentual de inibição da migração celular variou entre 67 a 88%. Cada valor representa a média  $\pm$  erro padrão. O percentual foi calculado de acordo com a equação: Atividade anti-inflamatória%=(m-m'/m)x100. **Discussão:** Todos os derivados testados exibiram inibição da migração leucocitária quando comparados ao grupo controle. Os derivados tiazolidinônicos 3,5-dissubstituídos LSPF/GQ-115, LSPF/GQ-125 e LSPF/GQ-192 estão sendo avaliados em outros modelos experimentais a fim de obter resultados que corroborem com os dados encontrados até o momento. **Apoio Financeiro:** CNPq

#### 04.087

Effects of the protein kinase inhibitor imatinib mesylate on silica-induced pulmonary fibrosis in mice. Farias-Filho FA<sup>1</sup>, Lucena SL<sup>1</sup>, Ramos TJF<sup>1</sup>, Ferreira TPT<sup>1</sup>, Queiroz APA<sup>2</sup>, Martins MA<sup>1</sup>, Cordeiro RSB<sup>1</sup>, Silva PMR<sup>1</sup> <sup>1</sup>IOC-FIOCRUZ - Fisiologia e Farmacodinâmica, <sup>2</sup>HEMORIO - Farmácia

**Introduction:** Silicosis is an occupational disease characterized by the presence of chronic fibrosis in the lungs. We previously showed an increase of stem cell factor (SCF) expression in the lung tissue of silicotic mice, a proteic factor that has been implicated in several fibrotic diseases. Mast cell numbers were also elevated in experimental silicosis. Imatinib mesylate is known to inhibit SCF/c-kit-associated tyrosine and profibrotic signaling in several experimental models of inflammation. Thus, in this study we investigated the effect of imatinib mesylate on silica-induced pulmonary fibrosis in mice. **Methods:** Swiss-Webster mice were intranasally instilled with silica particles (10 mg) and the imatinib mesylate (30 mg/kg, p.o.) was administered 3 times a day, from day 7 up to day 28 post-silica. The analyses were made on day 28. Pulmonary function was evaluated by invasive whole body plethysmography (Buxco system). Animals were killed and whole lung samples were prepared for biochemical and histological analyses. Lung morphology, collagen deposition and mast cell number were evaluated by means of specific staining techniques. Cytokines were quantified from homogenized lung extracts using an ELISA. Lung fibroblast proliferation was evaluated in primary cell culture system by [<sup>3</sup>H] thymidine incorporation. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (license 0213-4). **Results:** Morphological analysis revealed an extensive fibrotic response and the presence of numerous granulomas in the lungs of silicotic mice as compared to controls. Administration of imatinib mesylate had a suppressive effect on granulomatous area, collagen deposition as well as on the increased of the number of mast cells in silicotic mice. In parallel, the generation of cytokines associated with pulmonary fibrosis such as IFN- $\gamma$ , TNF- $\alpha$  and TGF- $\beta$  was significantly reduced in the lung tissue of silicotic treated animals. Moreover, imatinib mesylate inhibited silica-induced lung function failure as increased resistance and elastance. In another set of *in vitro* experiments, we noted that SCF-induced pulmonary fibroblast proliferation was significantly inhibited by imatinib mesylate. **Conclusion:** Altogether, our data show that silica-induced lung function failure and fibrotic response in mice was sensitive to treatment with imatinib mesylate, suggesting the involvement of SCF/c-kit in the disease. In addition, they indicate that imatinib mesylate may be considered as a potential therapeutic tool for treatment pulmonary fibrotic dysfunctions such as silicosis. **Financial support:** PAPES4/FIOCRUZ, CNPq and FAPERJ.

#### 04.088

Periodontal disease affects carrageenan-induced temporomandibular joint arthritis in rats. Campi P, Denadai-Souza A, Martins Porto R, Maia-Dantas A, Teixeira SA, Pires, AG, Gouvea IM, Costa SKP, Muscará MN ICB-USP - Farmacologia

**Introduction:** Periodontitis is a chronic inflammatory disease characterized by the impairment of the periodontal attachment apparatus secondary to the immunological reaction to colonizing bacteria. Periodontitis has been associated with a number of systemic diseases, including rheumatoid arthritis, but not temporomandibular joint arthritis. In this way, the objective of the present study was to evaluate the relationship between these two conditions. **Methods:** The experimental protocol was approved by our institutional Ethics Committee for Animal Experimentation (protocol number 154, page 24, book 2). Male Wistar rats (200-220 g) were anesthetized by the intraperitoneal administration of ketamine (80 mg/kg) and xylazine (20 mg/kg), and a cotton ligature was placed around the right mandibular first molar (group P). A simulated procedure was performed in the Sham (Sh) group. Seven days after the disease induction, the rats were anesthetized with the same mixture of ketamine and xylazine and received an intra-articular injection of carrageenan (10 ml of a 5% solution) into the left TMJ; the same volume of sterile saline was injected into the right TMJ (control). Twenty four hours later, plasma extravasation was measured by the accumulation of  $^{125}\text{I}$ -labeled BSA into the TMJ. In addition, the TMJ cavities were washed with 50  $\mu\text{L}$  (3 times) of heparinized saline solution, and the lavage fluids were pooled and collected for total and differential leukocyte counting and measurement of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  concentrations (ELISA). **Results:** All the inflammatory parameters evaluated were higher in the ipsilateral TMJ in comparison with the contralateral, demonstrating the efficacy of the carrageenan injection. Carrageenan-induced vascular permeability was lower in the P group (P:  $11.8 \pm 1.9$  vs. Sh:  $17.8 \pm 2.4$   $\mu\text{L}$ ,  $p < 0,05$ ), as well as the inflammatory cellular influx (P:  $5.6 \pm 1.7$  vs. Sh:  $11.5 \pm 3.3 \times 10^6$  cells/cavity,  $p < 0,05$ ). No differences between the experimental groups were observed for either IL-1 $\beta$  or IL-6 concentration in the TMJ lavage fluids; TNF- $\alpha$  was undetectable in all the samples. **Discussion:** These results show that the vascular and cellular components of the inflammatory response to carrageenan in the rat TMJ can be affected by pre-existing periodontitis independently of a differential production of IL-1 $\beta$  or IL-6. The participation of alternative inflammatory mediators is currently under investigation. **Financial support:** CNPq, Capes, FAPESP, Panthera Solutions Ltd.



#### 04.089

Avaliação da atividade anti-inflamatória do derivado da série 5-benzilideno-3-(4-fenil-benzil)-tiazolidina-2,4-diona (LPSF/GQ-24). Paula MJD<sup>1</sup>, Magalhães LR<sup>2</sup>, Santos E<sup>1</sup>, Silva TM<sup>1</sup>, Do Carmo RFR<sup>1</sup>, Silva FC<sup>1</sup>, Lima MCA<sup>1</sup>, Galdino SL<sup>1</sup>, Pitta IR<sup>1</sup>, Barbosa Filho JM<sup>2</sup>, Silva TG<sup>1</sup> <sup>1</sup>UFPE - Antibióticos, <sup>2</sup>UFPB - Tecnologia Farmacêutica

**Introdução:** As tiazolidinonas têm sido extensivamente estudadas por apresentarem uma variedade de atividades biológicas, entre as quais, anti-inflamatória, hipoglicemiante e analgésica. O mecanismo de ação dessa classe de moléculas se dá por meio da ativação de receptores nucleares denominados PPAR ("peroxisome proliferator-activator receptor"). Os ativadores dos PPAR  $\alpha$  diminuem os fatores produzidos e secretados pelo tecido adiposo, como o TNF $\alpha$ , que promovem resistência à inflamação vascular. Mais recentemente, várias pesquisas demonstraram que a ativação do receptor PPAR  $\alpha$  diminui a expressão de genes pró-inflamatórios, o que levou vários grupos de pesquisa a investigarem os efeitos Anti-inflamatórios dos agonistas PPAR. O presente trabalho teve como objetivo a avaliação anti-inflamatória do derivado LPSF/GQ-24, em diferentes doses. **Métodos:** A atividade anti-inflamatória foi avaliada com base na inibição da migração de leucócitos polimorfonucleares através do teste do bolsão de ar como modelo de inflamação aguda em camundongos (Air pouch), tendo como agente flogístico a carragenina (cg) 1%. Foram utilizados camundongos de ambos os sexos albinos Swiss (*Mus musculus*). Todos os procedimentos Experimentais foram aprovados pelo Comitê de Ética em Experimentação Animal da UFPE sob o nº Protocolo N°016269/2006-24. Os animais foram divididos em grupos de 6 animais. Foi inicialmente injetado ar estéril no dorso do animal (2,5 mL de ar) no dia zero e a segunda aplicação foi realizada 48 horas após a primeira. No sexto dia do ensaio, administrou-se o derivado (LPSF/GQ-24) nas doses de 0,03; 0,3; 3 e 10mg/kg via oral, piroxicam (3mg/kg v.o), droga anti-inflamatória não esteroideal, utilizada como padrão e grupo controle negativo recebeu somente o veículo (soro fisiológico contendo 2% de tween 80). Uma hora após a administração dos compostos, injetou-se 1mL de uma solução de carragenina a 1% dentro da bolsa de ar. Decorridas 6 horas após a aplicação de carragenina, os animais foram sacrificados por deslocamento cervical e as bolsas lavadas com 3 mL de solução salina com EDTA como líquido de arraste. O exsudato foi diluído em solução de Turk, e a contagem dos leucócitos polimorfonucleares (PMNL) foi feita em câmara de Neubauer. **Resultados:** A avaliação da atividade anti-inflamatória do derivado 5-benzilideno-2,4-diona (LPSF/GQ-24) apresentou-se promissora. Os resultados da contagem de PMNL / mL ( $\times 10^5$ ) nas doses testadas foram: 10 mg/kg ( $2,70 \pm 0,08$ ); 3 mg/kg ( $3,60 \pm 0,06$ ); 0.3 mg/kg ( $11,3 \pm 0,12$ ); 0.03mg/kg ( $19,0 \pm 0,17$ ); Piroxican 3mg/kg ( $17,4 \pm 0,08$ ); Controle com carragenina ( $41,3 \pm 0,57$ ). Os dados foram expressos como média do número de células  $\pm$  erro padrão da média. Os resultados foram significativos em relação ao controle com intervalo de confiança de 95%. A análise de variância entre os grupos foi feita pelo teste t pareado. **Discussão:** A atividade anti-inflamatória avaliada através do air pouch, demonstrou que o derivado LPSF/GQ-24 apresentou atividade anti-inflamatória promissora, nas doses testadas, dando destaque para a dose de 10 mg/kg, onde o perfil de atividade foi superior ao fármaco piroxicam. **Apoio Financeiro:** CNPq

#### 04.090

Molecular characterization of NF $\kappa$ B signaling in human colostrum mononuclear phagocytes – functional effect on peripheral melatonin synthesis. Lapa MAPC<sup>1</sup>, Pontes GN<sup>2</sup>, Ferreira ZS<sup>1</sup>, Markus RP<sup>1</sup> <sup>1</sup>IB-USP - Fisiologia, <sup>2</sup>USP - Imunologia das Mucosas

**Introduction:** Melatonin is produced in a rhythmic manner by the pineal gland and in a tonic way for extra-pineal tissues (Markus, *Neuroimmunomodul*, 14:126, 2007). The human colostrum phagocytes produce melatonin when activated by bacteria or opsonized zymosan (Pontes, *J Pineal Res*, 41:136, 2006). This production stops after the death of the bacteria, and continues in the zymosan presence. This molecule, from the cellular wall of *S. cerevisiae*, when opsonized activates Toll-like receptors 2 (TLR-2), Dectin-1 (Ikeda, *Biol. Pharm. Bull*, 31:13, 2008), or Fc $\alpha$ RI/CD89 (Bakema, *The Journal of Immunology*, 176:3603, 2006) which triggers the nuclear factor kappa B (NF $\kappa$ B) signaling pathway (Akira, *Nature*, 4:499, 2004). The aim of this study was to characterize the NF $\kappa$ B pathway on the melatonin synthesis in colostrum mononuclear phagocytes activated by zymosan. **Methods:** This project was approved by ethical committee (CEA/IB protocol 076/2007). Colostrum were obtained from healthy puerperae (48-72 h after delivery) from the Hospital Universitário - USP. Isolated mononuclear phagocytes were resuspended in RPMI 1640 medium ( $2.10^6$  cell/mL) and activated with opsonized zymosan with IgA (opZ, 10 mg/mL, 90 min) in the presence or absence of NF $\kappa$ B blockers PDTC (25 mM) or ALLN (50 mM) pre-incubated for 30 min. Melatonin was determined in the supernatant by ELISA kit (IBL, Germany) and the NF $\kappa$ B activity was assayed by electromobility shift assay (EMSA) in nuclear extracts from mononuclear cells. The NF $\kappa$ B subunits was assessed using a specific customized human immunoassay kit (Cayman Chemical, USA). The data was compared by ANOVA followed by Newman-Keuls test, accepting 5% probability. **Results and Discussion:** As previously shown, melatonin content was only measured in the supernatant of cells incubated with opZ ( $260.2 \pm 57.03$  pg/mL, n=3 different pools). The pre-treatment of these cells with PDTC and ALLN reduced the melatonin content by 75.63 % and 76.63%, respectively ( $63.41 \pm 31.94$  pg/mL and  $60.81 \pm 32.92$  pg/mL, n=3, different pools, p<0.001). NF $\kappa$ B basal activity was detected in nuclear extracts from control cells showing a pattern of two complexes (C1 and C2) and the presence of the subunits p50 and p65 were confirmed by the immunoassay. In the presence of opZ a different pattern of nuclear translocation was observed with an increase in the translocation of C1 and an almost complete reduction on translocation of C2. PDTC (25 mM) and ALLN (50 mM) had no effect on nuclear translocation of NF $\kappa$ B in control cells but reduces the increase in the translocation of the NF $\kappa$ B complex observed in cells incubated with opZ. Here we show the peripheral melatonin synthesis by activated human colostrum mononuclear phagocytes with immunoglobulin A opsonized zymosan. The machinery of this synthesis involves the NF $\kappa$ B pathway, which was functionally and molecularly demonstrated. The peripheral melatonin synthesis by the colostrum mononuclear cells and the NF $\kappa$ B signaling characterization in this cellular model links the innate immune response with the chronobiotic system by a humoral mediated interface in the organisms. Support: FAPESP (2007/07871-6), CAPES, CNPQ.

#### 04.091

Estudo da reatividade de fibroblastos pulmonares de camundongos silicóticos: análise em sistema de cultura de células em 3D. Guimarães-Silva AM, Trentin PG, Dalzy DV, Perez SAC, Martins MA, Silva PMR IOC/FIOCRUZ - Fisiologia e Farmacodinâmica

**Introdução:** A silicose é uma doença pulmonar crônica causada pela inalação de partículas de sílica cristalina, caracterizada por ser de evolução lenta e progressiva, com caráter fibrogênico. Os fibroblastos são considerados alvos cruciais em doenças de natureza fibrótica, sendo o desenvolvimento de sistemas que permitam avaliar a reatividade destas células de fundamental importância na busca por terapias antifibróticas. Desta forma, neste trabalho tivemos o objetivo de avaliar a reatividade de fibroblastos pulmonares provenientes de camundongos normais e silicóticos, utilizando o sistema de cultura de células em 3D (esferóide) *in vitro*. **Métodos:** Camundongos da cepa Swiss-Webster foram instilados por via intranasal com sílica cristalina (10 mg[A1][A1] /50 µL) ou com igual volume de salina. Após 7 dias, os pulmões foram perfundidos com PBS e submetidos à dissociação enzimática para obtenção dos fibroblastos. As células foram cultivadas em meio DMEM + soro fetal bovino (10%), e após atingirem o estado de confluência submetidas à tripsinização até a terceira passagem. Em seguida, foram adicionadas a placas de 96 poços, em sistema de agarose, na densidade de  $1,25 \times 10^4$  células/poço. A análise foi realizada no período de 1-4 dias e incluiu os parâmetros de tamanho/diâmetro médio e proliferação celular avaliados através de captura e análise de imagem (programa Image - Pro Plus) e incorporação de [ $H^3$ ] timidina, respectivamente. Todos os procedimentos experimentais foram previamente aprovados pelo Comitê de Ética de Uso de Animais da FIOCRUZ ( Licença 0213-4). **Resultados:** A análise da cinética revelou que os esferóides obtidos a partir de fibroblastos de animais normais, apresentaram em tempos iniciais (1-2 dias), característica de menor densidade e tamanho/diâmetro maiores quando comparados a tempos posteriores (3-4 dias). Na condição das células provenientes de animais silicóticos, verificamos a ocorrência de decréscimo progressivo de tamanho/diâmetro ao longo do tempo, porém com valores sempre superiores aqueles referentes aos normais. A taxa de proliferação celular mostrou-se aumentada nos esferóides silicóticos em comparação aos normais. Quando ambas as populações de esferóides foram submetidas à estimulação com a citocina pró-fibrótica IL-13 (40 ng/mL), por um período de 1 a 4 dias, verificamos um claro estado de ativação atestado pelo aumento no tamanho/diâmetro e na taxa de proliferação em relação aqueles incubados apenas com meio. É importante ressaltar que os esferóides silicóticos apresentaram sempre tamanho/diâmetro e taxa de proliferação superiores aos dos esferóides normais. **Conclusão:** Em conjunto, nossos achados mostram que os fibroblastos pulmonares de camundongos adultos são passíveis de serem cultivados em sistema de cultura 3D, são responsivos à estimulação com IL-13 e que os esferóides silicóticos apresentam características de ativação em comparação aos normais. Em adição, este modelo mostrou-se reprodutível e promissor no que tange a possibilidade de utilização futura na busca por compostos com atividade anti-fibrótica. **Suporte financeiro:** FIOCRUZ, CNPq, FAPERJ.

#### 04.092

Perfil anti-inflamatório e anti-hipernociceptivo de derivados *N*-acilidrazônicos indólicos otimizados a partir do protótipo LASSBio-651. Santos EAP<sup>1</sup>, Alves FRS<sup>1</sup>, Miranda ALP<sup>2</sup>, Fraga CAM<sup>3</sup>, Barreiro EJ<sup>4</sup> <sup>1</sup>LASSBio-FF-UFRJ - Fármacos

**Introdução:** A inflamação é um complexo processo fisiopatológico envolvendo interações entre inúmeros mediadores que resultam na formação de eritema, calor, edema e infiltração celular (Goulett, J. J. Immun., 173, 1321, 2004). Dentre outros fatores, o edema ocorre em virtude do aumento da permeabilidade vascular induzido pela ação de mediadores químicos, como serotonina e prostanoídes, os quais também contribuem com a hipernocicepção a partir da sensibilização de nociceptores que é caracterizada como uma resposta aumentada frente a estímulos normalmente dolorosos (Michel, C. Physiol. Rev., 79, 703, 1999; Verry, W. Pharm. Ther., 112, 116, 2006). A literatura descreve a participação e o papel fundamental do neutrófilo na resposta hipernociceptiva. O indol e a função *N*-acilidrazona (NAH) são conhecidas como estruturas privilegiadas e esta última apresenta caráter farmacofórico para as atividades anti-inflamatória e antinociceptiva (De Sá Alves, F. R. Mini-Rev. Med. Chem., *in press*, 2009; Fraga, C. A. M., Curr. Med. Chem., 13, 167, 2006). O objetivo deste trabalho consiste na determinação do perfil anti-inflamatório e anti-hipernociceptivo de uma série de derivados NAH indólicos homologados, otimizados a partir do protótipo LASSBio-651, apresentando importante atividade anti-inflamatória (Silva, B, 34º Congresso SBFTE, 04.093, 2002). **Métodos:** Avaliou-se as atividades anti-inflamatória e anti-hipernociceptiva através dos ensaios de edema de pata de rato induzido por carragenina (Cg 1%), ensaio da placa quente modificado e quantificação da mieloperoxidase (MPO) das patas dos animais. Realizou-se a triagem de 16 derivados administrados uma hora antes da Cg, por via oral na dose de 100 µmol/kg em suspensão de goma arábica 5%. O edema e a hipernocicepção foram avaliados a cada hora após a administração intraplantar da Cg por um período de 4h. As patas dos animais foram retiradas após 6h para dosagem de MPO em um aparelho de ELISA. Os resultados foram aferidos a partir da comparação entre os grupos tratados com os compostos e o controle (\**p*<0,05; *n*=6-10). Os protocolos experimentais foram submetidos à CEUA-CCS/UFRJ. **Resultados e Discussão:** A maior atividade antiedematogênica dos compostos NAH indólicos foi observada durante a 2ª h do experimento. Nestas condições, os compostos LASSBio-1245, LASSBio-1248 e LASSBio-1249 se destacaram inibindo a formação do edema em 41%\*, 36%\* e 51%\*, respectivamente. A maioria dos compostos apresentou atividade anti-hipernociceptiva associada à atividade antiedematogênica. Os derivados LASSBio-1249 e LASSBio-1248 apresentaram atividade pronunciada neste modelo inibindo a hipernocicepção em 35% a 62%, durante todo o experimento. Além disso, estes já tinham se apresentado como potentes derivados antinociceptivos (Dos Santos, E. A. P., 40º Congresso SBFTE., 07.060, 2008). LASSBio-1248 também inibiu a migração celular verificada através da redução da MPO em torno de 50%. Este resultado sugere que a atividade anti-hipernociceptiva do LASSBio-1248 pode estar relacionada com a diminuição de neutrófilos no local da inflamação. LASSBio-1166 e LASSBio-1247 inibiram a hipernocicepção de 40% a 60% sem contudo apresentar efeito significativo na formação do edema. O conjunto destes resultados indica alvos de ação distintos para estes derivados na dor inflamatória. Constituem perspectivas deste trabalho avaliar o efeito dos derivados NAH indólicos na produção de TNF- $\alpha$  e PGs. Apoio financeiro: CNPq, FAPERJ, IM-Inofar #420.013/05-1, PRONEX #E26/171.532/2006.

#### 04.093

Disfunção vascular em fêmeas com obesidade induzida pela administração neonatal de glutamato monossódico. Hagihara GN\*, Lobato NS, Tostes RCA, Carvalho MHC, Fortes ZB ICB-USP - Farmacologia

**Introdução:** A obesidade é considerada um dos principais fatores de risco para o desenvolvimento de doenças cardiovasculares. Estudos experimentais indicam que o glutamato monossódico (MSG) pode induzir lesões hipotalâmicas e resistência à leptina, influenciando o balanço energético e contribuindo para o desenvolvimento da obesidade. A associação entre MSG e obesidade foi recentemente demonstrada em humanos. A maior parte dos estudos avalia os efeitos do MSG em machos. Portanto, o objetivo deste estudo foi avaliar o efeito da obesidade induzida pelo MSG sobre a reatividade vascular a agentes vasoconstritores e vasodilatadores em leito arteriolar mesentérico de fêmeas Wistar. **Métodos:** Para este estudo, ratas Wistar neonatas receberam injeções subcutâneas de MSG nos dias 2,3,4,5 e 6 após o nascimento, e foram utilizadas para o estudo com 16 semanas de idade. Para a caracterização da obesidade, avaliaram-se: índice de Lee (peso corpóreo (g) / [comprimento naso-anal (cm)]<sup>1/3</sup>); peso relativo das gorduras perigonadal e retroperitoneal (g / 100 g de peso corpóreo); peso dos músculos sóleo e gastrocnêmio (g / 100 g de peso corpóreo). Para o estudo da reatividade vascular, curvas concentração efeito à noradrenalina (10 nM a 100 µM), à angiotensina II (10 nM a 100 µM) e à bradicinina (1 nM a 100 µM) foram avaliadas em leito arteriolar mesentérico isolado e perfundido. As respostas contráteis à noradrenalina e angiotensina II foram verificadas por aumento da pressão de perfusão (mmHg). O relaxamento à bradicinina foi avaliado em preparações contraídas com noradrenalina (10 µM) e foi expresso como porcentagem da redução dessa contração. **Resultados:** Ratas obesas MSG apresentaram aumento do Índice de Lee (C: 29,26 ± 0,35, n = 7 vs MSG: 30,31 ± 0,22, n = 13; p < 0,02) às controles. Maior acúmulo de gorduras perigonadal (C: 1,81 ± 0,40, n = 7 vs MSG: 4,70 ± 0,31, n = 13; p < 0,0001) e retroperitoneal (C: 0,57 ± 0,10, n = 7 vs MSG: 1,69 ± 0,10, n = 9; p < 0,0001) e redução do peso dos músculos gastrocnêmio (C: 0,54 ± 0,02, n = 7 vs MSG: 0,48 ± 0,01, n = 13; p < 0,03) e sóleo (C: 0,04 ± 0,01, n = 7 vs MSG: 0,04 ± 0,01, n = 13; p < 0,01) também foram observados em ratas MSG quando comparadas às do grupo controle. No estudo da reatividade vascular, verificamos que ratas MSG apresentaram aumento da resposta máxima vasoconstritora (mmHg) à noradrenalina (C: 119,70 ± 9,26, n = 10 vs MSG: 200,50 ± 9,87, n = 14; p < 0,0001) e à angiotensina II (C: 102,90 ± 7,28, n = 6 vs MSG: 133,50 ± 10,66, n = 4; p < 0,04). A resposta vasodilatadora dependente do endotélio à bradicinina (%) também foi significativamente aumentada em fêmeas MSG quando comparadas às ratas controles (C: 48,51 ± 5,13, n = 8 vs MSG: 64,77 ± 5,02, n = 8; p < 0,04). **Conclusão:** A obesidade induzida pela administração neonatal de glutamato monossódico promove alterações na função vascular em fêmeas, caracterizadas pelo aumento da resposta vasoconstritora mediada pela noradrenalina e pela angiotensina II. O aumento da vasodilatação dependente de endotélio à bradicinina observado em ratas MSG pode constituir mecanismo compensatório frente às alterações observadas na contração vascular. **Apoio Financeiro:** FAPESP/CNPq \*Bolsista de Treinamento Técnico da Pró-Reitoria de Pesquisa da USP. N° CEEA:007/04

#### 04.094

Estudo do efeito do corticóide flunisolida sobre miofibroblastos pulmonares *in vitro*. Ramos TJF, Perez SAC, Pires ALA, Martins MA, Silva PMR FIOCRUZ - Inflamação

**Introdução:** A silicose é uma doença fibrótica pulmonar grave causada pela inalação de partículas de sílica cristalina. Caracteriza-se pela ocorrência de intenso processo granulomatoso, não havendo até o presente momento uma terapia eficaz. Previamente demonstramos que o tratamento curativo com glucocorticóide flunisolida foi eficaz em inibir marcadamente o componente inflamatório e fibrótico no sistema de silicose experimental murina. Considerando-se que os fibroblastos são células cruciais para a resposta de fibrose tecidual, neste estudo investigamos o potencial efeito da flunisolida sobre a reatividade de fibroblastos *in vitro*. **Métodos:** Os fibroblastos foram obtidos a partir de pulmões de camundongos Swiss-Webster normais ou instilados intranasalmente com sílica (10 mg), em 7 (fase aguda) e 28 dias (fase crônica) após, mediante a dissociação mecânica e digestão enzimática com colagenase 1A. As células foram mantidas em cultura e, após a terceira passagem, foram realizados ensaios de imunocitoquímica para caracterização do fenótipo celular, proliferação por incorporação de [<sup>3</sup>H] timidina e ELISA para quantificação de mediadores pró-fibróticos avaliados a partir do sobrenadante. As análises foram realizadas 24 horas após a estimulação com sílica (0,1 – 10 µg/mL) ou rmIL-13 (1-20 ng/mL), citocina com reconhecida atividade pró-fibrótica. O tratamento com flunisolida constou da incubação das células com a droga ( $10^{-8}$  -  $10^{-5}$ M) por um período de 1 hora antes da estimulação. Os procedimentos utilizados foram aprovados pelo Comitê de Ética de Uso de Animais (CEUA) da FIOCRUZ (Protocolo 0213-4). **Resultados:** Inicialmente, a caracterização fenotípica das células mantidas em cultura revelou que as mesmas apresentaram marcação negativa para a proteína citoqueratina e positiva para a proteína  $\alpha$ -actina de músculo liso, caracterizando claramente o fenótipo de miofibroblasto. Em seguida, verificamos que os miofibroblastos provenientes de animais silicóticos apresentaram maior taxa de proliferação basal em comparação à das células normais. A estimulação com sílica falhou em induzir proliferação de miofibroblastos, porém levou à produção de MCP-1 e TGF- $\beta$  apenas por células silicóticas (7 dias). IL-13 induziu resposta proliferativa e geração de TGF- $\beta$  em miofibroblastos normais e silicóticos (7 dias), porém não apresentou qualquer atividade sobre as células silicóticas de fase crônica (28 dias). O tratamento com flunisolida, 1h antes da estimulação com IL-13, não interferiu com a proliferação dos miofibroblastos normais, porém reduziu significativamente a resposta dos miofibroblastos silicóticos. **Conclusão:** Nossos achados mostram que os miofibroblastos silicóticos apresentaram nível basal de proliferação superior àquele das células normais e sendo, também, responsivos à estimulação com sílica no que tange à geração de fatores fibróticos. Mais ainda, mostram que a estimulação com IL-13 foi capaz de ativar os miofibroblastos de fase aguda, sendo esta resposta sensível ao tratamento com flunisolida. Isto é indicativo de ser este composto esteroidal uma ferramenta terapêutica antifibrótica promissora de aplicação na condição da silicose. **Apoio Financeiro:** PAPES 4/FIOCRUZ, CNPq, FAPERJ e UNESCO.

#### 04.095

The intracellular pattern recognition receptor NOD2 is crucial for development of arthritis. Vieira SM<sup>1</sup>, Pinto LG<sup>1</sup>, Lemos HP<sup>1</sup>, Cunha TM<sup>1</sup>, Lima JB<sup>2</sup>, Talbot J<sup>1</sup>, Almeida SCL<sup>3</sup>, Verri WA, Jr<sup>1</sup>, Louzada Jr P<sup>3</sup>, Zamboni DS<sup>2</sup>, Ferreira SH<sup>1</sup>, Cunha FQ<sup>1</sup> <sup>1</sup>FMRP-USP - Farmacologia, <sup>2</sup>FMRP-USP - Biologia Celular, Molecular e Bioagentes Patogênicos, <sup>3</sup>HC-FMRP-USP - Clínica Médica

**Introduction:** Nucleotide-binding oligomerization domains containing 1 and 2 (NOD1 and NOD2) are intracellular sensor proteins belonging to the pattern recognition receptors family, which play an important role in the immune response. The NOD-like receptors can sense pathogens, products of damaged cells or endogenous metabolites and could potentially be involved in the initiation, amplification and progression of the inflammatory response in rheumatic diseases. **Methods:** Synovial and peripheral blood mononuclear cells (SPBMCs) from RA and osteoarthritis (OA) patients were taken for real-time RT-PCR or stimulated with MDP (a Nod2 agonist) for ELISA, Caspase-1 activation and western blot assays. Adult C57BL/6 (WT), *Nod1*<sup>-/-</sup>, *Nod2*<sup>-/-</sup>, *Caspase-1*<sup>-/-</sup> and *RIPK2*<sup>-/-</sup> mice weighing 18-23 g were used. Firstly, for the development of experimental arthritis model, mice were immunized with methylated bovine serum albumin (mBSA) and complete Freud's adjuvant through subcutaneous (s.c.) injection. Twenty-one days after the initial injection, arthritis was induced in the immunized mice by intra-articular (i.a.) injection of mBSA dissolved in PBS. Synovial membranes of arthritic and non-arthritic WT and knockout mice were taken to real-time RT-PCR. Migration assays, mechanical hypernociception and histological analysis were used to evaluate neutrophil (NØ) recruitment to knee joints, decrease of nociceptive withdrawal threshold and cartilage loss, respectively. Proteoglycan content of cartilage was measured by dimethylmethylene blue assay of papain digests. ELISA assays were used to evaluate cytokines production in joint of arthritic and non-arthritic WT and knockout mice. This study was approved by Ethics Committee of FMRP/USP (nº. 127/2008). **Results:** Real-time RT-PCR analysis shows that NOD2, RIPK2 and IL-1 $\beta$  mRNA expression is augmented in SPBMCs from RA patients when compared with cells from OA patients. MDP induced *in vitro* high levels of Caspase-1 activation as well as increase in IL-23, IL-1 $\beta$ , TNF- $\alpha$  and CXCL8 release by SPBMCs of RA patients in comparison with OA patients' cells. mBSA-induced arthritis, NØ migration, mechanical hypernociception and cartilage loss were completely abrogated in *Nod2*<sup>-/-</sup> and markedly reduced in *RIPK2*<sup>-/-</sup> and *Caspase-1*<sup>-/-</sup> mice in comparison with WT or *Nod1*<sup>-/-</sup> mice. mBSA challenge into joints increased Nod2 mRNA expression in synovial membrane of WT arthritic mice in comparison with non-arthritic mice. Joint synovial membrane mRNA expression of IL-6, IL-23 and IL-1 $\beta$  and the levels of IL-17, IL-23, IL-1 $\beta$ , TNF- $\alpha$  and CXCL1 were significantly reduced in *Nod2*<sup>-/-</sup> mice in comparison with WT or *Nod1*<sup>-/-</sup> mice. The levels of IL-17, IL-1 $\beta$  and CXCL1 were also significantly reduced in *Caspase-1*<sup>-/-</sup> mice after mBSA-challenge. **Discussion:** These results strongly suggest that NOD2 signaling, but not NOD1, plays a fundamental role in the pathogenesis of RA. Furthermore, RIPK2/Caspase-1 pathway seems to be the downstream signaling of NOD2 in joint inflammation and might favor Th17 immune response. Together, it is reasonable to propose NOD2 signaling as a novel target to development of new drug to control RA. **Financial support:** FAPEAM, FAPESP and FAEPA.

#### 04.096

Dissection of the modulatory role of nitric oxide on neutrophil migration reveals soluble guanylate cyclase as a therapeutic target in sepsis. Neto H<sup>1</sup>, Alves-Filho JC<sup>2</sup>, Souto FO<sup>3</sup>, Spiller F<sup>2</sup>, Amêndola R<sup>1</sup>, Freitas A<sup>2</sup>, Cunha FQ<sup>2</sup>, Barja Fidalgo TC<sup>1</sup> <sup>1</sup>UERJ - Farmacologia, <sup>2</sup>FMRP-USP - Farmacologia, <sup>3</sup>FMRP-USP – Cirurgia e Anatomia

**Introduction:** During severe sepsis neutrophils present a state of unresponsiveness to chemotactic stimuli and impaired migratory function. This correlates with poor prognosis and may collaborate to worsen the clinical outcome in sepsis by precluding neutrophil influx to the primary infectious focus and an efficient bacterial clearance. Experimental data have implicated nitric oxide (NO) in these phenomena. NO synthesis inhibition in sepsis restored neutrophil migratory function but paradoxically increased mortality. This could be at least in part due to an inhibition of the microbicidal role played by NO, suggesting that a better understanding of the roles of NO in sepsis is required. In several cell types and cellular settings, NO effects are dependent on the activation of the soluble guanylate cyclase (sGC) and the downstream effector cGMP-dependent protein kinase (PKG). We then sought to investigate the involvement of the NO-sGC-PKG signaling pathway on the establishment of neutrophil hyporesponsiveness to chemotactic stimulation in sepsis. **Methods:** We stimulated purified human neutrophils with Toll-like receptor ligands, lipopolysaccharide (LPS, 10 µg/mL) and lipoteichoic acid (LTA, 10 µg/mL) which resulted in decreased chemotactic responsiveness. The involvement of the NO-sGC-PKG pathway in this process was addressed by using selective pharmacological inhibitors to each component of this signaling pathway. We then used FACS analysis, western blotting and immunofluorescence techniques to study chemokine receptor dynamics in neutrophils stimulated with LPS. Finally, we used cecal ligation and puncture (CLP) as an experimental model of sepsis (ethics committee number: 181/2008) to confirm our *in vitro* findings and establish their *in vivo* implications. **Results and Discussion:** Neutrophils stimulated with LPS presented diminished responses to leukotriene B<sub>4</sub>, interleukin-8 and fMLP, and increased cGMP intracellular levels. Treatment of neutrophils with 1400W, a selective iNOS inhibitor (30 µM), ODQ, a selective sGC inhibitor (10 µM), or KT-5823, a selective PKG inhibitor (3 µM), blocked the effects of LPS and restored neutrophil migration. LPS stimulation induced CXCR2 internalization and treatment with the above mentioned inhibitors recovered CXCR2 membrane expression. LPS also increased G protein-coupled receptor kinase (GRK)-2 expression. GRK-2 mediates CXCR2 internalization and the LPS-induced increase in GRK-2 expression may account for the observed CXCR2 internalization. Based on these findings we reasoned that sGC would be a better therapeutic target in sepsis since it would restore neutrophil migration without interfering with NO synthesis and NO-dependent microbicidal activity. In fact, we found that ODQ treatment *in vivo* (5 µmol/kg) increased survival, restored neutrophil migration, and increased bacterial clearance in an experimental model of sepsis. Thus our results suggest a mechanism by which NO interferes with neutrophil chemotaxis and point to sGC as a promising therapeutic target in sepsis. Financial support: CAPES, CNPq, FAPERJ and FAPESP.



#### 04.097

Mediators of febrile response induced by *S. aureus* in rats. Martins JM<sup>1</sup>, Soares DM<sup>2</sup>, Malvar DC<sup>1</sup>, Figueiredo MJ<sup>1</sup>, Souza GEP<sup>2</sup> <sup>1</sup>FMRP-USP - Farmacologia, <sup>2</sup>FCFRP-USP - Física e Química

**Introduction:** Various cell types activated in the damaged or infected tissue release many soluble mediators, including cytokines and chemokines. It has been shown that the cytokines, IL-6 and TNF- $\alpha$ , and the chemokines MIP-1, CINC and RANTES induce fever in rats. In that way a large body of evidence supports the view that recognition of an exogenous pyrogens (Gram negative LPS or others) by the host results in fever via the formation of these endogenous pyrogens EPs (cytokines and chemokines) <sup>[1,2,3,4]</sup>. Nevertheless, few studies have been performed concerning the participation of EPs during fever induced by live Gram positive bacteria <sup>[5]</sup>. In view of that we investigated the participation of pyrogenic mediators on fever induced by i.p. injection of live *S. aureus*. **Methods:** Male Wistar rats (200g) received anti-IL-6 (5 mg, i.c.v., 30 min before), rsTNF (500 ng, i.c.v., 15 min. before), anti-MIP-1 $\alpha$  (10 ng, i.c.v., 1 h (1 $\mu$ l) e 15 min (1 $\mu$ l) before), Met-RANTES (100  $\mu$ g/kg, i.v., 15 min. before) before the i.p. injection (2 ml) of *S. aureus* (10<sup>10</sup> UFC/cavity). Anti-CINC (10 ng, i.h.) was co-injected with the i.p. injection of *S. aureus* and injected again 1 h after. Body temperature ( $^{\circ}$ C) was measured every 30 minutes for up to 12h by radio-telemetry system. Commission ethical protocol n<sup>o</sup> 06.1.1281.53.1 – CEUA-Ribeirão Preto/USP. **Results:** The pre-treatment with anti-MIP-1 $\alpha$  (2.5 h: Sal / *S. aureus*: 38.94  $\pm$  0.19 $^{\circ}$ C, anti-MIP-1 $\alpha$  / *S. aureus*: 37.75  $\pm$  0.18 $^{\circ}$ C; 5 h: Sal / *S. aureus*: 39.3  $\pm$  0.13 $^{\circ}$ C, anti-MIP-1 $\alpha$  / *S. aureus*: 38.0  $\pm$  0.36  $^{\circ}$ C) and Met-RANTES (5 h: Sal / *S. aureus*: 38.96  $\pm$  0.11 $^{\circ}$ C; Met-RANTES / *S. aureus*: 38.13  $\pm$  0.2  $^{\circ}$ C), but not with anti-IL-6, rsTNF and anti-CINC, reduced significantly the febrile response induced by *S. aureus*. **Discussion:** These data suggest that MIP-1 $\alpha$  and RANTES are strongly involved in the fever induced by i.p. injection of *S. aureus* (10<sup>10</sup> UFC/cavity) while IL-6 and TNF- $\alpha$  seem do not. These data also point out new strategies for treatment of fever or other signs of the acute phase response during infections induced by these bacteria. <sup>[1]</sup>Roth et al., *Immunol Allergy Clin North Am*, v. 29, p. 229, 2009; <sup>[2]</sup> Melo Soares et al., *Brain Res.*, v. 1109, p. 83, 2006; <sup>[3]</sup> Soares et al., *Brain Res.*, v.1233, p.79, 2008; <sup>[4]</sup> Machado et al. *Brain Research*, v.1161, p.21, 2007; <sup>[5]</sup> Longhi et al. Livro de Resumos, SBFTE, p. 41, 2006. **Support:** FAPESP

#### 04.098

Anti-allodynic effects of N-acetyl-xylopine, a semi-synthetic alkaloid derived from *Magnolia ovata*. Mori LS<sup>1</sup>, Kassuya CAL<sup>1</sup>, Stefanello MEA<sup>2</sup>, Zampronio AR<sup>1</sup> <sup>1</sup>UFPR - Farmacologia, <sup>2</sup>UFPR - Química

**Introduction:** Preliminary studies in our laboratory have shown that crude extract from *Magnolia ovata* (A. St. Hil.) Spreng has anti-allodynic properties. We identified and isolated an unstable alkaloid from this plant named xylopine, and produced a more stable form, N-acetyl-xylopine (AXYL, Stefanello et. al., unpublished observations). The aim of this study was to evaluate the anti-allodynic action of AXYL and the mechanisms involved in this action. **Methods:** Male Swiss mice (25-35g) received AXYL (0.015-0.15mg/kg by oral route or 0.3-300 ng in the paw) or vehicle (10 ml/kg, p.o. or 20µL/paw) 1h or 30 min, respectively, before the nociceptive stimulus. Acetic acid-induced writhing (0.8%) and formalin-induced nociception (1%) were evaluated for 20 or 30 min, respectively, after the injection. Mechanical allodynia in ipsi or contralateral paw was measured after carrageenan (Cg, 300µg/paw) intraplantar injection after 4 h using Von Frey filaments and the mechanical threshold was expressed in mg using the up-down paradigm as described previously (Chaplan SR, J Neurosci Methods, 53:55, 1994). Animals were also submitted to rota-rod and hot plate tests. For the analysis of the possible mechanism of action, AXYL (3ng/paw) was injected 15 min before bradykinin (BK, 500ng/paw), tumor necrosis factor-α (TNFα, 1pg/paw), interleukin-1β (IL1β, 0.5pg/paw), cytokine-induced neutrophil chemoattractant-1 (CINC-1, 100pg/paw), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>, 100 ng/paw) and dopamine (DOPA, 10µg/paw) and the mechanical threshold was measured as described before. The procedures were approved by the Institutional Ethics Committee (protocol 271). **Results and Discussion:** Oral administration of AXYL inhibited the acetic acid-induced nociception and phase II of formalin-induced nociception in a dose-dependent manner with a maximal inhibition of 50 ± 11% and 71 ± 10%, respectively. Also, AXYL significantly inhibited Cg-induced mechanical allodynia when given orally (82 ± 8%) or in the ipsilateral paw 102% but not when injected in the contralateral paw. Nociceptive and motor responses of the animals in hot-plate and rota-rod tests remained unaltered at maximal doses of AXYL tested. Local administration of AXYL significantly inhibited BK, TNFα, IL1β, PGE<sub>2</sub>-induced mechanical allodynia (99.9%; 54.67%; 83.4%; 81.1%, respectively) but not CINC-1- and DOPA-induced mechanical allodynia. **Discussion:** These results suggest that the stable derivative AXYL possess analgesic properties and that AXYL acts mainly peripherally and in the prostaglandin component of the sensitization of peripheral nociceptors but not in the sympathetic component. **Acknowledgements:** CNPq and CAPES.

#### 04.099

Papel regulatório da proteína anexina-1 no modelo experimental de asma murina. Souza DM<sup>1</sup>, Trentin PG<sup>1</sup>, Arantes ACS de<sup>1</sup>, Ferreira TPT<sup>1</sup>, Santos TPO<sup>1</sup>, Pires ALA<sup>1</sup>, Flower RJ<sup>2</sup>, Perretti M<sup>2</sup>, Martins MA<sup>1</sup>, Silva PMR<sup>1</sup> - <sup>1</sup>FIOCRUZ - Inflamação, <sup>2</sup>William Harvey Research - Biochemical Pharmacology

**Introdução:** A asma é uma doença inflamatória crônica das vias aéreas, caracterizada por hiperreatividade das vias aéreas, secreção de muco e eosinofilia. No decorrer do estabelecimento do processo inflamatório, há a liberação de mediadores endógenos com o propósito de limitar a evolução do quadro patológico e garantir a manutenção da homeostasia. Dentre estes recebem destaque os hormônios glicocorticóides, reconhecidos por sua potente atividade anti-inflamatória, que por sua vez depende, pelo menos em parte da liberação de fatores intermediárias como a proteína anexina-1. Desta forma, neste estudo objetivamos investigar o papel regulatório da anexina-1 sobre o modelo de asma experimental murina. **Metodologia:** Camundongos Anx1<sup>+/+</sup> (Balbc) e depletados do gene codificante para a anexina-1 (Anx1<sup>-/-</sup>) foram sensibilizados com 50 ug de ovoalbumina (OVA) e 5 mg de hidróxido de alumínio, por via subcutânea. Após 14 dias, os animais receberam um reforço por via intraperitoneal e passado 7 dias foi realizado o desafio antigênico (OVA - 25 mg), por 3 dias consecutivos. As análises foram realizadas 24 h após o último desafio e incluíram: i) função pulmonar (resistência e elastância) e hiperreatividade das vias aéreas à metacolina (3 – 27 mg/ml) utilizando-se sistema de pletismografia de corpo inteiro invasiva (Buxco System); ii) alterações morfológicas através de técnicas histológicas clássicas (coloração com hematoxilina-eosina - H&E); iii) quantificação do colágeno pelo ensaio colorimétrico de Sircol e iv) quantificação de mediadores inflamatórios através de ELISA. Os procedimentos utilizados foram aprovados pelo Comitê de Ética de Uso de Animais (CEUA) da FIOCRUZ (Protocolo 0213-4). **Resultado:** Verificamos que os camundongos ANX1<sup>+/+</sup> sensibilizados e estimulados com OVA apresentaram aumento nos níveis basais de resistência e elastância pulmonar e da resposta de hiperreatividade das vias aéreas frente à estimulação com o agonista colinérgico metacolina. Além disso, detectamos um intenso infiltrado inflamatório disperso no parênquima pulmonar e com aumento expressivo no número de eosinófilos localizados na região peribronquiolar. O conteúdo de colágeno, assim como os níveis de citocinas (IL-13 e IL-4) e de quimiocina (eotaxina-1) mostraram-se elevados no pulmão dos animais alérgicos desafiados. Considerando-se os camundongos Anx1<sup>-/-</sup>, vimos uma exacerbação da resposta de hiperreatividade das vias aéreas, no que tange tanto resistência como elastância pulmonar, assim como no número de eosinófilos peribronquiais e na deposição de colágeno quando comparados aos animais Anx1<sup>+/+</sup>. Os níveis IL-13, porém não os de IL-4 e eotaxina apresentaram-se exacerbados nos camundongos ANX1<sup>-/-</sup> em comparação aos ANX1<sup>+/+</sup>. **Conclusão:** Em conjunto esses resultados mostram que os camundongos nocautes para o gene da anexina-1 mostraram-se mais responsivos à estimulação antigênica, em comparação aos animais selvagens, o que foi indicativo de que a anexina-1 parece exercer um papel regulatório importante sobre a resposta inflamatória alérgica murina. **Suporte financeiro:** FIOCRUZ, CNPq, FAPERJ.

#### 04.100

Characterization of immune-pineal axis: lipopolysaccharide activates NF- $\kappa$ B translocation to nucleus and induces TNF production in rat pineal gland. Cruz-Machado SS, Ferreira ZS, Markus RP IB-USP Fisiologia

**Introduction:** The pineal gland, a pivotal structure in the time organization, is outside the blood-brain barrier, being exposed to all substances present in the blood. Lipopolysaccharide (LPS), the main component of gram-negative bacteria wall, is a potent activator of nuclear factor kappa B (NF- $\kappa$ B) and inducer of cytokines. *In vivo* it was demonstrated that LPS diffuses into pineal parenchyma after intravenous injection and promotes morphological alterations in the gland (Jiang-Shieh et al, J. Pineal Res. 38:17-26, 2005). Taking into account that pineal gland constitutively express NF- $\kappa$ B (Cecon et al., personal communication), our aim was to evaluate the effect of LPS on NF- $\kappa$ B nuclear translocation and the production of TNF in pineal gland *in vitro*.

**Methods:** This project was approved for ethical committee (CEA/IB protocol 045/2007). Pre-pubertal female wistar rats kept in light/dark cycle (12:12 h) was killed between 1 and 3 h after lights on. Denervated rat pineal glands (48 h; CO<sub>2</sub> 5%; O<sub>2</sub> 95%, 37°C) incubated in BGJb medium plus 2 mM glutamine, 100 U/mL penicillin and 10 ug/mL streptomycin was stimulated in the absence or presence of LPS (0.1 and 1 ug/mL) for 30 min to 6 h. The NF- $\kappa$ B translocation was assayed in the pineal nuclear extracts by Electromobility Shift Assay (EMSA). The TNF production by the gland was measured in the medium using a commercial ELISA kit. **Results:** Our results show that LPS activates NF- $\kappa$ B translocation to the nucleus in a time-dependent manner, with a rapid and transient activation in both concentrations used. The specificity of the NF- $\kappa$ B/DNA complexes detected was confirmed by competition with specific and non-specific unlabeled oligonucleotides. LPS at both concentrations tested increases the content of TNF which peaks at 4 hours of incubation (0.1 ug/mL: 21.87 $\pm$ 5.06 pg/mL and 1 ug/mL: 31.38 $\pm$ 9.766 pg/mL). The Two-way ANOVA test followed with Bonferroni's test revealed a significant time-dependent effect of LPS-induced TNF production in rat pineal glands *in vitro* (P<0.001) without significance between the doses. **Discussion:** The presented data shows that rat pineal glands are targets to LPS which activates the translocation of NF- $\kappa$ B to nucleus. LPS also induces TNF production in the gland, may as a consequence of NF- $\kappa$ B activation. Previous results of our group showed that TNF has a negative correlation with melatonin production in humans and inhibits melatonin synthesis in rat pineal *in vitro*, pointing to the concept of the existence of an immune-pineal axis (Markus et al, Neuroimmunomodulation, 14: 126-133, 2007). NF- $\kappa$ B is a transcription factor involved in response to a high range of stimulus. Our group showed that this factor is expressed constitutively in the gland and that is functional, because can be activated with TNF and inhibited with corticosterone. In conclusion, here we show that rat pineal glands are responsive to LPS which promotes activation of NF- $\kappa$ B nuclear translocation. Furthermore, we showed for the first time that pineal gland produces TNF *in vitro*. We hypothesize that the TNF production by the pineal acts as an autocrine modulator of neuroendocrine activities in the gland. **Financial Support:** FAPESP (Grant 2007/06444-7 and Grant 2007/07871-6), CNPq, CAPES.

#### 04.101

Efeito da rosiglitazona sobre a perda óssea alveolar secundária à periodontite induzida em ratos. Martins Porto R<sup>1</sup>, Teixeira SA<sup>1</sup>, Campi P<sup>1</sup>, Maia-Dantas A<sup>1</sup>, Zanet TG<sup>2</sup>, Herrera BS<sup>4</sup>, Fenyó-Pereira M<sup>3</sup>, Spolidório LC<sup>5</sup>, Costa SKP<sup>1</sup>, Muscará MN<sup>1</sup> <sup>1</sup>ICB-USP - Farmacologia, <sup>2</sup>FO - Radiologia, <sup>3</sup>FO-USP - Estomatologia, <sup>4</sup>FO-UNESP - Patologia, <sup>5</sup>UNESP - Patologia

**Objetivo:** A rosiglitazona (Ros) é um agonista de receptor ativado por proliferadores de peroxissomo (PPAR)-gama, amplamente empregado no tratamento do *diabetes mellitus*. O objetivo deste trabalho foi estudar os efeitos de doses terapêuticas antihiperlicemiantes de Ros sobre a perda óssea alveolar (POA) secundária à periodontite induzida em ratos. **Métodos:** Ratos Wistar machos (180-220 g) receberam Ros (1, 2, 5, 10 ou 15 mg/kg/dia) ou veículo (carboximetilcelulose – CMC à 0.5%, 1 mL/kg) por via oral, durante os 14 dias prévios à indução de periodontite, e até o dia do sacrifício (protocolo aprovado pelo Comitê de Ética em Experimentação Animal sob numero 077, fls. 33, livro 2). Sob anestesia com hidrato de cloral (7 mg/kg, i.p.), a periodontite foi induzida pela colocação de ligadura de algodão entorno da cervical do primeiro molar inferior direito (grupos P). Nos ratos controle (Sham - S), a ligadura foi imediatamente removida. Assim, os seguintes grupos ficaram definidos: S, Per tratado com CMC (P) ou com Ros nas doses de 1 (P1), 2 (P2), 5 (P5), 10 (P10) ou 15 mg/kg/dia (P15). Após 7 ou 14 dias, grupos de animais foram sacrificados, as mandíbulas foram removidas e radiografadas para medida de POA, e o fêmur para avaliação da densidade óssea femoral (DOF). Amostras de sangue foram coletadas para análise da concentração sérica de íon cálcio e das atividades séricas de fosfatase alcalina (FA) e fosfatase ácida resistente a tartarato (TRAP) como respectivos marcadores de atividades osteoblástica e osteoclástica. **Resultados:** A POA nos grupos de animais P5 ( $0,71 \pm 0,06$ ,  $p < 0,05$ ), P10 ( $0,90 \pm 0,10$ ,  $p < 0,01$ ) e P15 ( $0,85 \pm 0,13$ ,  $p < 0,01$ ) foi maior do que a do grupo P ( $0,55 \pm 0,10$ ) 7 dias após a indução da periodontite. No dia 14 não observamos alterações de POA devidas aos tratamentos. A DOF no grupo P5 ( $99,9 \pm 3,4$ ) foi menor do que nos grupos S ( $120,3 \pm 1,4$ ;  $p < 0,05$ ) ou P ( $117,6 \pm 6,6$ ;  $p < 0,05$ ) 7 dias após a indução da Per, mas permaneceu inalterada no dia 14. Não foram observadas diferenças de FA, TRAP ou cálcio sérico entre os grupos em quaisquer dos tempos estudados. **Discussão:** O tratamento com Ros potencializa a POA e a diminuição da DOF decorrentes da periodontite, evidenciando o envolvimento do PPAR-gama nas alterações de metabolismo ósseo durante a fase inicial da periodontite. Apoio financeiro: CNPq, CAPES, FAPESP.

## 04.102

Early-lifetime exposure to 1,2-naphthoquinone (1,2-NQ) interact to increase asthma susceptibility in juvenile mice. Florenzano J, Santos KT, Teixeira SA, Barreto MAA, Muscará MN, Costa SKP ICB-USP - Farmacologia

**Introduction:** Previous research has documented effects of prolonged exposure to environmental pollution (eg. diesel exhaust particles [DEP]) on childhood asthma<sup>1</sup>. However, none of these studies examined how the novel DEP contaminant, 1,2-naphthoquinone (1,2-NQ) and their derivatives, can be a potential candidate for the deleterious effects of DEP in juvenile asthma. This study aimed to test interactions between early-lifetime and acute exposure to 1,2-NQ in predicting exacerbation of asthma clinical outcomes in female and male juvenile mice. **Methods:** Neonate male and female C57Bl/6 mice (2-5 g) were used, under a protocol approved by our Institutional Ethics Committee (Number 113/07/CEEA). Animals were nebulized with 1,2-NQ (100 nM; 10 mL) or corresponding vehicle (PBS:Tween 80:DMSO) at days 6, 8 and 10 for 15 min. Seventeen days later, mice exposed to 1,2-NQ or its vehicle were sensitized by OVA (10 µg/0.2 ml PBS, s.c.) or vehicle (1.6 mg Al(OH)<sub>3</sub>/0.2 ml PBS) for 2 days. After seven days, they were stimulated with OVA 1% or vehicle. After 24 h, both the BUXCO noninvasive lung function detection was performed to examine the airway hyperresponsiveness (Penh value). The quantification of inflammatory biomarkers was assessed in the bronchoalveolar lavage fluid (BALF). Data are mean ± SEM. Stats were performed by ANOVA followed by Bonferroni's *t*-test. P<0.05 was taken as significant. **Results:** The total BALF cells, neutrophils, macrophages, lymphocytes and eosinophils in allergic male mice previously submitted to 1,2-NQ (1,2-NQ + OVA) were significantly higher when compared with non-allergic group treated with pollutant vehicle (vehicle + non-allergic). In contrast, the total BALF cells, neutrophils, macrophages and eosinophils in 1,2-NQ + OVA female group did not significantly differ when compared with vehicle + non-allergic or 1,2-NQ + non-allergic female groups. In 1,2-NQ + OVA male group, the early exposure to 1,2-NQ also enhanced allergen-induced BALF neutrophils, lymphocytes and eosinophils when compared to 1,2-NQ + non-allergic group or vehicle + OVA group. The early exposure to 1,2-NQ reduced allergen-induced BALF neutrophils and lymphocytes in allergic female group. The Penh value in 1,2-NQ + non-allergic male group was significantly higher (216 ± 42 AUC) than in vehicle + OVA (86 ± 18 AUC) or 1,2-NQ + OVA male groups (46 ± 11 AUC). The Penh value in 1,2-NQ + OVA female group is similar (112 ± 11 AUC) to Penh value in allergic female group (95 ± 21 AUC). In the BALF of 1,2-NQ + OVA male group increased concentration of Th2 (IL-4, IL-5 and IL-13) and Th1 (IFN-γ) cytokines was found as compared to all groups. In contrast, BALF concentrations of Th1 and Th2 cytokines in 1,2-NQ + OVA female group were lower than in vehicle + OVA female group. **Discussion:** the early-lifetime exposure to 1,2-NQ in male neonate mice increased the prevalence of allergic inflammatory signs at a juvenile stage as compared to female mice and control male group. Thus, the impact of air pollution such as DEP may put boys at a higher risk of respiratory diseases than girls. **Acknowledgements:** Fapesp, CNPq and CAPES for financial support. We thank Panthera solutions for providing the BUXCO equipment. **References:** 1. Bråbäck et al. Environ Health. 2009 Apr 16;8:17.

#### 04.103

Effect of *Lonomia obliqua* venom on human neutrophils. Moraes JA<sup>1</sup>, Rodrigues GS<sup>2</sup>, Nascimento-Silva V<sup>3</sup>, Guimarães JA<sup>4</sup>, Barja Fidalgo TC<sup>3</sup> <sup>1</sup>UERJ - Farmacologia Bioquímica e Celular, <sup>2</sup>UERJ - Farmacologia e Psicobiologia, <sup>3</sup>UERJ - Farmacologia, <sup>4</sup>UFRGS

**Introduction:** Lonomism is the envenomation promoted by caterpillar *Lonomia obliqua*. Accidents usually occur when the victim touch tree trunks containing a lot of caterpillars, leading to the contact with the caterpillar's bristles. *Lonomia obliqua* venom rapidly induces local inflammation and also reaches blood circulation, leading to a pro-inflammatory profile. The understanding of the *Lonomia obliqua* venom effect on the prominent leukocyte on circulation, polymorphonuclear (PMN), could contribute to the development of new drugs to lonomism treatment. Thus, the present study was carried out to evaluate the *in vitro* effects of *Lonomia obliqua* venom on PMN and the molecular mechanisms underlying these processes. **Methods:** *L.obliqua* caterpillar bristles extract (LOCBE) was obtained as described (Bohrer at al., 2007). The effects of LOCBE (1-10 µg/mL) were evaluated *in vitro* on isolated human blood PMN of healthy donors (Percoll gradient). Chemotaxis assays were performed in Boyden chambers (1h); Apoptosis was evaluated by morphological analysis (20h). Cell adhesion was measured by CMFDA (30min) and was accomplished on Collagen IV, Fibrinogen and Fibronectin. ROS production was quantified by lucigenin. COX-2, 5-LO, FAK phosphorylation and NFκB nuclear translocation were evaluated by immunoblotting. Finally IL-8 expression was analyzed by RT-PCR. **Results and Discussion:** Firstly we observed that LOCBE was able to induce PMN migration in a bell shape manner (3mg/mL). Corroborating these data, FAK, a key kinase involved in cell migration, was rapidly and strongly phosphorylated in cells treated with the venom (5-60min). As expected, LOCBE increased PMN adhesion to collagen IV, but, curiously, not to fibrinogen and fibronectin. LOCBE also promoted oxidative burst in PMN, a well-know event in inflammatory process. Beyond these effects, after 30min LOCBE induced COX-2 and 5-LO expression, pivotal proteins in pro-inflammatory process. LOCBE rapidly induced NFκB nuclear translocation (30min-1h), which could lead to pro-inflammatory cytokines transcription. Then we observed that LOCBE, after 2h, induced IL-8 expression in PMN. Among different effects induced by IL-8 and other proteins involved in pro-inflammatory process, is the PMN survival. Finally, we showed that LOCBE induced strongly PMN survival after 20h, a crucial effect to the maintenance of the pro-inflammatory profile. In resume, our data show that *L. obliqua* venom can activate PMN, inducing different effects. Thus, we demonstrated that LOCBE acts as a potent pro-inflammatory agent, as well the molecular mechanisms underlying the affected processes. Financial Support: CAPES, CNPq, FAPERJ

#### 04.104

Diminuição da resposta edematogênica induzida por carragenina e não por dextran na pata em modelos de doença inflamatória intestinal em ratos. Barbosa ALR<sup>1</sup>, Torres JNL<sup>2</sup>, Medeiros J-VR<sup>2</sup>, Soares PMG<sup>2</sup>, Lima-Júnior RCP<sup>2</sup>, Gomes AS<sup>2</sup>, Lucetti LT<sup>2</sup>, Ribeiro RA<sup>2</sup>, Souza MHL<sup>2</sup> <sup>1</sup>UFPI - Fisioterapia, <sup>2</sup>UFC - Fisiologia e Farmacologia

**Introdução:** Recentemente foi demonstrado que portadores de Doença de Crohn apresentam uma redução da resposta inflamatória aguda, caracterizada por uma diminuição na infiltração neutrofílica e da produção IL-8 (Marks D.J et al., *Lancet*. 2006 Feb 25;367-9511:668-78). Tendo em vista poucos dados experimentais que evidenciem a influência das doenças inflamatórias sobre o processo inflamatório agudo, o presente trabalho tem como objetivo avaliar no desenvolvimento experimental das doenças inflamatórias intestinais o edema de pata induzido por carragenina ou por dextran 14 dias após a indução dessas doenças. **Métodos:** Protocolo foi aprovado pelo comitê de ética ( protocolo 63/07). As doenças inflamatórias intestinais (colites) foram induzidas por TNBS (20mg/animal, modelo de Crohn), iodoacetamida (IODO;6%, modelo de retocolite ulcerativa) ou álcool (50%, colite inespecífica) por via transanal. Quatorze dias após a indução da colite, animais com ou sem colite receberam Carragenina (Cg;500µg/pata direita) ou Dextran (Dx; 500µg/pata direita) injetadas na região intraplantar. O edema foi avaliado 1, 2, 3 e 4 hs após administração da Cg ou 30', 1, 2, 3 e 4 hs após a administração do Dx por pletismometria. No final o cólon dos animais foram retirados para análise macroscópica e mensuração do peso. **Resultados:** Nas colites induzidas por TNBS, iodoacetamida e álcool observou-se uma lesão colônica, com achados macroscópicos pontuados em escores (TNBS=1,0(0,0-1,0), IODO=1,0(1,0-1,0), Etanol=1,0(1,0-1,0) e aumento do peso (TNBS=161,87 ± 6,08mg/cm de lesão, IODO=200,08 ± 10,28mg/cm de lesão, Etanol=184,5 ± 8,50/cm de lesão ) em comparação ao grupo controle (Escore=0(0-0) , peso=124,5 ± 4,83/cm de lesão). Na colite por TNBS (Cg+TNBS - 1<sup>a</sup>h;0,124±0,041 ml e 3<sup>a</sup>h;0,236±0,053 ml), mas não na colite por iodoacetamida (Cg+IODO - 1<sup>a</sup>h;0,200±0,043 ml e 3<sup>a</sup>h;0,325±0,079ml) ou por etanol (Cg+etanol - 1<sup>a</sup>h;0,336±0,039ml e 3<sup>a</sup>h;0,425±0,063ml), foi observado uma menor resposta edematogênica da Cg, quando comparado ao grupo controle (Controle 1<sup>a</sup>h;0,350±0,07 ml e 3<sup>a</sup>h;0,516±0,256ml). Não houve diferença significativa entres os grupos quando se avaliou a resposta edematogênica do Dx em todos os tempos em estudo. **Discussão:** Em modelos experimentais de doença inflamatória intestinal em ratos existe uma diminuição da resposta edematogênica na pata induzida pela carragenina mas não da induzida pelo Dextran, podendo assim inferir que a curso da doença inflamatória intestinal pode reduzir a resposta inflamatória aguda dependente do estímulo da Cg, em sítios distantes do cólon. **Apoio Financeiro:** Funcap e CNPq.



#### 04.105

Evaluation of anti-inflammatory activity of thiazolidine derivative. Santos IBV, Campos IA, Lima SMA, Botelho SPS, Lima MCA, Galdino SL, Pitta IR, Silva TG UFPE - Antibióticos

**Introduction:** Inflammation is a condition of morbidity that accompanies all infectious processes that affect man, including cancer and chronic diseases. It is, by definition, the reaction of vascularized living tissue to a lesion site. The role of inflammation is to contain and isolate the damage, destroy the invading microorganisms, inactivate toxins and initiate processes of healing and repair. However, these processes can be potentially harmful, causing a hypersensitivity reaction that threatens the life of the affected, and injury and progressive fibrosis of organs. Anti-inflammatory drugs are used in the treatment and relief of symptoms of inflammatory diseases such as rheumatoid arthritis, rheumatic fever, osteoarthritis, psoriatic arthritis, Lupo eritmatoso, among others. In this context, the synthesis and biological activities of thiazolidinadionas have been extensively reviewed, and identified as carriers of significant anti-inflammatory activity. **Methodology:** The animals used for research were Swiss albino mice (*Mus musculus*) of both sexes, weighing between 20 and 25g and 60 days old. They were submitted to fasting for 6 hours, and after that period, received orally, the substance test GQ-130 (10 $\mu$ M/kg) and saline (control group). After 1 hour of treatment, was given 0.5 mL of a solution of thioglycolate (3% in saline - p / v) by intraperitoneal route. After 4 hours, the animals were euthanized and immediately have the peritoneal fluid (exudate) removed by suction, using 2mL of phosphate buffer solution of heparinized (10 IU heparin / mL). The exudate was diluted in Turk solution, and counting of polymorphonuclear leukocytes (PMNL) made in a Neubauer chamber. The indomethacin (28mmol/kg) was used as the reference drug. The protocol used was approved by the Ethics Committee in Animal Experimentation (EAEC), Federal University of Pernambuco (no. 23076, 0040.13 / 2008-35). The number of PMNL was analyzed statistically by analysis of variance (ANOVA) and Bonferroni test with a confidence interval of 95%, using the software Origin 7.0. **Results and discussion:** The animals treated with compound thiazolidine LPSF/GQ-130 showed  $7,7 \pm 0,5 \times 10^5$  PMNL/ cavity, since the control group and the indomethacin group showed  $26.2 \pm 1.5 \times 10^5$  and  $6.5 \pm 0.5 \times 10^5$  PMNL/cavity, respectively. The data obtained showed that the LPSF/GQ-130 reduced the migration of polymorphonuclear leukocytes in the abdominal cavity of animals, which stimulates the trials continue with more specific anti with possible development of a prototype candidate for anti-inflammatory agent. **Bibliography:** Gayathri, B. *Int. Immunopharmacol.* v.7, 473. 2007. Széles, L; Töröcsik,D; Nagy, L. *Biochimica et Biophysica Acta*, v.1771 1014, 2007. Santos, L. C, et al. *Heterocyclic Communications*, V.11 (2), 121-128. 2005. **Financial support:** UFPE / CNPq

#### 04.106

A new model of steatohepatitis induced by the cancer chemotherapeutic agent irinotecan in mice. Costa MLV, Lima-Júnior RCP, Medeiros RP, Marques Neto RD, Leite LL, Nunes LG, Souza MHL, Ribeiro RA UFC - Fisiologia e Farmacologia

**Introduction:** Steatohepatitis is a recognized complication of cancer chemotherapy treatment. With the development of new neoplastic agents, particularly active against colorectal cancers, a significant increase in its incidence became evident. The chemotherapy regimens including irinotecan, a topoisomerase I inhibitor, seems to be associated to a particular increased risk. With the frequent use of a neoadjuvant approach in the treatment of hepatic metastasis, the issue of whether steatohepatitis may complicate the clinical course of patients submitted to hepatic resection, by decreasing hepatic functional reserve, was not fully answered. In order to address this issue, a more complete understanding of the physiopathology of this toxicity is necessary. The development of irinotecan induced steatohepatitis animal model will allow the discovery of the possible mechanisms and inflammatory mediators involved in this disease. **Objective:** to establish a model of Irinotecan-induced steatohepatitis in mice. **Methods:** Swiss male mice (n=8-10) were divided into 5 groups and treated with saline (5ml/kg, i.p.) or irinotecan (25, 50, 75 or 100mg/kg, i.p.), three times a week for 7 weeks. Once a week the animals were weighed and blood samples were collected via the retro orbital plexus to measure the serum concentration of hepatic enzymes ALT and AST (U/L) as markers of liver damage. By the end of the seventh week the animals were sacrificed by cervical dislocation and the liver was removed by carefully dissection. Liver weight was measured and samples were excised to perform myeloperoxidase assay (MPO, U/L) as well as histopathological analysis. A survival curve was also obtained. Statistical analysis was performed with ANOVA/Student Newman Keul or Kruskal Wallis/Dunn as appropriate. The survival rate was expressed as the percentage of live animals and the Kaplan-Mayer log rank test was used to determine the differences between survival curves. Statistical significance was set at  $p < .05$  (CEPA: Protocol 02/04). **Results:** Animals treated with irinotecan (25, 50 mg/kg) presented a significant ( $p < .05$ ) weight loss, which seems to be related to the significant reduction on the survival rate ( $p < .05$ ). Liver weight was significantly increased ( $p < .05$ ) on irinotecan treated animals: 25mg/kg ( $1462 \pm 17.98$ ), 50mg/kg ( $1718 \pm 156.2$ ) when compared to saline groups ( $1110 \pm 66.1$ ). The treatment with irinotecan (50mg/kg) showed significantly ( $p < .05$ ) increased serum concentrations of hepatic enzymes ALT ( $95,99 \pm 4,136$ ) and AST ( $138,7 \pm 9,521$ ) when compared to saline group (ALT:  $44,58 \pm 12,03$  ; AST:  $84,16 \pm 8,122$ ), characterizing a typical liver damage. Irinotecan also showed a significant ( $p < .05$ ) increasing on MPO activity ( $2.19 \pm 0.56$ ) in comparison to saline treated group ( $0.06 \pm 0.03$ ). Histopathological analysis evidenced an important presence of periportal neutrophil infiltration, tissue renewal (larger cells and nuclei) and micro-vesicular steatosis. **Conclusion:** This is the first report of liver injury due to Irinotecan injection in mice which mimics clinical findings. This study provides further perspectives on the knowledge of steatohepatitis mechanisms induced by chemotherapeutic agents. Financial support: CAPES/CNPq.

#### 04.107

Inhalation of hydroquinone impairs LPS-induced leukocyte migration to the lung. Shimada ALB<sup>1</sup>, Ribeiro ALT<sup>1</sup>, Silva PCB<sup>1</sup>, Hebeda CB<sup>1</sup>, Farsky S<sup>1</sup>, Tavares de Lima W<sup>2</sup>  
<sup>1</sup>FCF-USP - Análises Clínicas e Toxicológicas, <sup>2</sup>ICB-USP - Farmacologia

**Introduction:** Hydroquinone (HQ) is an important phenolic compound of cigarette, medicines and foods. Moreover, it is obtained from benzene (BZ) metabolism and it is heavily used in petroleum refining, petrochemical and chemical industries, contributing to the environmental contamination. Our research group has demonstrated that male Wistar rats intraperitoneally exposed to HQ (5 or 10 mg/kg/day) presented reduced numbers of mononuclear (MN) and polymorphonuclear (PMN) leukocytes in the bronchoalveolar lavage (BAL) 24 hours after instillation of LPS of *Salmonella abortus* or inhalation of ovalbumin in animals previously sensitized. In addition, the impaired leukocyte migration into inflamed lung did not reflect alterations on number of circulating cells. Most current regulatory agencies (CETESB, OSHA and ACGIH) determine that occupational exposure limits is 2mg/m<sup>3</sup> (8h - time weighted average threshold limit values for occupational air exposed). To amplify our knowledge, here we investigated the effects of inhalation of HQ on lung leukocyte recruitment induced by LPS. **Methods:** Male Swiss mice (20-25g) were exposed to aerosolized HQ using different schedules: 1) 0,75mg/60mL/1h (12,5ppm); 1,5mg/60mL/1h (25ppm) or 3mg/60mL/1h (50 ppm), during 5 days, once a day; 2) 1,5mg/60mL/1h (25ppm), one day. Control animals received the same amount of vehicle (saline with 5% ethanol). Lung inflammation was induced by inhalation of LPS (*E. coli*, sorotype 026:B6; 0,1 mg/mL; 10 min) 1 or 72 hours after the last exposures and BAL was collected three hours later. Number of circulating and recruited leukocytes to the BAL was determined in a Neubauer chamber. Differential count was performed in staining smears. The experiments were conducted according to Ethics Committee in Animal Experiments n.53/2008 – Protocol n.196. **Results:** Exposure to HQ during five days in all doses employed reduced the number of leukocytes in the BAL (12,5 ppm= 43,6% vs control; 25 pp= 53,1% vs control; 50 ppm= 61,7% vs control). When inflammation was induced 72 hours after the last HQ exposure (25 ppm), the impaired leukocyte migration was abolished. A single HQ exposure (25 ppm) did not alter leukocyte recruitment to the BAL. Number of circulating leukocytes was not modified in all schedules of HQ exposures. **Discussion and Conclusions:** Results presented show that inhaled HQ impairs leukocyte recruitment into lung induced by LPS and that this effect depends on period of exposure and may be reversed after stopping *in vivo* exposure. Financial Support: FAPESP (Process n°08/55382-7), Capes and CNPq.

#### 04.108

*In vivo* confirmation of the endocrine arm of the immune-pineal axis. Fernandes PACM, Tamura EK, Ferreira ZS, Monteiro AWA, Markus RP IB-USP - Fisiologia

**Introduction:** Melatonin released at night by the pineal gland is an important chronobiological signal that modulates several physiological functions. Nocturnal concentrations of this hormone are capable, for example, to decrease the neutrophil migration through the endothelial layer (LOTUFO, Eur J Pharmacol, 430:351, 2001). Rhythmic pineal melatonin synthesis is under the control of the central nervous system but, we have shown that while high levels of corticosterone increases (FERREIRA, J Pineal Res, 38:182, 2005), the early inflammatory cytokine tumor necrosis factor (TNF) decreases *in vitro* melatonin production (FERNANDES, J Pineal Res, 41:344, 2006). These data support the existence of an immune-pineal axis which postulates that, at the beginning of an inflammatory process, the production of TNF would lead to a decrease on nocturnal melatonin production (MARKUS, Neuroimmunomodulation, 14:126, 2007). Here we investigated whether administration of LPS to rats, besides increasing circulating levels of TNF, reduces pineal function. **Methods:** Male rats (2 month-old) kept in 12/12 h light/dark cycle (lights on at *zeitgeber time* -ZT- 0), injected at ZT 16 with LPS (0.5 mg/kg) or saline were killed after two hours (ZT 18). One group was injected with saline at ZT 4 and killed at ZT6 as a diurnal control. All animal procedures were performed according to approved institutional protocols (086/2008) for the proper use and care of experimental animals. The expression of the key enzyme arylalkylamine-N-acetyltransferase (AA-NAT) mRNA in the pineal glands was determined by quantitative real time RT-PCR. The results were normalized by the reference gene 18-S and expressed as folds of increase relative to diurnal saline group. Plasmatic melatonin and TNF were determined by commercial ELISA kits (Genway Biotech Inc, USA; eBioscience Inc, USA). All data are presented as mean  $\pm$  SEM. Statistical comparisons were performed using the Student's t-test. **Results:** The *in vivo* treatment with LPS induces the production of TNF measured in the plasma ( $659.40 \pm 49.79$  pg/mL, n=6). Saline injection (during the day or during the night) did not result on measurable plasmatic content of TNF. The expression of the *Aa-nat* mRNA was significantly reduced in the LPS group ( $88.74 \pm 15.58$ ; n=4) when compared to the saline group ( $166.20 \pm 14.23$ ; n=4). This effect was reflected in the nocturnal plasma melatonin content (saline =  $88.25 \pm 13.86$  pg/mL vs LPS =  $40.16$  pg/mL; n=6-8). **Discussion:** Our results confirm *in vivo* one arm of the immune-pineal axis hypothesis. As nocturnal melatonin production reduces cell migration through the endothelial layer (LOTUFO, 2001) the reduction of nocturnal melatonin concentration observed here was expected and predicted for the appropriate mounting of the inflammatory response. The data also confirms our expectation of an inverse correlation between pineal *Aa-nat* mRNA expression and TNF plasmatic levels since the treatment of cultured pineal glands with this cytokine leads to the same effect (FERNANDES, 2006). The knowledge derived from this emerging concept open the possibility to new therapeutic applications of melatonin during inflammatory responses. **Acknowledgments:** The technical support of Debora Aparecida de Moura is gratefully acknowledged. **Financial Support:** FAPESP (07/07178-06), CAPES and CNPq.

#### 04.109

Antipyretic effect of ibuprofen on LPS-induced fever is not related to cytokines synthesis inhibition. Soares DM<sup>1</sup>, Cristofolletti R<sup>2</sup>, Melo MCC<sup>2</sup>, Souza GEP<sup>2</sup> <sup>1</sup>USP - Farmacologia, <sup>2</sup>FCFRP-USP - Física e Química

**Introduction:** We previously reported that ibuprofen (IBU) inhibits PG-dependent and -independent fever, reduces the LPS-induced increase of CSF PGE<sub>2</sub> levels and its antipyretic effect is blocked by arginine vasopressin (AVP) receptors antagonist (1). Moreover, it seems that IBU exerts COX inhibition-independent effects as the blockade of the activation of nuclear factor-kB (NF-kB) and activator protein-1 (AP-1) and then inhibiting the transcription of genes encoding adhesion molecules, chemokines, COX-2 and pyrogenic cytokines (2). Here, we investigated if IBU could also display its antipyretic effect by inhibiting the synthesis of the pyrogenic cytokines IL-1b, IL-6 and TNF-a. **Methods:** Body temperature was measured every 30 minutes for up to 3 h by radio-telemetry system in male Wistar rats (200g). Rats were pre-treated i.p. with IBU (10 mg/kg) 30 min before i.v. injection of LPS (5mg/kg). 3 h later the animals were deeply anaesthetized and the blood was collected by cardiac puncture just prior the decapitation for hypothalamus extraction. The cytokines concentrations in the serum or hypothalamus were measured by Elisa and are expressed as pg/g of tissue from 4-5 animals. **Results:** LPS i.v. injected increased the IL-1b (SAL/SAL = 13330.6 ± 5750.0; SAL /LPS = 37413 ± 5929.7) and IL-6 (SAL/SAL = 6580.3 ± 2713.2; SAL/LPS = 22288.0 ± 4685.4) concentrations in the hypothalamic tissue. Ibuprofen did not alter the concentration of IL-1 (IBU/SAL = 20567.4 ± 2305.8; IBU/LPS = 29579.2 ± 3075.1) or IL-6 (IBU/SAL = 7169.4 ± 1367.6; IBU/LPS = 16411.1 ± 2130.8). Neither TNF-α was detected in the hypothalamus nor cytokines were found in the plasma. **Conclusions:** The fever induced by i.v. injection of 5µg/kg of LPS seems do not depend on an increase in the plasmatic concentration of IL-1b, IL-6 and TNF-a. Furthermore, the antipyretic effect of ibuprofen is not related to the synthesis inhibition of these cytokines in the hypothalamus. **Support:** FAPESP, CNPq. 1) Cristofolletti et al., 2004. Livro resumo SBFTE - 06.018 - Pagina 31; 2) Tegeuder I, Pfeilschifter J, Geisslinger G. FASEB J 2001; 15:2057-72.

#### 04.110

Effects of hydroquinone on the functional activity of endothelial cell. Pinedo F<sup>1</sup>, Cavalcanti DM<sup>1</sup>, Teixeira SA<sup>2</sup>, Muscará MN<sup>2</sup>, Hebeda CB<sup>1</sup>, Farsky S<sup>1</sup> <sup>1</sup>FCF-USP - Clinical and Toxicological Analysis, <sup>2</sup>ICB-USP - Pharmacology

**Introduction:** Hydroquinone (HQ) is a phenolic compound obtained after benzene endogenous metabolism, and it is a component of cigarette, medicines, photographic developer, and in some foods and medicinal herbs (Lee *et al.*, 2007; Silva *et al.*, 2003, RDC n.º 79, 2000, for review see Nordland *et al.*, 2006). Our research group has been shown that *in vivo* exposure to HQ to rats impairs leukocyte migration into lung during allergic or non-specific inflammation (Ferreira *et al.*, 2007; Macedo *et al.*, 2007). This experimental study aimed to investigate the role of HQ treatment on pro or anti-inflammatory properties of endothelial cells (EC), since this is the blood vessels and exerts key role in controlling the inflammatory response. **Methods:** Primary cultured EC was obtained from microcirculatory network of Male Wistar rats, and incubated with 10 or 100 µM of HQ, during two hours and after incubated with or without E. coli lipopolysaccharide (LPS; 2 µg/mL). Cell viability and adhesion molecules expression were quantified by flow cytometer; NO<sub>2</sub><sup>-</sup> and cytokines in the supernatant of cells were measured by Griess reaction and ELISA, respectively; gene expression of adhesion molecules, myeloperoxidase and CYP 2E1 by RT-PCR; nitric oxide synthases (NOS) by enzymatic. **Results and discussion:** Results obtained showed that HQ treatments did not induce apoptosis or necrosis in endothelial cells. HQ exposure to EC impaired NO production induced by LPS (50%) due to dependent and independent Ca<sup>+2</sup> NOS activity blockade (80%); enhanced the basal secretion of TNF-α (50%) e IL-1β (100%); and enhanced the synthesis and expression of ICAM-1 (50%), PECAM-1 (100%) e VCAM-1 (25%). These effects may not be dependent on HQ metabolism to reactive benzoquinones, as EC does not present myeloperoxidase and gene expression of CYP2E1 was not altered by HQ incubation. **Financial Support:** FAPEPS (nº 07/56299-3) / CEEA (nº 169).

#### 04.111

Oxidative changes in diaphragm muscles from rats submitted to intestinal ischemia and reperfusion. Bolonheis SM, Teixeira SA, Gouvea IM, Varriano AA, Costa SKP, Muscará MN USP - Farmacologia

**Introduction:** Contractile dysfunction (CD) of respiratory muscles is a common finding in the systemic inflammatory response syndrome (SIRS) caused by bacteria infection (i.e., sepsis). CD difficults weaning from mechanical ventilation, thus resulting in longer hospitalization periods in intensive care units and higher risk of secondary infections. However, there are no reports on the occurrence of CD in SIRS secondary to intestinal ischemia and reperfusion (IIR). In this way, we decided to study the inflammatory process in diaphragm muscle from rats submitted to IIR. **Material and Methods:** The experimental procedures were approved by our institutional Ethics Committee for Animal Experimentation (protocol 153, on page 24, book 2). Male Wistar rats (250-300g) were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg). After a medial abdominal laparotomy, the superior mesenteric artery was clamp-occluded during 45 min and followed by a 2 or 6 h-reperfusion period. Samples of diaphragm muscles were collected for analysis of nitric oxide synthase (NOS) isoforms, superoxide dismutase (SOD), myeloperoxidase activity (MPO), nitrotyrosine-containing proteins (NT), lipid peroxidation (TBARS) and cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10). In order to evaluate alterations in mitochondrial respiration, we also analyzed the oxygen consumption rate in slices of muscles (potentiometry). **Results:** Significantly increased MPO (115%), lipid peroxidation (67%) and Ca<sup>2+</sup>-dependent NOS (162%) activities occurred in diaphragms from IIR animals after 2 h reperfusion in comparison to Sham rats, as well as reduced nNOS (78% for mRNA and 59% for protein expression), protein eNOS (52%) and SOD-1 (22%). A slight increase in NT-containing proteins was found after 2 h. Increased Ca-independent NOS activity (717%) and mRNA for both iNOS (113%) and nNOS (109%) were found in diaphragms after 6 h reperfusion, but the expressions of either NT-containing proteins, SOD, nNOS or eNOS were unaltered. Increased levels of IL-1 $\beta$  and IL-6 were found at both time points. In addition, increased oxygen consumption rate (63%) occurred when mitochondrial complex I was stimulated with malate plus glutamate, which was reversed by pre-incubation of the slices with either L-NAME or aminoguanidine. **Discussion:** These results show that an oxidative response occurs in diaphragm muscles from rats submitted to IIR. Increased activity of mitochondrial respiratory chain could be a source of superoxide anion in IIR animals. Whether this time-dependent response involving NOS isoforms and protein modifications affects muscle contractility and functionality deserves further investigation. **Financial Support:** CNPq, FAPESP, Panthera Solutions Ltd.

#### 04.112

Remote Ischemic Preconditioning (RIPC): the anti-inflammatory effects are mediated by nitric oxide and carbon monoxide pathway via CXCR2. Simão AFL<sup>1</sup>; Souto FO<sup>2</sup>; Souza-Filho MVP<sup>3</sup>; Cunha FQ<sup>4</sup>; Ribeiro RA<sup>1</sup> <sup>1</sup>UFC - Fisiologia e Farmacologia; <sup>2</sup>FMRP-USP – Cirurgia e Anatomia; <sup>3</sup>UFC - Cirurgia; <sup>4</sup>FMRP-USP

**Introduction:** Remote ischemic preconditioning (RIPC) consists of short-term periods of ischemia followed by reperfusion, which induces protective anti-inflammatory effect on distant tissues and organs. Studies have shown that inflammatory parameters such as leukocyte migration and pH logistic signs are significantly reduced in preconditioned animals. However, the mechanism involved in this effect is not yet well established. This study sought out to evaluate the role of NO and CO in mediation this anti-inflammatory activity of RIPC. **Methods:** RIPC model was set up on tourniquet in right hind limb of a mouse for 10 minutes followed by 30 of reperfusion. Participation of NO and CO was investigated using inhibitors of iNOS (1400 W; 3 mg/kg or Aminoguanide; 50 mg/kg) and HO-1 (ZnPPIX;10 mg/kg) as pre-treatment 30 minutes before RIPC. Afterwards, inflammatory reaction was induced by intraperitoneal administration of Carragenin (500 µg/cav). Four hours after the peritoneal cavities were washed with saline and number of migrated leukocytes was evaluated. With this same procedure, animals were undergone to intravital microscopy towards to evaluate the NO and CO effects in real time upon blood flow of mesentery's vessels. Chemotaxis ability of neutrophils obtained from the mice under RIPC treated or not with the inhibitors was analyzed in Boyden Chamber, where the animal neutrophils were undertaken to a chemotaxis stimulus (KC; 30 µg/ml). Expression of CXCR2 and GRK2 in neutrophils was determined by flow cytometry and immunofluorescence, respectively. **Results:** CO and NO inhibitors prevented the inhibitory effect of RIPC upon Cg-induced neutrophil migration into peritoneal cavity of animals. ((Ctl:0.38 Sham:8.62 PCI:2.64 PCI(ZnPPIX):8.51 PCI(1400W):7.92)x10<sup>6</sup> neutrophils/field p<0.05). Furthermore, neutrophils obtained from preconditioned animals presented reduced chemotaxis in Boyden Chamber to the chemokine KC(Ctl:9.95 PCI:4.525 PCI(1400W):13.172 PCI(ZnPPIX):10.731x10<sup>6</sup>neutrophils/field p<0.05), finding that correlated with the decrease in CXCR2 receptor expression in the membrane of the neutrophils(M.F.I. Ctl:141.02 PCI:89.57 p<0.05) and the increase in GRK2 expression(M.F.I. Ctl: 10.08 Sham:19.05 PCI(1400W):14.39 PCI(ZnPPIX):11.79 p<0.05). Reduction of chemotaxis, increase in GRK2 and decrease in CXCR2 were not observed when neutrophils were obtained from animals pretreated with the gases inhibitors. As well, preconditioned animals presented reduction in neutrophils rolling and adhesion in Intravital Microscopy , prevented by pretreatment with CO- and NO-inhibitors.(Rolling:mean(Ctl:104.16 Cg:585.5 Cg(PCI):94.01 CgZnPPIX(PCI):524.3 CgNO(PCI):467.01)cells/100µm<sup>2</sup> p<0.05) Adhesion: mean (Ctl:0.21 Cg:1.36 CgPCI:0.41 CgZnPCI:1.42 CgNOPCI:1.67)cells/min p<0.05). **Discussion:** The HO-1/CO and NOSi/NO pathways mediate the anti-inflammatory effect of RIPC by via decrease of CXCR2 expression. Further studies are necessary to clarify the mechanisms underlie this down-regulation of CXCR2. Updated literature suggests several mechanisms, where internalization or degradation of this receptor could be the main explanation. **Financial support:** CNPq, CAPES e FAPESP. **Acknowledgement:** Patology and Lab assistants. **número da Licença de Autorização do Comitê de Ética:** 089/2006



#### 04.113

Avaliação da atividade anti-inflamatória e antinociceptiva do extrato bruto metanólico da *Litchi chinensis*. Castellain RCL<sup>1</sup>, Cechinel Filho V<sup>2</sup>, Souza MM<sup>1</sup>, Meyre da Silva C<sup>2</sup> <sup>1</sup>UNIVALI - Ciências Farmacêuticas, <sup>2</sup>NIQfar-CCS-UNIVALI

**Introdução:** *Litchi chinenses* (LC) conhecida como Lichia, é utilizada pela população para o tratamento de processos dolorosos e inflamatórios. O objetivo do presente trabalho foi de avaliar o efeito do extrato bruto metanólico (MeOH) desta planta em alguns modelos farmacológicos de dor e inflamação. **Metodologia:** Os protocolos experimentais foram submetidos ao CEP/UNIVALI e aprovados com parecer 291/08. Para todos os experimentos foram utilizados camundongos Swiss Webster (25-35g) machos. No modelo de dor induzido por formalina (MDF), os animais foram tratados via oral com o MeOH (25 mg/kg, 50 mg/kg e 100 mg/kg), indometacina (10 mg/kg), ou com veículo (salina, 0.1mL/10g peso). 60 min após cada tratamento, os animais receberam uma injeção intraplantar de formalina a 2,5% (25 µL/ pata) e imediatamente foi cronometrado o tempo de reação para a fases neurogênica (0-5) e fase crônica(15-30) representado pelo tempo gasto em lambar ou morder a pata injeta. No modelo de dor induzida por calor – ou modelo da placa quente (MPQ), os animais pré-selecionados 24hs antes foram colocados sobre uma placa de alumínio pré-aquecida (55 ± 0,5 °C) e o tempo (s) que cada animal gastou para retirar uma pata traseira da placa de alumínio e levá-la à boca foi cronometrado. Animais tratados via oral com MeOH (25 mg/kg, 50 mg/kg e 100 mg/kg), veículo (v.o) e Morfina (5 mg/kg; s.c.) foram utilizados nesse modelo. Dois grupos separados tratados com morfina e o MeOH (100 mg/kg) receberam pré tratamento com naloxona (5 mg/kg; i.p.). Para verificação do efeito Anti-inflamatório foi utilizado o modelo do edema de pata induzido pela injeção de carragenina (MEP)/Cg 300 µg/pata). Animais pré-tratados com MeOH (25 mg/kg, 50 mg/kg e 100 mg/kg), e veículo. 60 minutos após, receberam na região subplantar da pata direita Cg (300 µg/pata/0,50 µL), e na pata esquerda foi administrado o mesmo volume de salina. Dexametasona (0,5 mg/kg s.c) foi usada como controle positivo, sendo administrada 4hs antes do uso da Cg. A medida do edema foi feita pela diferença entre os volumes deslocados da pata direita e os da pata esquerda utilizando-se um pletismômetro, no tempo de 30', 60', 120', 240', 360'e 24hs. **Resultados e discussão:** No MDF, o MeOH produziu de forma dose-dependente inibição do processo doloroso induzido pela formalina nas duas fases neurogênica e inflamatória com IM (inibição máxima) calculadas de 62,9% e 61% respectivamente. No MPQ o tratamento com MeOH (100mg/kg) (15,36±1,46) e morfina (27,27±1,32) produziu aumento do limiar nociceptivo dos animais quando comparado com o controle (6,96±0,92). Sendo esse efeito revertido pela naloxona (14,27±1,23), sugerindo um possível efeito antinociceptivo através do sistema opióide. No MEP em todas as medições observou-se efeito antiedematogênico do extrato. Entretanto nos tempos 360 min. e 24hs o MeOH produziu redução do edema com todas as doses utilizadas com IM (23,52%; 35,29%;41,17%) para 360 min. e IM (37,03%;40,74%;44,44%) para 24hs, sugerindo assim uma possível ação anti-inflamatória. O MeOH, demonstrou ter efeitos antinociceptivo e antiedematogênico, nos métodos empregados validando pelo menos em parte os efeitos analgésicos e Anti-inflamatórios da planta. Apoio Financeiro: FAPESC/SC.

#### 04.114

Lipopolysaccharide *in vivo* decreases rat platelet aggregation: role of reactive-oxygen species (ROS) and NADPH-oxidase system. Pires MEL, Casarin AL, Antunes E, Marcondes S UNICAMP - Farmacologia

**Introduction:** Sepsis is a complex clinical syndrome resulting from a harmful host inflammatory response to infection, and activation of the coagulation cascade. A marked decrease in the circulating platelet counts is one of the earliest events in sepsis. Septic shock is known to increase the ROS production, but little is known on the role of ROS in modulating platelet functions under septic shock. Therefore, in this study we decide to determine the production of ROS in platelets of LPS-treated rats and the role of these species on platelet aggregation. **Methods:** Wistar rats (250-320 g) were injected i.p. with saline or LPS (from *E. coli*, 1 mg/kg). At 6 to 48 h thereafter, blood was collected in ACD-C (9:1 v/v). In a second experimental group, N-acetylcysteine (NAC) was injected i.p. (150 mg/kg) 30 min after LPS or saline injection. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min and the platelets were washed using citrated buffer (pH 6.0). Washed platelets were suspended in Krebs's solution adjusted to  $2 \times 10^8$  platelets/ml. Aggregation assays were carried out using ADP (3-10  $\mu$ M). Production of ROS in platelets was measured by flow cytometry using DCFH-DA (5  $\mu$ M). **Results:** Platelet aggregation induced by ADP (5 mM) was time-dependently reduced by the *in vivo* LPS treatment, as assessed at 6, 8 and 48 h after LPS injection (55%, 62% and 75% reduction, respectively). The inhibitory effect of LPS on platelet aggregation was higher when platelets were incubated *in vitro* with PEG-SOD 30 U/ml or PEG-catalase 300U/ml compared with controls. In contrast, *in vivo* treatment with NAC significantly reduced the inhibitory effect of LPS on platelet aggregation. The ROS production in non-stimulated platelets was similar in control or LPS groups. However, generation of ROS in ADP-stimulated platelets in LPS group was significantly higher than control animals (increase of 2.3-, 2.5- and 3.6-fold at 6, 8 and 48 h after LPS injection, respectively). On the other hand, NAC treatment nearly abolished the increased production of ROS in ADP-activated platelets from LPS-treated rats. Incubation of platelets from LPS-treated rats with the NADPH-oxidase inhibitor DPI (5 mM) significantly reduced the ROS generation in ADP-stimulated platelets (37% and 55% reduction at 8 and 48 h after LPS treatment, respectively). **Conclusion:** Our data indicate that *in vivo* treatment of rats with LPS increases ROS production in ADP-stimulated platelets by mechanisms involving NADPH-oxidase activation. It is likely that systemic or *in situ* production of ROS plays a modulatory role on platelet aggregation. **Supported by:** CAPES **License authorization of the Committee of Animal Ethics:** n° 1056-1 - UNICAMP

#### 04.115

Screening of new rolipram analogues using a long-term murine model of asthma. Cardozo SVS<sup>1</sup>, Santos TPO<sup>2</sup>, Pires ALA<sup>2</sup>, Silva PMR<sup>2</sup>, Barreiro EJ<sup>3</sup>, Lima LM<sup>3</sup>, Martins MA<sup>2</sup> - <sup>1</sup>UFRJ - Farmácia, <sup>2</sup>FIOCRUZ - Fisiologia e Farmacodinâmica, <sup>3</sup>LASSBio-UFRJ - Farmácia

**Introduction:** Asthma is a complex lung disease characterized by airway hyperreactivity, chronic eosinophilic inflammation, mucus hypersecretion and airflow obstruction. The enzyme phosphodiesterase (PDE) 4 is thought to play a critical role in the pathogenesis of asthma, and several PDE4 inhibitors are under evaluation in advanced clinical trials for asthma treatment. In the current study, we have investigated the effects of the PDE4 inhibitor rolipram and several analogues, namely LASSBio 448, LASSBio 959 and LASSBio 983 in a long-term murine model of asthma. **Methods:** Mice of the strain A were actively sensitized with a mixture of ovalbumin (OVA) and Al(OH)<sub>3</sub> at the days 0 and 7 and, one week later, subjected to a sequence of 4 consecutive weekly provocations with OVA (50 µg/25 µl), administered via the intranasal route. Oral treatments with the compounds or vehicle were performed 1 h before the third and fourth allergen provocation, when pathological changes were already noted. Airway hyperreactivity to methacholine was performed by invasive methodology 24 h after the last provocation. Other measurements included tissue eosinophil infiltration (assessed by eosinophil peroxidase activity), mucus secretion, collagen deposition (lung histology) and lung tissue cytokine generation (ELISA). All procedures were approved by the Ethics Committee for Use of Animals (CEUA) from FIOCRUZ (protocol 0213-4). **Results:** We found that rolipram (10 mg/kg) abolished allergen-evoked airway hyperreactivity, whereas LASSBio 965 and LASSBio 983 (100 mg/kg) were inactive. In contrast, LASSBio 448 and LASSBio 959 (100 mg/kg) clearly inhibited airway hyperreactivity (~ 87% and 88%, respectively), eosinophil accumulation (~ 58% and 43%, respectively), mucus secretion (~ 69% and 37 %, respectively) and fibrogenesis (~ 100% and 41%, respectively). IL-4 and IL-13 production triggered by allergen were also prevented by LASSBio 448 under conditions where eotaxin generation remained unaltered. Similar profile was seen following LASSBio 959 except for the lack of effect upon IL-13 production. **Conclusion:** Our results indicated that LASSBio 448 was clearly the most active compound studied, followed by LASSBio 959, being both substances able to reverse crucial features of severe asthma including eosinophilic infiltration, airway hyperreactivity, mucus and peribronchial fibrogenesis. LASSBio 448 and LASSBio 959 should be considered as molecular templates in drug discovery for asthma therapy. Financial support: CNPq, FAPERJ and PRONEX.

#### 04.116

The use of 3D lung myofibroblast culture system for screening anti-fibrotic drugs. Dalzy DV, Guimarães-Silva AM, Ferreira TPT, Silva PMR, Perez SAC, Martins MA FIOCRUZ - Fisiologia e Farmacodinâmica

**Background and Aim:** Lung fibrosis observed in airway of asthmatic patients is controlled by myofibroblast mainly because they are considered the major cell type producing extracellular matrix components (ECM). The establishment of *in vitro* model that better reflects lung fibrosis observed *in vivo* has become an important topic for screening anti-fibrotic drugs, especially because the current therapy for lung fibrosis is not efficient. In this view, this study was undertaken in order to establish a system of three-dimensional culture with lung myofibroblasts for the study of mechanisms of fibrogenesis and screening of new drugs with anti-fibrotic and anti-asthmatic activity.

**Methods:** Lung myofibroblasts of naïve Balb/c mice were grown in flat monolayers after mechanic and enzymatic lung dissociation. After the third trypsinization, 95% of pure myofibroblasts were obtained. Multicellular spheroids were formed using myofibroblast plated in agarose-coated 96 U-well plates. Analyses were performed by phase and confocal microscopy, ELISA and Sircol for measuring fibronectin, eotaxin and collagen secretion, respectively. All the procedures involving care and use of laboratory animals were approved by the Animal Ethics Committee of the Fiocruz (CEUA-FIOCRUZ, Prot. 0509/08). **Results:** We found that pulmonary myofibroblasts were able to reorganize in cellular spheroids and to respond with increased volume and production of fibronectin and collagen after rmlL-13 stimulation within 4 days of culture. Interestingly, IL-13-activated myofibroblast spheroids produced eotaxin release dose-dependently, under conditions which TGF- $\beta$  was ineffective. Moreover, we observed that treatment with the non anesthetic lidocaine analogue JM25-1 (30  $\mu$ M) abolished the increased production of collagen in IL-13-activated-myofibroblast spheroids, under conditions which the steroid dexamethasone was clearly inactive. **Conclusion:** Our findings indicate that the three-dimensional culture system of murine lung myofibroblasts seems to be an appropriate model for the study of fibrogenesis *in vitro*. In addition, the compound JM25-1 has the potential to control fibrogenesis in the asthmatic response confirming previous data obtained in an *in vivo* system. Financial Support: CAPES, FAPERJ, CNPq, PAPES and PDTIS.

#### 04.117

Role of cyclooxygenase-1 and -2 in prostacyclin production induced by the sPLA<sub>2</sub> subunit of crotoxin from *Crotalus durissus terrificus* snake venom in endothelial cells. Matsubara MH<sup>1</sup>, Lima SA<sup>1</sup>, Moreira V<sup>1</sup>, Soares AM<sup>2</sup>, Teixeira CFP<sup>1</sup> <sup>1</sup>Instituto Butantan - Farmacologia, <sup>2</sup>FCFRP-USP - Análises Clínicas Toxicológicas e Bromatológicas

**Introduction:** Crotoxin (CTX), the major component of *Crotalus durissus terrificus* snake venom (CdtV) contains two subunits: crotopotin (CA) and phospholipase A<sub>2</sub> (CB). The CB subunit exerts neurotoxic and myotoxic effects and inhibits macrophages functions. The phospholipase A<sub>2</sub> (PLA<sub>2</sub>) enzymes catalyze the cleavage of arachidonic acid (AA) from the *sn*-2 position of phospholipids with subsequent conversion of free AA into prostaglandin H<sub>2</sub> by two distinct isoforms of cyclooxygenases (COX-1 and COX-2); newly formed PGH<sub>2</sub> is then converted into distinct prostaglandins by terminal synthases. Prostacyclin (PGI<sub>2</sub>), a potent vasodilator and inhibitor of platelet aggregation, is the predominant prostaglandin synthesized by vascular endothelial cells (ECs). Recent data from our laboratory demonstrated the ability of CB to release PGI<sub>2</sub> from endothelial cells *in vitro*. **Objective:** In this study, the role of COX-1 and -2 enzyme systems on CB-induced prostacyclin production by endothelial cells was evaluated. **Methods:** ECs from murine endothelioma cell line (tEnd) were cultured in RPMI-1640 medium with 10% FBS and seeded on 96 well microplates for formation of monolayers. After reaching confluence ECs monolayers were incubated with CB (0.4 μM) or RPMI-1640 (control) by selected periods of incubation. Inhibition of COXs activities was performed by the pre-incubation of ECs with indomethacin (non-selective COX inhibitor) or valeryl salicylate (selective COX-1 inhibitor) or etoricoxib (selective COX-2 inhibitor). Production of PGI<sub>2</sub> was measured using enzyme immunoassay (EIA) and COXs protein expression evaluated by *western blotting* analysis. **Results:** Pre-incubation of ECs with indomethacin or valeryl salicylate or etoricoxib significantly decreased the prostacyclin production induced by CB subunit (78%, 42% and 61%, respectively) (p≤0.05). This toxin also up-regulated COX-2 protein expression (53%) whereas 4-bromophenacyl bromide (BPB), an inhibitor of phospholipases A<sub>2</sub> enzyme activity, abrogated this effect. However, the COX-1 pattern of protein expression was not modified by CB. **Discussion:** These data indicate that COX-1 and COX-2 activities are involved in CB-induced prostacyclin production from endothelial cells. In addition, up-regulation of COX-2 protein expression is relevant for the effect induced by CB. Moreover, the catalytic activity of CB is essential for the stimulatory effect of this phospholipase A<sub>2</sub> on biosynthesis of PGI<sub>2</sub>. Finally, these findings indicate novel regulatory mechanisms for venom secretory PLA<sub>2</sub> in endothelial cells. **Financial Support:** FAPESP e CNPq

#### 04.118

Efeito do LASSBio-897 sobre a inflamação pulmonar causada por sílica em camundongos. Arantes ACS de<sup>1</sup>, Ferreira T<sup>2</sup>, Trentin PG<sup>1</sup>, Pires ALA<sup>1</sup>, Cordeiro RSB<sup>1</sup>, Barreiro EJ<sup>3</sup>, Martins MA<sup>1</sup>, Fraga CAM<sup>3</sup>, Silva PMR<sup>1</sup> <sup>1</sup>IOC-FIOCRUZ - Fisiologia e Farmacodinâmica, <sup>2</sup>UNICAMP - Farmacologia, <sup>3</sup>LASSBio-UFRJ - Farmácia - LASSBio

**Introdução:** A silicose é uma doença de caráter ocupacional causada pela inalação prolongada de partículas de sílica livre e cristalina, e que a cada ano leva ao óbito milhões de trabalhadores em todo o mundo. Caracteriza-se pela ocorrência uma intensa resposta inflamatória e formação de nódulos fibróticos no tecido pulmonar. Como até o presente momento não há uma terapia eficaz, justifica-se a busca por novos compostos com a habilidade de controlar esta doença. Neste estudo, investigamos o efeito do derivado acilidrazônico LASSBio-897 sobre a resposta fibrótica em camundongos silicóticos. **Métodos:** Camundongos Swiss-Webster foram anestesiados e instilados via intranasal com sílica (10 mg/50 µL), com animais controles recebendo igual volume de salina. As análises realizadas 28 dias pós-sílica. Os animais receberam administração diária do LASSBio-897 (1, 2 e 5 mg/kg, v.o), durante 7 dias, iniciando-se 21 dias após a estimulação com sílica. A avaliação da função pulmonar (resistência e elastância) foi realizada através do sistema de pletismografia de corpo inteiro invasivo (Buxco System), sendo analisada a hiperreatividade pulmonar ao agente broncoconstrictor metacolina (3 - 27 mg/mL). Foram realizadas análises morfológicas por meio de histologia clássica e morfometria tecidual, quantificação do colágeno por Sircol e quantificação de citocinas e quimiocinas por ELISA. Todos os procedimentos utilizados foram aprovados pelo Comitê de Ética de Uso de Animais (CEUA) da FIOCRUZ (Protocolo 0213-4). **Resultados:** Observamos que os camundongos silicóticos apresentaram um aumento da resistência e elastância das vias aéreas, além do fenômeno de hiperreatividade pulmonar quando da aerolização com metacolina. Além disso, a análise histológica revelou que os animais silicóticos mostraram um intenso infiltrado inflamatório, presença de nódulos fibróticos e aumento na deposição de colágeno quando comparados aos controles. Em paralelo, vimos um aumento na produção das quimiocinas KC e MIP1- $\alpha$  e das citocinas TNF- $\alpha$  e IL-6 no tecido pulmonar dos animais silicóticos. O tratamento dos animais silicóticos com o composto LASSBio-897 determinou uma inibição significativa das alterações da função pulmonar, bem como da resposta de hiperreatividade das vias aéreas à metacolina, incluindo tanto resistência como elastância. Verificamos, ainda, diminuição do infiltrado inflamatório, assim como a redução da área do parênquima pulmonar ocupada por granuloma e do aumento na deposição de colágeno pelo composto LASSBio-897. A geração dos mediadores KC, TNF- $\alpha$  e IL-6, porém não de MIP1- $\alpha$ , foi sensível ao tratamento com o composto. **Conclusão:** Nossos resultados mostram que tanto o comprometimento da função pulmonar como a resposta fibrótica induzidos pela instilação de sílica em camundongos foram inibidos pelo derivado LASSBio-897, indicando que este composto coloca-se como de aplicação promissora na terapia de doenças de caráter fibrótico com a silicose. **Suporte financeiro:** FIOCRUZ, PRONEX, CNPq, FAPERJ.

#### 04.119

Inflammatory response triggered by Influenza A (H1N1 WSN/33) infection in a murine model. Tavares, LP<sup>1</sup>, Garcia CC<sup>1</sup>, Carvalho RT<sup>2</sup>, Russo RC<sup>1</sup>, Machado AMV<sup>3</sup>, Sousa LP<sup>2</sup>, Teixeira MM<sup>1</sup> <sup>1</sup>UFMG - Bioquímica e Imunologia, <sup>2</sup>COLTEC-UFMG - Patologia Clínica, <sup>3</sup>UNIFESP - Bioquímica

**Introduction:** Flu is a respiratory illness of great world-wide relevance, causing annual epidemics and great number of deaths and hospitalizations. The immune reaction against the Influenza virus starts with a huge neutrophil infiltration in lung parenchyma followed by effector T lymphocytes that then migrate to the airway space. The neutrophilic phase is known to be associated with the clinical symptoms and the exacerbation of inflammation might cause the higher rates of mortality in pandemic flu. Our aim was to establish a murine model of Influenza A infection, focusing in the inflammatory response. **Methods:** C57 BL6J male mice were infected with the mouse adapted Influenza A strain – H1N1 WSN/33 – intranasally with a lethal ( $10^6$  PFU) and sub lethal inoculums ( $10^4$  PFU), or PBS only (Mock). Weight loss and lethality were accompanied from the first day of infection.  $10^4$  PFU infected mice were sacrificed after 1, 4, 7 and 10 days of infection;  $10^6$  PFU infected mice were sacrificed at 1, 3 and 5 days after the infection (d.a.i.). Blood were collected to hematocrit analysis; bronchoalveolar lavage (BAL) was performed to evaluate cell influx to airways, chemokine levels by ELISA and total protein amounts; lungs were collected to evaluate neutrophil and macrophages amounts through MPO and NAG assays and chemokine and cytokine levels by ELISA. All experiments were proceeded under approval of UFMG Ethics Committee (203/08). **Results:**  $10^4$  PFU infected mice started to lose weight at day 4 after infection and loses were higher at 7 and 10 d.a.i. Hematocrit levels increased in the same manner as weight loss, starting at day 4. BAL neutrophils peaked at day 4 with progressive reduction observed at day 7 and returning to basal levels at day 10. The mononuclears peak was at day 7. Increased levels of total protein in BAL were found at days 7 and 10. MPO levels were higher at day 4 and 7 and NAG levels were higher from day 4 to 10. Neutrophil attractive chemokines KC and MIP-2 had different profiles. While MIP-2 levels were elevated from day 1 to 7 in BAL and lungs, KC had an increase just at day 4. Monocyte chemoattract protein 1 (MCP-1) were higher from day 4 to 10. The lethal inoculum  $10^6$  PFU seems to aggravate the disease and enhances neutrophil influx. Most of the parameters were already higher in the first day of infection – hematocrit, neutrophil influx to BAL and lungs, KC levels in BAL and lungs and MCP-1 levels in lungs. The pro-inflammatory cytokine IL-6 was in higher levels in serum in the first day of infection. **Discussion:** In this study we established the kinetics of the inflammatory parameters that follows the Influenza A infection in a murine model. We could make a correlation between weight loss and hematocrit increase, an indicative of plasma extravasation, one of the cardinal signals of inflammation. It was also shown the indirect systemic damage caused by the great neutrophil influx. Strategies to decrease neutrophil influx have been used in our lab with the aim to achieve a reduction of mortality associated with the disease. **Financial support:** CAPES and CNPq

#### 04.120

Inhibition of the vascular permeability for new thiazolidine derivative (LPSF/GQ-130). Santos IBV, Lima SMA, Galvão CM, Botelho SPS, Galdino SL, Lima MCA, Silva TG, Pitta IR UFPE - Antibióticos

**Introduction:** Inflammation is the reaction of tissue to an aggression, characterized by the reaction of blood vessels, leading to the accumulation of fluid and leukocytes in the site attacked. These characteristics have the following objectives: locate, isolate and destroy the harmful agents. This is a complex process, involving a wide variety of cells of the immune system, including chemical mediators produced by these cells. These mediators are related to phenomena such as pain, migration of neutrophils, peripheral vasodilation and increased body temperature. Various treatments to combat the inflammatory process of diseases such as rheumatoid arthritis, rheumatoid fever, osteoarthritis, lupus erythematosus, among others, have been widely studied in order to develop new molecules able to reduce inflammation. The thiazolidine substances are widely studied because of their important pharmacological properties, such as anti-inflammatory, anticonvulsant, hypoglycemic, anticancer, antifungal, antimicrobial and others. Thus, the purpose of this study was to evaluate the anti-inflammatory activity of thiazolidine derivative LPSF/GQ-130, to contribute to the discovery and development of new prototype candidates for anti-inflammatory. **Method:** The anti-inflammatory activity was evaluated by testing of vascular permeability induced by acetic acid in mice, which aims to investigate the release of mediators of the 1st phase of inflammation and consequent formation of edema. The protocol used was approved by the Ethics Committee in Animal Experimentation (EAEC), Federal University of Pernambuco (no. 23076, 0040.13 / 2008-35). Thus, initially the adult albino mice of both sexes were left in water fast and with the desire for a period of 4 hours. After this period, animals were pretreated with LPSF/GQ-130 at a dose of 30 $\mu$ mol/kg orally. After 1 hour of the injection treatment was performed in the retro-orbital plexus (0.2 mL / mouse) of the Evans blue dye 1%, then administered to the acetic acid solution 1% in the peritoneum of animals, to cause peritonitis acute. Each animal received 0.5 mL. After an interval of 30 minutes, the mice were sacrificed under anesthesia with xylazine/ ketamine. As a net drag was used 0.9% NaCl solution (2mL/cavity). The peritoneal exudate was collected and centrifuged at 200  $\times$  g for 10 min. The absorbance of the supernatant was read to 610 nm using the ELISA (Thermo plate). The indomethacin (28 $\mu$ mol/kg) was used as the reference drug. **Results and Discussion:** After the test were obtained means and standard errors of the absorbance of the experimental groups, control group - 0.337  $\pm$  0.06; indomethacin - 0.206  $\pm$  0.06; LPSF/GQ-130 - 0.246  $\pm$  0.03. The LPSF/GQ-130 was efficient, as well as indomethacin, to inhibit vascular permeability when compared to the control group. Thus, opportunities arise to continue with more specific anti-inflammatory tests. **Bibliography:** Gayathri, B. *Int. Immunopharmacol.* v.7, 473. 2007. Whittle, B.A. *British Journal of Pharmacology* v.22, 246.1964. **Financial support:** UFPE / CNPq



#### 04.121

Therapeutic attenuation of silica-induced pulmonary fibrosis by immunotoxin chimeric molecule IL-13PE. Ferreira TPT<sup>1</sup>, Arantes ACS de<sup>1</sup>, Rocco PRM<sup>2</sup>, Puri R<sup>4</sup>, Hogaboam C<sup>5</sup>, Martins MA<sup>1</sup>, Silva PMR<sup>1</sup> <sup>1</sup>FIOCRUZ - Fisiologia e Farmacodinâmica, <sup>2</sup>UFRJ - Investigação Pulmonar, <sup>4</sup>CBER, <sup>5</sup>UMICH - Patologia

**Introduction:** IL-13 appears to contribute to the development of pulmonary fibrosis, presumably due to its ability to induce irreversibly fibroblasts activation, thus representing an attractive target in the case of fibrotic diseases. Inhalation of crystalline silica particles leads to silicosis, a chronic lung disease characterized by granulomatous inflammation and fibronodular response. Since there is no effective treatment that can effectively abolish the risk of the disease, in this study we examined the curative effect of the fusion protein IL-13PE on the experimental model of silica-induced pulmonary in mice. **Methods:** Swiss-Webster mice were nasally instilled with silica particles (10 mg/50µL) and IL-13PE was administered intranasally, every other day, starting on day 21 up to day 28 post silica provocation. Twenty four hours later, pulmonary function (resistance and elastance) and airways hyperreactivity to aerosolization with the bronchoconstrictor agent methacholine were evaluated by invasive whole body plethysmography (Buxco system). All animals were killed and whole lung samples were prepared for biochemical and histological analyses. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (license 0213-4). **Results:** We showed that intranasal administration of IL-13PE had a significant therapeutic effect on the increased pulmonary resistance and elastance as well as on the airways hyperreactivity to methacholine in silicotic mice. IL-13PE reduced the inflammatory infiltrate, fibrotic response and granuloma formation. Coherently, the expression of extracellular matrix components including collagen, laminin and fibronectin was sensitive to IL-13PE. In parallel, the levels of inflammatory and profibrotic chemokines (MIP-2, JE, MIP-1a, TARC and MDC) and cytokines (TGF- $\beta$  and TNF- $\alpha$ ) were clearly suppressed in the lung tissue of silica-challenged mice treated with IL-13PE. In addition, by means of immunohistochemical technique, we evidenced a less intense labeling for F4/80 and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) which indicated a decrease in the presence of macrophages and myofibroblasts in the lung tissue, respectively. Also, a reduction of the IL-13a2 receptor-positive cells was noted in silicotic mice under condition of IL-13PE treatment. **Conclusion:** Our findings show that treatment with the immunotoxin IL-13PE effectively inhibited silica-induced lung inflammation and fibrosis, indicating that it seems to constitute a promising therapeutic approach in the case of chronic inflammatory lung diseases such as silicosis. **Financial Support:** FIOCRUZ, CNPq, FAPERJ (Brazil), Global REACH Michigan University (USA), UNESCO (France).

#### 04.122

Periodontal disease alters saliva composition in rats. Maia-Dantas A, Martins Porto R, Teixeira SA, Campi P, Costa SKP, Muscará MN USP - Farmacologia

**Introduction:** Periodontitis is the most prevalent chronic disease in the human dentition and is mainly related to both the presence of bacteria associations and their products, and tissue destruction caused by the host defense cells. Considering that saliva is an important entrance barrier to oral microorganisms, and periodontitis is also associated to systemic alterations, the aim of the present work was to study the early effects of ligature-induced periodontitis in rats on saliva secretion rate and composition.

**Methods:** Male Wistar rats (200-220 g) were anesthetized by the intraperitoneal administration of ketamine (80 mg/kg) and xylazine (20 mg/kg), and a cotton ligature was placed around the right mandibular first molar. A simulated procedure was performed in the Sham group. Three days after the disease induction, salivation was stimulated by the administration of the muscarinic agonist pilocarpine (1 mg/kg, i.p.; Allergan®); salivation rate was measured 15 minutes after pilocarpine injection, and saliva and blood samples were collected for analysis of amylase activity, calcium and protein concentrations. These procedures were approved by our institutional Ethics Committee for Animal Experimentation (protocol 020, on page 29, book 2 ). **Results:** No statistical differences were observed between the experimental groups in terms of salivation rate ( $115.7 \pm 12.8$  vs.  $100 \pm 12.4$  % of sham). In comparison with the sham group, serum amylase concentration was augmented in the periodontitis group ( $108 \pm 7$  vs.  $147 \pm 20$  mU/mL), in addition to the salivary concentration and production (calculated as concentration X salivation rate) of calcium (concentration:  $4.3 \pm 0.2$  vs.  $3.2 \pm 0.3$  mg/dL,  $p < 0.01$ ; production:  $0.29 \pm 0.03$  vs.  $0.19 \pm 0.03$  mg/min,  $p < 0.05$ , respectively), amylase (concentration:  $2330 \pm 158$  vs.  $2068 \pm 117$  mU/mL; production:  $120 \pm 14$  vs.  $67 \pm 9$  mU/min,  $p < 0.01$ ) and total proteins (concentration:  $1543 \pm 83$  vs.  $1125 \pm 143$  pg/mL,  $p < 0.01$ ; production:  $70 \pm 5$  vs.  $34 \pm 4$  pg/min,  $p < 0.001$ ). However, the specific activity of salivary amylase (relative to the total protein contents) was lower in the periodontitis group ( $1.5 \pm 0.1$  vs.  $2.1 \pm 0.2$  mU/mg protein,  $p < 0.01$ ). **Discussion:** These results demonstrate that salivary glands are another important target of periodontitis; the physiological mechanisms affected for each of the measured salivary components and the mediators involved are under current investigation. Financial support: CNPq, Capes, FAPESP

#### 04.123

Bradykinin activates human eosinophils: role of endogenous PGE<sub>2</sub> in induction of lipid body biogenesis. Bakker-Abreu I<sup>1</sup>, Ferreira-Souza L<sup>1</sup>, LUNA TGS<sup>1</sup>, Mesquita-Santos FP<sup>2</sup>, Bozza PT<sup>2</sup>, Diaz BL<sup>1</sup>, Scharfstein J<sup>1</sup>, Bandeira-Melo C<sup>1</sup> <sup>1</sup>IBCCF-UFRJ, <sup>2</sup>FIOCRUZ - Fisiologia e Farmacodinâmica, <sup>3</sup>IBCCF-UFRJ - Imunobiologia

**Introduction:** Eosinophils are subtle regulators of immune responses that massively infiltrate and affect tissues during chronic inflammations, such as asthma and rhinitis, conditions frequently associated with increased kinin levels. Here, we studied whether bradykinin (BK) is able to activate eosinophils by eliciting lipid body (LB) biogenesis and eicosanoid-synthesizing function. **Methods:** Human eosinophils were isolated from peripheral blood of volunteers with a negative selection kit (StemCell Technologies; human studies approved by 052/09 CEP UFRJ/HUCFF). Cells were stimulated for 4 min (shape change assay) or 1 h (all other assays), 37 °C with physiological (1 – 100 nM) BK concentrations. Eosinophil shape change was analyzed by flow cytometry, LB biogenesis by osmium staining, and PGE<sub>2</sub> levels by EIA. **Results:** First, we show that BK (10 nM) did not induce eosinophil shape change – an indicative of cell kinesis. On the other hand, BK (1 – 100 nM) was able to selectively induce LB biogenesis in eosinophils, but not in neutrophils, whereas arachidonic acid and PAF induced LB formation in both cell types. Eosinophil LB biogenesis induced by BK (10 nM) was fully dependent on bradykinin type 2 receptor (B<sub>2</sub>R) activation. This phenomenon was partially sensitive to pertussis toxin, and involved participation of PKA and cAMP. BK-induced LB biogenesis appears to be mediated by endogenous prostanoids, since it was reduced by cyclooxygenase type 1 and 2 inhibitors. Consistent with these findings, eosinophils stimulated by BK were able to synthesize and secrete prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). Since PGE<sub>2</sub> (1 nM) itself was also capable of eliciting LB biogenesis when applied in physiological concentrations, our data suggest that B<sub>2</sub>R activates an autocrine/paracrine PGE<sub>2</sub> loop initiated by BK. **Discussion:** Our data clearly show that BK is able to elicit specific eosinophil responses through mechanisms involving (1) direct interaction with B<sub>2</sub>R expressed on these cells; (2) stimulation of cAMP/PKA-driven signaling pathways; and (3) an autocrine/paracrine loop mediated by endogenous PGE<sub>2</sub>. **Financial support:** CAPES, CNPq, FAPERJ.

#### 04.125

Estudo do mecanismo de ação anti-inflamatória e anti-hipernociceptiva de LASSBio-1135 e LASSBio-1141: papel da MAPK p38 e dos receptores de adenosina. Lima CKF<sup>1</sup>, Lacerda RB<sup>1</sup>, Fraga CAM<sup>1</sup>, Quintas LEM<sup>2</sup>, Barreiro EJ<sup>1</sup>, Miranda ALP<sup>1</sup> - <sup>1</sup>FF-LASSBio-UFRJ - Fármacos, <sup>2</sup>UFRJ - Farmacologia Básica e Clínica

**Introdução:** Os derivados imidazopiridínicos LASSBio-1135 e LASSBio-1141 apresentaram atividades anti-inflamatória e anti-hipernociceptiva em modelos de triagem farmacológica e foram selecionados para investigação de seus mecanismos de ação. Estes foram planejados para serem inibidores duais de MAPK p38 e PGHS-2, dois alvos terapêuticos importantes do processo inflamatório. Os receptores de adenosina (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> e A<sub>3</sub>) são expressos em células do sistema imune e exercem papel fundamental no controle da resposta inflamatória. A semelhança estrutural dos derivados imidazo piridínicos com substâncias moduladoras de receptores de adenosina despertou o interesse na investigação do papel destes no mecanismo de ação de LASSBio-1135 e LASSBio-1141. **Métodos:** A atividade inibitória de LASSBio-1141 sobre a migração de neutrófilos foi avaliada no modelo de atividade mieloperoxidase (MPO) (BRADLEY, P.P.; et al. *J. Invest. Dermatol.*, 78, 206, 1982) em patas de ratos estimuladas com carragenina 1% 6 horas após o estímulo, o composto foi administrado na dose de 100mmol/kg por v.o. Atividade sobre receptores de adenosina foi avaliada no modelo de agregação plaquetária em plasma rico de coelhos estimulados com colágeno (5mg), os compostos foram avaliados na concentração de 100mM assim como o DDA (G.V. R. BORN & M. J CROSS. *J. Physiol.*, 168, 178, 1963). A atividade sobre PGHS-2 foi avaliada no modelo de sangue humano total estimulado com LPS por 6h seguido pela dosagem de TXB<sub>2</sub> (PATRIGNANI, P. et al. *J. Pharmacol. Exp. Ther.*, 271, 1705 1994) Os protocolos experimentais foram submetidos a CEUA-UFRJ e aprovados aguardando apenas a confirmação do número de protocolo. **Resultados e Discussão:** O composto LASSBio-1141 foi avaliado no modelo de edema de pata de rato induzido por carragenina 1% e apresentou DE<sub>50</sub> igual a 14,33µmol/kg e quanto a atividade anti-hipernociceptiva no modelo de hipernocicepção térmica induzida por capsaicina LASSBio-1135 mostrou-se mais eficaz do que LASSBio-1141 (91% e 51%, respectivamente, de inibição) (40° Congresso SBFTE, 2008). LASSBio-1141 inibiu em 59,8% (n=4, p<0,05) o infiltrado de neutrófilos no local da inflamação. Quanto a atividade sobre a PGHS-2 LASSBio-1135 apresentou IC<sub>50</sub>= 18,49µM (n=3) com eficácia máxima de 42,20% de inibição da produção de TXB<sub>2</sub> sem apresentar atividade sobre PGHS-1 até 100µM, LASSBio-1141 não apresentou atividade sobre ambas isoformas. A atividade sobre a MAPK p38 está sendo avaliada e resultados preliminares de Western Blot em macrófagos peritoneais murinos estimulados com LPS mostram que LASSBio-1135 seria capaz de inibir a fosforilação da MAPK p38 enquanto que LASSBio-1141 não parece interferir neste processo. A atividade anti edematogênica de LASSBio-1141 foi revertida pela teofilina, um antagonista não específico de receptores de adenosina (SBFTE, 2008). Neste estudo empregamos o modelo de agregação plaquetária em plasma de coelho estimulado com colágeno, já que plaquetas apresentam apenas um subtipo deste receptor, o A<sub>2A</sub>. LASSBio-1135 e LASSBio-1141 apresentaram atividade antiagregante plaquetária com 87,2% e 92,2% (n=4, p<0,05) de inibição, respectivamente. O efeito de LASSBio-1141 foi revertido pelo inibidor de adenilato ciclase, didesoxiadenosina (DDA), enquanto que o de LASSBio-1135 não foi alterado. LASSBio-1135 parece exercer seu efeito por inibição dual da MAPK p38 e da PGHS-2, enquanto LASSBio-1141 se comporta como um modulador de receptores de adenosina. **Apoio Financeiro:** PRONEX, CAPES, CNPq, FUJB e INCT.

#### 04.126

Comparison between topical and *per os* treatments from *Caryocar coriaceum* Wittm. fruit pulp fixed oil on murine inflammation. Saraiva RA<sup>1</sup>, Araruna MKA<sup>1</sup>, Menezes KDP<sup>1</sup>, Oliveira RC<sup>1</sup>, Leite GO<sup>1</sup>, Souza HHF<sup>1</sup>, Fernandes CN<sup>1</sup>, Costa JGM<sup>2</sup>, Campos AR<sup>3</sup>, Menezes IRA<sup>1</sup> <sup>1</sup>URCA - Química Biológica, <sup>2</sup>URCA - Ciências Biológicas e da Saúde, <sup>3</sup>UNIFOR

**Introduction:** Ethnobotanical studies show the application of *pequi* (*Caryocar coriaceum* Wittm.) oil on respiratory troubles and inflammation in Brazilian Northeastern. So, we aimed to evaluate and compare the possibly anti-inflammatory effect from this natural product by topical and *per os* treatments in pre-clinical bioassays. **Methods:** The extraction of *C. coriaceum* pulp fruit fixed oil (CCPFO) was obtained by hot-extraction method, using Soxhlet apparatus and ethyl-acetate as solvent. The topical anti-inflammatory effect was evaluated by croton-oil-induced ear edema in *Swiss* mice (n=6/group, 20-30 g, both sexes), pre-treated topically with 20  $\mu$ L of saline solution in Tween 80 2% (control), dexamethasone 0,08 mg/ear, CCPFO in Tween 80 2% in 1, 2, 4 and 8 mg/ear concentrations and CCPFO pure (20  $\mu$ L). The inflammation percentage was calculated from the weight of the 6 mm discs obtained from the ears after 6 h of the croton-oil application. The *per os* treatment anti-inflammatory effect was known by the carrageenan-induced paw edema in *Wistar* rats (n=6/group, 185-280g, both sexes), pre-treated orally with saline in Tween 80 2% (control), indomethacine 10 mg/kg or CCPFO in 200 and 400 mg/kg concentrations. The volume of the right paw edema was evaluated by pletismometry at time 0 (before carrageenan administration) and 1, 2, 3 and 4 h after carrageenan administration. All the procedures were submitted and approved by Research Ethics Committee of Faculty of Medicine of Juazeiro, under number 2009-0218. Mean  $\pm$  SEM of inflammation percentages were calculated and the results were submitted to ANOVA and compared to Student-Newmann-Keuls test at P < 0.05. **Results and Discussion:** There was no significant difference between the volume of paw edema from CCPFO-treated and control group (P>0,05). Only the indomethacine decreased the inflammation compared to control group, showing the best reduction percentages at time 3 h (16.3 $\pm$ 1.5% vs 51.0 $\pm$ 7.0%) and 4 h (20.5 $\pm$ 6.7% vs 53.8 $\pm$ 4.9%). In relation to ear edema, compared to control (167.3 $\pm$ 9.6%), the topical treatment of dexamethasone (24,50 $\pm$ 2.7%), CCPFO 8 mg/ear (119.7 $\pm$ 9.5%) and CCPFO pure (113.8 $\pm$ 14.4%) showed significant decrease (p<0.05). These results suggest that CCPFO has local action. More studies should be conducted in order to understand its local mechanism of action. **Support:** Funcap, CNPq.

#### 04.127

Evaluation of anti-inflammatory effect of LQFM002, a new candidate of prototype drugs derived from pyrazole. Lino RC<sup>1</sup>, Gomes, M. N.<sup>2</sup>, Nascimento MVM<sup>1</sup>, Galdino PM<sup>1</sup>, Florentino FI<sup>1</sup>, Menegatti R<sup>2</sup>, Costa EA<sup>1</sup> <sup>1</sup>ICB-UFG - Farmacologia de Produtos Naturais, <sup>2</sup>UFG - Química Farmacêutica Medicinal-FF-UFG

**Introduction:** In the search for new anti-inflammatory agents that have fewer side effects, new nucleus pyrazole derivatives have been highlighted as possible prototypes of drugs (Eur Méd. Chem. 40:850, 2005). The aim of this work was to evaluate the anti-inflammatory activity of the pyrazole derivative LQFM002 obtained by the Laboratory of Pharmaceutical Chemistry-FF-UFG. **Methods:** To evaluate the anti-inflammatory activity of LQFM002 were used the following methodologies: acetic acid-induced writhing (1.2% v/v) (Koster et al., 1959) and carrageenan-induced pleurisy tests (Vinegar et al., 1973) in mice. The inhibitory activity of phospholipase A2 was evaluated *in vitro* using the snake venom as enzyme source and egg yolk as substrate source, the halo formed was measured and used as indicative of PLA<sub>2</sub> enzymatic activity (GUTIERREZ et al., 1988; FORTES-DIAS et al., 1999). All the experimental with animals (n = 8, per group) were carried out in accordance with the Research Ethics Committee of UFG (Prot. No. 104/08). **Results:** LQFM002 (50 mg/kg, p.o.) reduced the writhes at 14.25 ± 3.89%, when compared to control group (DMSO 3%, 10 mL/kg p.o.): 87.60 ± 2.87. On Carrageenan-induced pleurisy, the previous treatments with LQFM002 (25, 50 and 100 mg/kg, p.o.) reduced the number of leukocytes migrated to the pleural cavity at 37.5 ± 10.78%, 64.10 ± 8.12 and 68.7% ± 2.65%, respectively, compared to control group (DMSO 3%, 10 mL/kg p.o.): 6.4 ± 2.0 x 10<sup>6</sup> leukocytes/mL, dexamethasone (2 mg/kg, p.o.), used as positive control, reduced the migration at 82.0 ± 5.15%. The pretreatment with LQFM002 (25, 50 and 100 mg/kg, p.o.) also reduced the Evan's blue concentration in pleural exudates at 30.11 ± 6.40%, 34.28 ± 5.74% and 40.1 ± 6.40%, respectively, when compared to control (DMSO 3%, 10 mL/kg p.o.): 4.5 ± 0.65 g/mL, dexamethasone (2 mg/kg) reduced the Evan's blue concentration at 57.59 ± 3.09%. At the concentrations of 250, 500, 1000, 2000 µg/mL, LQFM002 reduced the halo area formed by phospholipids hydrolysis at 14.43 ± 6.28%, 16.28 ± 2.45%, 23.61 ± 2.62%, 37.06 ± 3.25%, respectively, compared to control (3% DMSO) 518.33 ± 6.86 mm<sup>2</sup>. The phospholipase A2 inhibition could explain the inhibition in the leukocyte migration and also the anti-inflammatory effects, because there are several phospholipase A2 inhibitors in the literature that shown anti-inflammatory activity, like monoalide, p-bromo bromofenacil, propanosulfônico acid, among others. **Conclusion:** LQFM002, a pyrazole derivative, showed analgesic and anti-inflammatory activities. The PLA2 activity inhibition seen on *in vitro* methodology could explain the *in vivo* observed effects. **Financial Support:** CAPES, CNPQ and FUNAPE/UFG

#### 04.128

Human eosinophils synthesize PGD<sub>2</sub>: autocrine function in eosinophil activation. Luna TGS<sup>1</sup>, Magalhães KG<sup>1</sup>, Bakker-Abreu I<sup>1</sup>, Mesquita-Santos FP<sup>2</sup>, Weller PF<sup>3</sup>, Bozza PT<sup>2</sup>, Diaz BL<sup>1</sup>, Bandeira-Melo C<sup>1</sup> <sup>1</sup>IBCCF-UFRJ, <sup>2</sup>FIOCRUZ - Fisiologia e Farmacodinâmica, <sup>3</sup>Harvard - Medicine

**Introduction and Objectives:** Prostaglandin (PG) D<sub>2</sub> is a key mediator of allergic inflammatory diseases, like asthma. This eicosanoid is a cyclooxygenase product synthesized mainly by mast cells that constitutively express high levels of the terminal enzyme involved in PGD<sub>2</sub> synthesis, the hematopoietic PGD synthase (PGDS). Recently, it was shown that eosinophils infiltrating nasal polyps express this enzyme (Arch Otolaryngol Head Neck Surg. 133(7):693-700, 2007). Therefore, we hypothesized that eosinophils may be able to synthesize PGD<sub>2</sub> under proper stimulation. **Methods and Results:** First, we detected the expression of PGDS in human eosinophils isolated from peripheral blood of healthy volunteers (licensed by Committee of Human Ethics/UFRJ – HUCFF 052/09) by PCR and Western blotting. Increased expression of PGDS was observed in eosinophils stimulated with arachidonic acid (AA; 10 μM). Levels of PGD<sub>2</sub> found in supernatants of properly stimulated human eosinophils were quantified by EIA. First, we found that human eosinophils stimulated for 15 min with the calcium ionophore A23187 (0,5 – 5 μM) produce and release PGD<sub>2</sub> in a dose-dependent manner (n = 3). Of note, PGD<sub>2</sub> was not detectable in supernatants of non-stimulated cells. Physiological stimuli, such as AA (10 μM) and human eotaxin (100 ng/ ml) were also able to induce PGD<sub>2</sub> production by human eosinophils. Such PGD<sub>2</sub> production was inhibited by HQL-79 – a specific inhibitor of PGD synthase (n=3). In addition, the ability of both AA and eotaxin to trigger eosinophil activation was completely inhibited by pre-treatment with HQL-79 (10 μM) - a specific inhibitor of PGD synthase. Specifically, HQL-79 decreased both acute eosinophil shape change (analyzed by flow cytometry within 4 min of stimulation, n=3) induced by eotaxin, and lipid body biogenesis (viewed by light microscopy of osmium-stained eosinophils within 1 h, n = 3) in human eosinophils stimulated with eotaxin, AA or bradykinin (BK), thus indicating an autocrine effect of endogenous PGD<sub>2</sub> derived from human eosinophils. **Discussion and Conclusion:** Altogether, our results reveal that human eosinophils are indeed able to synthesize PGD<sub>2</sub>, which functions as an autocrine signal for eosinophil activation induced by eotaxin, AA and BK. **Financial Support:** FIOCRUZ, CAPES, CNPq and FAPERJ.

#### 04.129

Salivary gland extract (SGE) of *Lutzomyia longipalpis* and LJM 111 protein, component from SGE, inhibited inflammatory parameters on experimental arthritis model. Grespan R<sup>1</sup>, Lemos HP<sup>1</sup>, Oliveira CJF<sup>2</sup>, Valenzuela J<sup>3</sup>, Teixeira C<sup>3</sup>, Cunha FQ<sup>1</sup> <sup>1</sup>USP - Pharmacology, <sup>2</sup>USP - Immunology, <sup>3</sup>NIH - Malaria and Vector Research

**Introduction:** Several studies have pointed out the immunomodulatory properties of the Salivary Gland Extract (SGE) from *Lutzomyia longipalpis*. In this context, we aimed to evaluate the role of SGE and such component (s) in the inflammatory parameters observed in antigen-induced arthritis (AIA) model. **Methods:** Migration assay was used to evaluate neutrophil recruitment to peritoneal from OVA-challenged immunized mice treated or not with plasmids containing genes that codify for proteins from SGE. The antiartritic activities of SGE and its component LJM 111 protein were investigated on AIA by measuring the mechanical hypernociception using an electronic pressure meter, the neutrophil migration into synovial cavity and the levels of IL-17, TNF-alpha and IFN-gamma released by lymph nodes cells stimulated with mBSA using enzyme-linked immunosorbent assay (ELISA). Additionally, it was evaluated the effect of SGE and LJM 111 protein on co-stimulatory molecules expression (MHC-II and CD-86) on bone marrow dendritic cells (BMDCs), by flow cytometry and TNF-alfa and IL-10 production, by ELISA. The means of different treatments were compared by ANOVA, followed by Bonferroni's *t* test. The protocol number 004/2009 regarding this study was approved by the ethical commission of ethics in animal research (CETEA). **Results:** Plasmid containing gene that codify for protein LJM 111, but not for others proteins, inhibited neutrophil migration to peritoneal cavity in immunized mice. Both SGE and the LJM 111 protein inhibited neutrophil migration and pain sensitivity in AIA. In addition, SGE and LJM 111 reduced IL-17, TNF-alpha and IFN-gamma levels when compared with cells obtained from lymph nodes of immunized mice and stimulated with mBSA without treatment. Thereafter, we sought to investigate how SGE and LJM 111 protein was reducing the cytokine release. Thus, we observed that SGE and LJM 111 reduced the co-stimulatory molecules expression (MHC-II and CD-86) from BMDCs stimulated with LPS when compared without treatment. Besides that, SGE and LJM 111 reduced TNF- $\alpha$  and increased IL-10 levels in supernatant of cultured BMDC stimulated with LPS. **DISCUSSION:** These results suggests that one of the possible mechanisms used by SGE and LJM-111 to inhibit the inflammatory response induced by mBSA could be by the modulation of DC function and maturation. These findings showed that the SGE and LJM 111 protein reduced inflammatory parameters in experimental arthritis model. These results together suggest that peptides from this protein are viewed as potential therapeutic immunointervention in the rheumatoid arthritis. **Supported by:** FAPESP, FAEPA.



#### 04.130

TNF induces iNOS expression by pineal cells through a mechanism dependent of NFkB. Sousa CEC, Fernandes PACM, Tamura EK, Petrilli CL, Markus RP IB-USP - Fisiologia

**Introduction:** Although the well known chronobiological function of melatonin, it has also been implicated in the modulation of several immune processes. Our group showed that melatonin alters the inflammatory response by impairing immunocompetent cells migration to the endothelial layer (Lotufo, *Eur J Pharmacol*; 430: 351, 2001) and inhibiting the eNOS in endothelial cells (Tamura, *J Pineal Res*; 41: 267, 2006). On the other hand mediators of inflammation like cytokines and glucocorticoids can modulate pineal function, leading to the inhibition (proinflammatory cytokines) or potentiation (glucocorticoids) of nocturnal melatonin synthesis. We have shown in a human acute inflammation model and in the rat pineal gland *in vitro* that TNF acts inhibiting melatonin production, notably decreasing the expression of the key enzyme arylalkylamine-N-acetyltransferase (AA-NAT) and the production of the melatonin precursor, N-acetylserotonin (NAS), by a mechanism that seems to be dependent on NF-kappaB (Fernandes, *J Pineal Res*, 41: 34, 2006). The exactly machinery by which TNF exerts its effect on the pineal function is unclear. Since cytokines and lipopolysaccharides (LPS) are known to induce iNOS expression in several tissues and cells type and that pineal gland produces NO in the presence of LPS (unpublished) we decided to use the expression of this inflammatory enzyme as a work model to follow the intracellular pathways activated by TNF. In the present work we aimed to investigate the NF-kappaB pathway on TNF-induced iNOS expression on cultured pinealocytes. **Methods:** Pinealocytes were obtained from pre-pubertal female Wistar rats. Animal procedures were performed according to approved institutional protocols (081/2008). Pineal glands were dissociated by trypsinization and cultured in DMEM medium in an 8-well culture plate ( $0.5-1 \times 10^5$  cells/ well). Cells were incubated in the presence of increasing concentrations of TNF (10– 80 ng/mL, for 2 hs). The NF-kappaB blocker, PDTC, (12.5 or 25 mM) was used 30 min before TNF (80 ng/mL) stimulation. Cells were fixed and incubated overnight with anti-iNOS TRITC-conjugated and the fluorescence was analyzed by confocal microscopy. The data are expressed as % relative to the non-stimulated group (100%). **Results:** TNF increased the pinealocyte iNOS fluorescence in a dose-dependent manner. The maximal response was obtained with the dose of 80 ng/mL ( $390.08 \pm 166.12\%$ ). PDTC completely inhibited the TNF-induced iNOS expression with both doses (80 ng/mL:  $210.87 \pm 58.29\%$ ; 80 ng/mL TNF + 12.5 mM PDTC:  $79.21 \pm 17.89\%$ ; 80 ng/mL TNF + 25 mM:  $80.9 \pm 11.9\%$ ). **Discussion:** The present work shows that TNF induces the expression of iNOS on pineal cells by a mechanism dependent on NF-kappaB activation. The NO produced by the iNOS may have a protective effect for the pineal gland during inflammatory processes. Taking into account that TNF-induced NF-kappaB activation also modulates AA-NAT expression it seems that this pathway is central on the TNF modulation of pineal metabolism. This work shows others mechanisms by which pineal responds to inflammatory modulators and amplifies our understanding about the role of this gland on the immune-pineal axis context. **Financial support:** FAPESP, CNPq, CAPES.

#### 04.131

Female sex hormones influence on the acute lung inflammation after an intestinal ischemic insult. Breithaupt-Faloppa AC, Ramos MMA, Vitoretti LB, Ligeiro de Oliveira AP, Sudo-Hayashi LS, Oliveira-Filho RM, Vargaftig BB, Tavares de Lima W ICB-USP Farmacologia

**Introduction:** Besides its effects on the reproductive tract, female sex hormones seem to be involved with the course of inflammatory process such as asthma. In addition, menopausal women upon hormonal therapy replacement experience more pronounced asthma deterioration. Acute lung inflammation is also observed during intestinal ischemic trauma and estradiol seems to be a protective agent in male rats upon intestinal ischemia reperfusion (I/R). Thus in this study we evaluated the influence of sex hormones on the acute lung inflammation caused by I/R. Studies regarding the involvement of nitric oxide on the lung inflammation due I/R were also carried out.

**Methods:** Anesthetized female Wistar rats were subjected to ovariectomy (OVx) and 7 days later, upon anesthesia, the superior mesenteric artery was occluded (45 min). Rats were euthanized 2 h after the intestinal reperfusion and lung neutrophil (MPO activity) recruitment and microvascular permeability (Evans blue dye extravasation) were investigated. Groups of rats were treated with 17 $\beta$ -estradiol (280 mg/kg) and/or progesterone (200 mg/kg) 24 hours before ischemia induction and treated or not with NOS inhibitors, aminoguanidine (50 mg/kg) or L-NAME (5 mg/kg) 1 hour prior ischemia establishment. **Results:** OVx-rats upon intestinal I/R significantly increased the MPO activity and microvascular permeability, compared with intact-I/R rats. Estradiol and/or progesterone treated OVx-I/R rats showed reduced lung microvascular permeability, whereas MPO activity was not affected by the treatments. L-arginine treatment of OVx rats before I/R, reduced the Evans blue dye extravasation but it did not change by iNOS inhibitor, aminoguanidine, treatment. Lung MPO activity was not affected by L-arginine, but was exacerbated by aminoguanidine. Aminoguanidine plus estradiol treated Ovx-rats prevented the exacerbated lung microvascular permeability.

**Conclusion:** The data allowed us to suggest that sex hormones mediate the systemic inflammatory response involving iNOS activation, that might play a differential role on lung neutrophil recruitment and increased vascular permeability followed the gut trauma. Financial support: CNPq and FAPESP. Ethics Committee number: 78/07/CEEA – ICB(USP)

#### 04.132

Lectin of *Lonchocarpus araripensis* seeds inhibits paw edema induced by carrageenan, sodium nitroprusside and phospholipase A<sub>2</sub> in mice. Pires AF<sup>1</sup>, Sousa PL<sup>1</sup>, Fernandes, DC<sup>1</sup>, Rodrigues NVFC<sup>1</sup>, Marques-Domingos GFO<sup>1</sup>, Marinho MM<sup>2</sup>, Pereira MG<sup>1</sup>, Cavada BS<sup>2</sup>, Alencar NMN de<sup>3</sup>, Assreuy AMS<sup>1</sup> <sup>1</sup>UECE - Ciências Biomédicas, <sup>2</sup>UFC - Bioquímica e Biologia Molecular, <sup>3</sup>UFC - Fisiologia e Farmacologia

**Introduction:** It has been demonstrated that the seed lectin from *Lonchocarpus sericeus* presents anti-inflammatory activity in the models of rat paw edema and peritonitis. The paw edema induced by carrageenan in animals is an excellent *in vivo* model to investigate anti-inflammatory drugs. This edema possesses a biphasic nature and is associated with prostaglandins and nitric oxide (NO) production. We evaluated the anti-inflammatory effect of *L. araripensis* lectin (LaL) on the mice paw edema induced by carrageenan, sodium nitroprusside, a NO donor, and phospholipase A<sub>2</sub> (PLA<sub>2</sub>), activate production of prostaglandins (PGs) and leukotrienes. **Methods:** Swiss mice (25 – 30g) were maintained and handled in accordance with the principles recommended by our Institutional Ethical Committee (UECE N<sup>o</sup>. 0559924-4). In order to evaluate the lectin anti-inflammatory effect, animals were pre-treated with LaL by intravenous route (i.v.) at 0.1, 1 and 10 mg/kg, 30 min before induction of paw edema by carrageenan (300 mg/paw; s.c.). The involvement of NO and prostaglandins in this effect was investigated via i.v. injection of the lectin most active dose, 30 min before sodium nitroprusside (10 mmol/kg; s.c.) or phospholipase A<sub>2</sub> (1mg/paw; s.c.), respectively. Positive controls received local application of carrageenan or sodium nitroprusside and negative controls, sterile saline (NaCl 0.9%; 50µL), L-NAME (10mg/kg; i.v.), an NO synthase inhibitor, or indomethacin, a ciclooxigenase inhibitor, 30 min before inflammatory stimuli. Paw edema was measured immediately before subcutaneous injection of stimuli (zero time) and at selected time intervals (1, 2, 3 and 4h) there after by hydroplethysmometry. Results were expressed as Mean ± S.E.M. of the increase in paw volume (mL) calculated by subtracting the basal volume measured at zero time or in arbitrary units (area under the time-course curve - AUC). Statistical comparisons were done using analysis of variance (ANOVA) followed by Bonferroni's test and p<0.05 was fixed as significant. **RESULTS AND DISCUSSION:** LaL, administered at 0.1, 1 and 10 mg/kg, dose-dependently inhibited the paw edema induced by carrageenan (AUC: 15.69 ± 1.58) at all times by 32% (AUC: 10.68 ± 1.37), 52% (AUC: 7.45 ± 0.36) and 69% (AUC: 4.84 ± 0.41), respectively. At 10 mg/kg (AUC: 1.84 ± 0.58) LaL also inhibited the edema induced by sodium nitroprusside (AUC: 10.76 ± 1.08) by 83%, at all times, similar to L-NAME (AUC: 2.36 ± 0.68). At the same dose, the lectin (AUC: 6.21 ± 0.86) reduced the PLA<sub>2</sub>-edematogenic effect (AUC: 12 ± 0.89) by 48% at all times, similar to indomethacin (AUC: 6.62 ± 1.11). The antiedematogenic effect of the seed lectin from *L. araripensis* involves NO and PGs, showing an important application of these molecules in studies of the physiopathological inflammatory processes. **SUPPORT:** CNPq, CAPES and FUNCAP

#### 04.133

Study of calamine, allantoin and silver sulfadiazine as skin wound healing drugs. Salomão ACS, Riveros BS, Batista SD, Silva CD<sup>1</sup>, Pietrovski EF, Otuki MF, Cabrini DA UFPR - Farmacologia

**Introduction:** The skin serves as a protective barrier, assuring the body homeostasis. Therefore, any fault must be rapidly and efficiently restored. Wound healing is a dynamic process that involves reactions and interactions among cells and inflammatory mediators. There are a great amount of studies concerning wound healing understanding and treatment, however a lack of information about clinically used drugs is still an issue, mainly about their efficacy. Thus the present study aimed to evaluate the wound healing effectiveness of calamine, allantoin and silver sulfadiazine in mice.

**Methods:** An excision wound was executed on the back of Swiss mice (n=5-8), removing all the skin layers using a *punch* (6 mm). The animals were topically treated (12/12h, 100 mg/g) during 15 days using calamine, allantoin, silver sulfadiazine, dexamethasone or Irujol®. The edge of the wound was daily measured and the data was analyzed using Image Tool 3.0. At the end of the experiment, animals were killed and samples were collected for hydroxyproline analysis, which can indicate the amount of collagen at the wound site. All animal procedures were approved by the Comissão de Ética em Experimentação Animal da UFPR (nº127). **Results:** Calamine and allantoin topical application reduced the wound area in 6.5% and 3.5%, respectively, on the 9<sup>th</sup> day of treatment. At the 11<sup>th</sup> day, the area of the calamine treated group was 0.5% smaller when compared to control group, while allantoin treatment presented only a reduction of 0.3% of the lesion. Both drugs calamine and allantoin promoted an increase in the collagen content of 30.7% and 108.7%, respectively. The topical application of silver sulfadiazine did not interfere on the collagen synthesis, and curiously extended the healing time in 3-4 days. Irujol® enhanced the collagen synthesis in 39% and reduced the time of healing comparable to calamine and allantoin, while dexamethasone decreased collagen synthesis in 20.2% and presented a 95% opened wound on the 15<sup>th</sup> day. **Discussion:** Our results suggest that the topical treatment using calamine and allantoin can improve the wound healing. However, silver sulfadiazine did not present healing efficacy in the studied model. These results present the importance of an extension for this study, encouraging for a deeper search about the mechanisms of action of these drugs. **Support:** CNPq

#### 04.134

Hydrogen peroxide induces neutrophil apoptosis via NFκB inhibition and activation of bAX and caspase-3. Lopes F<sup>1</sup>, Sousa LP<sup>2</sup>, Coelho FM<sup>3</sup>, Costa VV<sup>3</sup>, Teixeira MM<sup>3</sup>, Pinho V<sup>3</sup> <sup>1</sup>UFMG - Morfologia, <sup>2</sup>COLTEC-UFMG - Patologia Clínica, <sup>3</sup>UFMG - Bioquímica e Imunologia, <sup>4</sup>UFMG - Bioquímica e Imunologia

**Introduction:** During an inflammatory response, leukocytes produce and secrete great amount of substances, such as reactive oxygen species that contribute to the initiation, maintenance and resolution of inflammation. In the present study, we investigated the role of hydrogen peroxide in apoptosis and its impact on inflammatory resolution in an antigen induced arthritis. **Methods:** In the present study, antigen induced arthritis (AiA) was induced by the injection of methylated bovine serum albumin (mBSA) into the knee joint of pre-immunized mice. The role of hydrogen peroxide was investigated, by the administration of SOD (0.3mg/kg) and catalase (1.2mg/kg) 12 hours after the induction of AiA. Several techniques were used to evaluate neutrophils apoptosis, such as morphological aspects and activation of caspase 3 and bax by western blot (All procedures were approved by the Ethics Committee – CETEA 166/06 – UFMG). **Results:** The intra-articular administration of SOD 12 hours after the mBSA challenge increased the pool of hydrogen peroxide and reduced the amount of neutrophils in the joint cavity and periarticular tissue. The concomitant administration of catalase and SOD reversed the effect of SOD alone. The decrease of neutrophils was associated with an increase in the amount of apoptotic events in the joint cavity, inhibition of transcription factor (NFκB) translocated to the nucleus, and activation of Bax and Caspase-3. **Discussion:** The increase of hydrogen peroxide induced by the treatment with SOD can inhibit the NFκB transcription factor and activate important molecules such as Bax and Caspase-3 involved in the resolution of the inflammatory response. This work was sponsored by CNPq and FAPEMIG.

#### 04.135

CCR2 drives neutrophil migration into the joint and determines severity of experimental arthritis. Bianchini FJ<sup>1</sup>, Nascimento DCB<sup>2</sup>, Souto FO<sup>3</sup>, Pinto LG<sup>1</sup>, Alves-Filho JC<sup>1</sup>, Cunha FQ<sup>1</sup> <sup>1</sup>FMRP-USP - Pharmacology, <sup>2</sup>FMRP-USP - Biochemistry and Basic Immunology, <sup>3</sup>FMRP-USP - Surgery and Anatomy

**Introduction:** Blood neutrophils (PMN) trafficking during inflammation is a complex process which involves endothelial and PMN adhesion molecules and involvement of several types of chemotactic factors which may include lipids, complement activation products and especially CXC chemokines. In general, CXC chemokines, particularly macrophage inflammatory protein (MIP)-2 and KC, appear to be involved in mediating PMN influx into tissues, while CC chemokines interact predominantly with monocytes and macrophages. However, recent findings suggest that under certain inflammatory conditions, PMN may also directly interact with CC chemokines, such as MCP-1 and MIP-1 $\alpha$ . Here, we investigate the role of the immunization process on the modulation of CCR2 expression and responsiveness in neutrophil and the consequence of this regulation during an experimental model of autoimmune disease. **Methods:** WT and CCR2<sup>-/-</sup> mice were sensitized (s.c.) with 500  $\mu$ g of mBSA in a emulsion containing complete Freund's adjuvant. Booster injections of mBSA in incomplete Freund's adjuvant were given 7 and 14 d after the first injection. Arthritis was induced in the immunized mice 21 d after the initial injection by intra-articular injection of mBSA (10  $\mu$ g/cavity) or MCP-1 (50ng/cavity). Neutrophil migration was assessed 6 h after intra-articular challenges. Articular hypernociception was evaluated using an electronic version of the von Frey test. TNF- $\alpha$  determination in intra-articular 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. Neutrophil chemotaxis in response to MIP-2 (30ng/ml) or MCP-1/CCL2 (1ng/ml) was performed using a 48-well Boyden-modified microchamber. CCR2 expression in blood neutrophils was determined by immunofluorescence. This experiment was approved by Ethics Committee (n<sup>o</sup> 181/2008). **Results:** Results show that resting murine neutrophils do not express CCR2 receptor or respond to CCL2 chemokine. However, CCL2 was able to induce neutrophil chemotaxis *in vitro* and intra-articular migration *in vivo* in immunized mice. The response of neutrophil to CCL2 was blocked by treatment with CCR2 antagonist or by using CCR2<sup>-/-</sup> neutrophil. Interestingly, the intra-articular migration of neutrophil induced by mBSA was significantly reduced in CCR2<sup>-/-</sup> mice or by treatment with CCR2 antagonist. As a consequence, TNF-alpha production and articular hypernociception induced by intra-articular injection of mBSA in immunized mice was substantially reduced in the CCR2<sup>-/-</sup> mice. **Discussion:** Our findings suggest that the immunization process induces CCR2 expression and CCL2 responsiveness in murine neutrophils, and that this expression profile in neutrophils is involved in the detrimental infiltration of these cells in the joints. Therefore, we envisage that CCR2 blockage is a potential strategy for human rheumatoid arthritis treatment. **Financial Support:** FAPESP/CNPq/FAEPA

#### 04.136

The role of endogenous glucocorticoids and annexin-A1 on neutrophil mobilization from bone marrow to circulation. Cavalcanti DM<sup>1</sup>, Dalli J<sup>2</sup>, Perretti M<sup>2</sup>, Farsky S<sup>1</sup> <sup>1</sup>FCF - Clinical and Toxicological Analysis, <sup>2</sup>WHRI - Biochemical Pharmacology

**Introduction:** Using *in vivo* blockade of glucocorticoid receptor (GR) by treatment with RU 38486, we have shown that endogenous glucocorticoids (GE), through GR, physiologically control rolling behavior by acting on neutrophils, modulating the expression of L-selectin and firm adherence by modulating immunoglobulins endothelial adhesion molecules. The molecular mechanism induced by activated GR seems to be different in each cell, as NF Kappa B nuclear translocation was enhanced only in endothelial cells (Cavalcanti *et al.*, Br. J. Pharmacol., 2007). **Aim:** Now we evaluated the participation of annexin-1, protein induced by glucocorticoids, on neutrophil mobilizations from the bone marrow to peripheral compartment and L-selectin expression. **Methods:** Male Balb/c mice (20g) wild type (WT) or annexin-1 knockout (KO) were treated with synthetic ACTH (5ug,i.p) or vehicle (PBS, control) and employed for the following experiments: determination of bone marrow segmented cells or circulating neutrophils numbers; expression of L-selectin, on bone marrow or circulating leukocytes by flow cytometry. All the experiments were conducted according to Ethics Committee in Animal Experiments (CEEA) n° 20. **Results:** Annexin-1 KO animals present elevated numbers of neutrophils in the circulation (54% vs WT) and in the bone marrow compartment (46% vs WT). ACTH treatment decreased the number of granulocyte in the bone marrow (43% vs control) and enhanced the number of circulating neutrophils in WT animals (83% vs control). On the other hand, ACTH treatment did not evoke any alteration in number of cells in the bone marrow compartment and in the circulation. Annexin-1 KO animals present reduced (65% vs WT) and elevated (32% vs WT) expression of L-selectin in bone marrow and circulating cells, respectively; ACTH treatment reversed these effects. **Conclusion:** Annexin-1 is involved in the control of glucocorticoids on neutrophils mobilization from bone marrow to blood and control L-selectin expression on cells from both compartments. However, an additional control on L-selectin is exerted by ACTH or GE, independently of annexin-1. Financial Support: FAPESP (05/59753-1)

#### 04.137

Edematogenic activity of polysaccharidic extracts from *Caesalpinia ferrea* in rats. Pereira LP<sup>1</sup>, Sousa AAS, Martins PA, Assreuy AMS<sup>1</sup>, Pereira MG ISCB-UECE

**Introduction:** Plant extracts and its active principles have been used as therapeutic agents. *C. ferrea* Mart., Known as "pau-ferro" or "Jucá" is widely distributed in the North and Northeast of Brazil and popularly used for treatment of wounds and bruises, rheumatism, relief of chronic cough and asthma, enterocolitis, diarrhea, diabetes and as antipyretic. Some activities have being described for *C. ferrea* extracts, including anti-inflammatory, analgesic, anti-ulcer and anti-cancer. Studies have pointed plant polysaccharides as important biological tools and ideal therapeutic candidates with anti-inflammatory, immunomodulatory and antitumor properties. The objective was to evaluate the edematogenic action of polysaccharide extracts from bark, leaves and pods of *C. ferrea*. **Methods:** The dried parts were macerated (5 g), suspended in methanol (250 mL) and homogenized (2 h, 76 °C). The procedure was repeated (2x) and then filtered. Residue was suspended (250 mL, 0.1 M NaOH), mixed (2 h, 97 °C) and centrifuged (3500 rpm, 15 min.) - repeated (3x). Supernatants were pooled, neutralized (1 M HCl), precipitated (4 volumes of ethanol, 4 °C, 24 h) and centrifuged (3500 rpm, 15 min.). The pellet was dialyzed, centrifuged and the supernatant lyophilized (named total polysaccharides-TLP (Yoon, Thromb Res, 106, 51, 2002), with modifications. Wistar rats (150-200g) were handled in accordance with the principles established by the Ethic Committee of UECE (N° 0559924-4). Paw edema was induced by subcutaneous (s.c.) intraplantar injection of TPL at 0.01, 0.1 and 1 mg / kg. Paw edema was measured immediately before subcutaneous injection of stimuli (zero time) and at selected time intervals (30min., 1-8h) thereafter by hydroplethysmometry. The control group received sterile saline (NaCl 0.9%, 100 mL/100g, s.c). Results were expressed as Mean  $\pm$  S.E.M. (n = 6) of the increase in paw volume (ml) calculated by subtracting the basal volume measured at zero time or in arbitrary units (area under the time-course curve-AUC). Statistical comparisons were done using analysis of variance (ANOVA) followed by Bonferroni test and p<0.05 was fixed as significant. **Results and Discussion:** The total polysaccharide extracts from *C. ferrea*, except those obtained from pods at the 6<sup>th</sup>h, induced paw edema at all doses tested, starting at 30 min. after stimuli and maintained until the 7<sup>th</sup> h. In all groups treated with the TPL, the most potent pro-inflammatory activity was observed at 1mg/kg, but showing maximal effects at different times: the peak of activity produced by the bark extract occurred at 30 min (TPL: 0.75  $\pm$  0.05 mL *versus* saline: 0.45  $\pm$  0.04 mL) and those of pods and leaves at 120 min. (pod: 0.57  $\pm$  0.05 mL *versus* saline: 0.15  $\pm$  0.02 mL and leaves: 0.38  $\pm$  0.04 mL *versus* saline: 0.08  $\pm$  0.03 mL). The best TPL edematogenic effect was observed from the pod extracts (ASC: 150  $\pm$  18.8) at 1mg/kg, followed by shell (ASC: 139  $\pm$  11.2) and leaves (ASC: 118.75  $\pm$  9.40) compared to controls (AUC: 25.75  $\pm$  5.1). It was concluded that the extracts of bark, leaves and pods of *C. ferrea* present edematogenic action. The best dose dependent effect was observed for the TPL of the pod, which show potential interest to be exploited as immunostimulating molecules. **Supported:** FUNCAP, CNPq



#### 04.138

Anti-inflammatory effect of hydrogen sulphide (H<sub>2</sub>S) on ifosfamide-induced hemorrhagic cystitis in mice. Lima-Júnior RCP, Wong DVT, Cavalcante-Neto JS, Medeiros RP, Marques Neto RD, Leite LL, Nunes LG, Brito GAC, Souza MHL, Ribeiro RA UFC - Fisiologia e Farmacologia

**Introduction:** Ifosfamide (IFO) is an alkylating agent with a broad spectrum of antineoplastic activity. Hemorrhagic cystitis (HC) is a common complication that limits the use of IFO. Our group (Ribeiro et al, J Urol., 167(5):2229-34, 2002) has demonstrated the role of the gaseous inflammatory mediator nitric oxide (NO) in the pathogenesis of HC. H<sub>2</sub>S is another gaseous mediator. It is found in several mammalian tissues, where it is generated during cysteine metabolism by the activity of two enzymes: cystathionine  $\gamma$ -lyase (CSE) and cystathionine  $\beta$ -synthetase (CBS) (Moore et al., Trends Pharmacol Sci 24: 609-611, 2003). **Objective:** Once H<sub>2</sub>S and NO share a number of activities in pathological conditions we aimed to evaluate the role of H<sub>2</sub>S on IFO-induced hemorrhagic cystitis in mice. **Methods:** Swiss female mice (n=6) were pretreated by gavage with sterile saline or Lawessen's reagent (Law, a H<sub>2</sub>S donor, 9, 27 or 81mmol/kg) or L-cysteine (Cys, 25, 50 or 100 mg/kg) or DL-propargylglycine (PPG 100 mg/kg, p.o)+L-cysteine 30 minutes before and 6h after the induction of HC with an injection of IFO (400 mg/kg, i.p). The control group received only sterile saline. Twelve hours after IFO treatment, animals were sacrificed, and the bladders were removed by careful dissection and had the urinary content emptied. Bladder wet weight (BWW) was measured and expressed as g/25 g body weight. Vesical vascular permeability (VVP) was evaluated by the i.v injection of 2.5% Evans blue (25 mg/kg) via the retro orbital plexus 30 minutes before the animals were sacrificed. Bladders were then excised, dissected and placed into glass tubes containing a formamide solution (1 mL/bladder) at 56°C overnight to extract the stain. The total extracted dye was determined by measuring the absorbance change at 630 nm (ELISA device) and plotted in a standard curve. The results were reported as mg/mg tissue. Vesical edema and hemorrhage were assessed macro and microscopically through scores. Statistical analysis was performed with ANOVA/Student Newman Keul or Kruskal Wallis/Dunn as appropriate. Statistical significance was set at  $p < 0.05$ . (CEPA: Protocol 06/09). **Results:** IFO induced significant ( $p < .05$ ) increasing in BWW ( $59.21 \pm 4.05$ ), VVP ( $0.52 \pm 0.05$ ), edema (3 [2-3]) and hemorrhage related scores (3 [1-3]) in comparison to saline treated group ( $16.26 \pm 1.64$ ;  $0.009 \pm 0.001$ ; 0[0-0]; 0[0-0], respectively). LR (9, 27 and 81 mmol) and Cys (50 and 100 mg/kg) prevented in a significant manner ( $p < .05$ ) the increasing of BWW (43%, 35%, 39%, 23%, 54% respectively), VVP (49%, 69%, 78%, 43%, 49% respectively), edema (1[1-2], 1[0-2], 1[0-3], 1[1-2], 1[0-2]) and hemorrhage related scores (1[0-2], 1[0-3], 1[0-2], 2[0-3], 1[0-3]). Additionally, PPG significantly reversed ( $p < .05$ ) the protective effect of Cys (50 and 100 mg/kg) upon BWW ( $58.69 \pm 5.35$ ;  $67.63 \pm 3.84$ , respectively) and VVP ( $0.59 \pm 0.03$ ;  $0.056 \pm 0.03$ , respectively) when compared with Cys treated animals only. However, only Cys 100 mg/kg was effective ( $p < .05$ ) on preventing microscopic alterations (1[0-2]) in comparison with IFO (2[2-2]). **Conclusions:** H<sub>2</sub>S seems to exert a partial protective role on IFO-induced hemorrhagic cystitis. Further studies are required to determine the possible mechanisms involved. **Financial support:** CAPES/CNPq.

#### 04.139

Effects of hydroquinone on the functional activity of neutrophil. Pinedo F<sup>1</sup>, Hebeda CB<sup>1</sup>, Ferreira ZS<sup>2</sup>, Markus RP<sup>2</sup>, Almeida SR<sup>1</sup>, Farsky S<sup>1</sup> <sup>1</sup>USP - Clinical and Toxicological Analysis, <sup>2</sup>ICB-USP - Physiology

**Introduction:** Hydroquinone (HQ) is a phenolic compound obtained after benzene endogenous metabolism, and it is a component of cigarette, medicines, photographic developer, and in some foods and medicinal herbs (Lee *et al.*, 2007; Silva *et al.*, 2003, RDC n.º 79, 2000, for review see Nordland *et al.*, 2006). Our research group has been shown that *in vivo* exposure to HQ to rats impairs leukocyte migration into lung during allergic or non-specific inflammation (Ferreira *et al.*, 2007; Macedo *et al.*, 2007). This study evaluated the effect of HQ on the treatment of neutrophils, since these are the first cells to be recruited in an inflammatory process. **Methods:** Peritoneal neutrophil obtained four hours after local injection of oyster glycogen 1% were incubated with 5 or 10 µM, during 1 hour, and after incubated with or without LPS (5 µg/mL). Control cells were incubated with equivalent volumes of HQ and LPS vehicle. Cell viability was quantified by flow cytometer; NO<sub>2</sub><sup>-</sup> and cytokines in the supernatant of cells were measured by Griess reaction and ELISA, respectively; NF κB translocation into nucleus by gel shift assay and *Candida albicans* phagocytic and killing activities were evaluated by optical microscopy. **Results and discussion:** Results obtained showed that HQ treatments did not induce apoptosis or necrosis in neutrophil. HQ-treated neutrophils presented reduced basal NO production, even at basal or LPS-stimulated conditions; decreased TNF-α, IL-6 and IL-1β secretion, maybe dependent on NF-κB translocation into nucleus; and impaired *Candida albicans* phagocytic and killing indexes. Our results indicate that HQ inhibits pro-inflammatory effects of neutrophils. Financial Support: FAPESP (nº 07/56299-3) / Comitê de Ética (CEEA nº 169).

#### 04.140

Anti-inflammatory activity of *Adiantum* SP extract (Pteridaceae, Pteridophyta). Nonato FR<sup>1</sup>, Nogueira TM<sup>1</sup>, Barros TAA<sup>1</sup>, Lucchese AM<sup>2</sup>, Ribeiro-dos-Santos<sup>1</sup>, Soares MBP<sup>1</sup>, Villarreal CF<sup>1</sup> <sup>1</sup>CPqGM-FIOCRUZ - Engenharia Tecidual e Imunofarmacologia (LETI), <sup>2</sup>UEFS - Exatas

**Introduction:** The pteridophytes have been considered an excellent source of medicines for many years, as Dioscorides and Galeno stated in their manuscripts. Despite this, there are few books that include pteridophytes, and in most of all, these plants are called merely ferns or “avenca”. Relatively little work concerning its medicinal properties are being conducted in oriental countries, in which traditional medicine employs a plethora of plant species that are used by many individuals. Several species of *Adiantum* are employed in folk medicine in different countries. For instance, the plants from the Northeastern Brazil have been used as diuretic or against infections. In the present study, we evaluate the anti-inflammatory activity of the methanolic extract of *Adiantum* sp. (MEA) in mice. **Methods:** The anti-inflammatory activity of MEA was evaluated in male Swiss mice (20-25g) using the carrageenan-induced paw edema and the arachdonic acid-induced ear edema. After the carrageenan (200 µg) or arachdonic acid (2 mg) injection the edema was measured in plethysmometer (%) or pachymeter (mm), respectively. In addition, the paw and ear IL1-β levels were evaluated by ELISA. This study was submitted to the Institutional Animal Care and Use Committee - FIOCRUZ 26/2009-1. **Results:** The pre-administration of MEA (100 and 200 mg/kg) 4 hours before the carrageenan injection promotes a significant inhibition of paw edema ( $0,04 \pm 2,4$  and  $4,512 \pm 2,82$ , respectively) as well as in the levels of pro-inflammatory cytokine IL1-β ( $21,2 \pm 10,4$  and  $17,0 \pm 4,97$ , respectively) 2 hours post-injection. Similarly, the treatment with MEA (100 and 200 mg/kg) reduced the ear edema induced by arachdonic acid ( $0,14 \pm 0,02$  and  $0,066 \pm 0,02$ , respectively) 1 hour post-injection. In contrast, we observed an impaired production of IL1-β in mice treated only with the high dose of MEA (100 and 200mg/kg;  $651,88 \pm 79,2$  and  $387,55 \pm 48,22$ ) 1 hour ater arachdonic acid injection. **Discussion:** In summary, we have demonstrated that the methanolic extract of *Adiantum* sp showed a significant anti-inflammatory activity. In addition, experiments will be performed to evaluate the mechanisms involved with this anti-inflammatory activity as well as the isolation of bioactive molecules from the extract. Financial supports: FAPESB, FIOCRUZ, CNPq

#### 04.141

ATLa, an aspirin-triggered lipoxin A<sub>4</sub> synthetic analog, prevents the inflammatory and fibrotic effects of bleomycin-induced pulmonary fibrosis. Martins V<sup>1</sup>, Valença SS<sup>2</sup>, Farias-Filho FA<sup>3</sup>, Silva PMR<sup>3</sup>, Hogaboam C<sup>4</sup>, Kunkel SL<sup>4</sup>, Fierro IM<sup>5</sup>, Canetti C<sup>6</sup>, Benjamim CF<sup>1</sup> <sup>1</sup>UFRJ - Farmacologia Básica e Clínica, <sup>2</sup>IBRAG-UERJ - Histologia e Embriologia, <sup>3</sup>FIOCRUZ - Fisiologia e Farmacodinâmica, <sup>4</sup>UMICH - Pathology, <sup>5</sup>UERJ - Farmacologia

**Introduction:** Pulmonary fibrosis is an interstitial disease characterized by diffuse chronic interstitial inflammation, increased fibroblast proliferation, and enhanced extracellular matrix synthesis and deposition (Gross, T. J. *N Engl J Med* 345:517.2001). Lipoxins (LX) are endogenously produced eicosanoids via lipoxygenase-lipoxygenase interactions and by aspirin-triggered acetylation of cyclooxygenase-2 and activation of 5-lipoxygenase forming 15-epimer LX or aspirin-triggered LX (ATL). Among the various ATL analogs studied, 15-epi-16-(*para*-fluoro)-phenoxy-LXA<sub>4</sub> (ATLa) and related molecules have been shown to be active *in vivo* in several models of inflammatory disease (Levy, B. D. *Drugs Today (Barc)* 39:373. 2003). **Methods:** For induction of pulmonary fibrosis, groups of mice C57Bl/6 (n=5-6) were administered by intratracheal (i.t.) route with bleomycin (BLM) (0.1U/mouse) or sterile saline (30 µl) as control. ATLa (1µg/mouse) was injected concomitantly with BLM (0.1U/mouse), or four days after BLM inoculation. Treatment with ATLa was boosted i.v. in the 7<sup>th</sup> and 14<sup>th</sup> days. For analysis, mice were sacrificed on day 21 after BLM administration and lungs removed for preparation of histological analysis, morphometry and immunohistochemistry. All experimental procedures were performed according to guidelines of the Committee on Ethical Use of Laboratory Animals of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (process code DFBCICB028). **Results:** Bleomycin-induced lung fibrosis was prevented by ATLa, which inhibited inflammation, matrix deposition and decreased α-actin expression. Furthermore, ATLa post-treatment (4 days after BLM) showed similar inhibitory effects on inflammation, matrix deposition, and fibroblast differentiation. **Discussion:** The present results elucidate the antifibrotic effect of an aspirin-triggered LX analog using a relevant *in vivo* model of lung fibrosis. It is noteworthy that in this report we showed the therapeutic effect of ATLa in addition to the early namely preventive effect, given that in the clinical use, the therapeutic effect is often more important when the fibrotic changes of various etiology are already apparent in the patient. This work was supported by Conselho Nacional de Pesquisa (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

#### 04.142

Alpha-1-acid glycoprotein decreases the endothelium-neutrophil interactions and increases the susceptibility of diabetic mice to sepsis. Spiller F<sup>1</sup>, Carlos D<sup>1</sup>, Souto FO<sup>1</sup>, Freitas A<sup>1</sup>, Vieira SM<sup>2</sup>, Mestriner FLAC<sup>3</sup>, Paula FJA<sup>3</sup>, Alves-Filho JC<sup>1</sup>, Cunha FQ<sup>1</sup>  
<sup>1</sup>FMRP-USP - Farmacologia, <sup>2</sup>COPE-INPA, <sup>3</sup>FMRP-USP - Clínica Médica

Defects in neutrophil-endothelium interplay contribute to the increased susceptibility to infections during diabetes. Despite the clinical importance of this problem, little is known about how diabetes reduces neutrophil migration and impairs the host defense. The objective of this study was to determine the mechanisms implicated in the reduction of neutrophil migration to infectious focus in alloxan-induced diabetic mice subjected to polymicrobial sepsis by cecal ligation and puncture. This experimental protocol was approved (n° 089/2006) by CETEA of FMRP - USP. The diabetic mice were highly susceptible to sepsis due to the reduction of neutrophil migration to infectious focus, thus resulting in the impairment of bacterial clearance in peritoneal cavity, bacteremia and elevated systemic inflammatory response. Moreover, in contrast to non-diabetic mice, diabetic mice subjected to mild sepsis presented reduction on rolling and adhesion of leukocytes to the endothelium of mesenteric microcirculation, which was accompanied by low levels of ICAM-1 expression on these cells, shedding of CD62L and high expression of CD11b on neutrophils. In addition, *in vitro* MIP-2-induced chemotaxis was reduced in neutrophils from diabetic mice after septic stimulus. In accordance, CXCR2 internalization on neutrophils from diabetic mice after sepsis correlated with increase of the G protein-coupled receptor kinase 2 expression (GRK2). Different of non-diabetic mice, diabetic mice submitted to mild sepsis displayed rise of acute phase protein alpha-1-acid glycoprotein (AGP) hepatic mRNA expression and serum protein levels. Administration of AGP in non-diabetic mice subjected to mild sepsis inhibited the neutrophil migration to infectious focus as well as induced L-selectin shedding and rise of CD11b on blood neutrophils. Insulin treatment of diabetic mice decrease the mortality rate by prevents the failure of neutrophil migration. Moreover, this treatment prevented the GRK2-mediated CXCR2 downregulation as well as decreased the generation of AGP. Finally, administration of AGP abolished the effect of insulin treatment in diabetic mice. Together, the presented data suggests that AGP might be a key role on failure of neutrophil migration in diabetic mice, which have a strict correlation to susceptibility of these mice to sepsis. Financial support: FAPESP, CAPES, CNPq.

#### 04.143

Phototherapy (PhT): attenuation of cholinergic hyperreactivity,  $\beta_2$ -adrenergic hyporesponsiveness and TNF- $\alpha$  mRNA expression in rat bronchi segments in *E. coli* lipopolysaccharide-induced airway inflammation by a NF-KB-dependent mechanism. Mafrá De Lima, F<sup>1</sup>, Albertini R<sup>2</sup>, Aimbire F<sup>2</sup> <sup>1</sup>UNIVAP, <sup>2</sup>UNICASTELO

**Introduction:** It is unknown if the decreased ability to relax airways smooth muscles in asthma and other inflammatory disorders, such as acute respiratory distress syndrome (ARDS), can be influenced by low level laser therapy irradiation. In this context, the present work was developed in order to investigate if PhT could reduce dysfunction in inflamed bronchi smooth muscles (BSM) in rats. **Methods:** A controlled ex vivo study was developed where bronchi from Wistar (n=7) rat were dissected and mounted in an organ bath apparatus with or without a TNF- $\alpha$  (10 ng/ml). In the experimental assays with the participation of TNF- $\alpha$ , the reactivity of BSM to cholinergic agonist (tension g/100 mg tissue) or to  $\beta_2$ -adrenergic agonist (% contraction with ACh) was analyzed. cAMP level (pmol/mg protein) was also investigated in BSM bathed or not with TNF- $\alpha$  and treated or not with PhT. In other series of experiment, the BSM from rat were incubated with LPS (20  $\mu$ g/ml) in order to investigate the PhT effect on TNF- $\alpha$  mRNA expression in BSM. **Results:** TNF- $\alpha$  caused cholinergic hyperreactivity ( $20.57 \pm 1.61$ ) and  $\beta_2$ -adrenergic hyporesponsiveness ( $78.0 \pm 2.21$ ) when compared to respective controls (BSM not stimulated with TNF- $\alpha$ ) (control ACh:  $10.38 \pm 1.1$ ; control  $\beta_2$ -adrenergic:  $33.0 \pm 2.37$ ). PhT administered perpendicularly to a point in the middle of the dissected bronchi with a wavelength of 655 nm and a dose of 2.6 J/cm<sup>2</sup>, partially decreased BSM hyperreactivity to cholinergic agonist ( $13.33 \pm 1.10$ ), restored BSM relaxation to isoproterenol ( $53.0 \pm 2.85$ ) and reduced the TNF- $\alpha$  mRNA expression. cAMP level diminished by TNF- $\alpha$  ( $12.61 \pm 2.1$ ) in comparison with isoproterenol ( $25.30 \pm 2.5$ ) was restored by PhT ( $22.30 \pm 2.1$ ). An NF-kB antagonist (BMS205820) blocked the PhT effect on dysfunction and cAMP level in inflamed BSM. **Discussion:** Although the BSM have been stimulated only with TNF- $\alpha$ , this exposure produces different agonist dependent- responses. Surprisingly, the PhT was efficient in attenuating either cholinergic hyperreactivity as  $\beta_2$ -adrenergic hyporesponsiveness by a mechanism in which the blocked of NF-kB activation seems to limit the efficiency of PhT in an *in vitro* experimental model of lung inflammation. **Financial Support:** FAPESP (2008/08048-4). **CEP:** A07/CEP/2008

#### 04.144

Caspase-1 deficient mice present increased resistance to severe sepsis. Sonogo F<sup>1</sup>, Alves-Filho JC<sup>1</sup>, Nascimento DCB<sup>2</sup>, Freitas A<sup>1</sup>, Zamboni DS<sup>3</sup>, Cunha FQ<sup>1</sup> <sup>1</sup>FMRP-USP - Farmacologia, <sup>2</sup>FMRP-USP - Imunologia e Bioquímica Básica, <sup>3</sup>FMRP-USP - Biologia Celular, Molecular e Bioagentes Patogênicos

**Introduction:** The establishment of a local inflammatory response with production of cytokines/chemokines and the consequent neutrophil recruitment are critical to control an infection. In this context, our group has demonstrated an impaired neutrophil migration toward infectious focus in severe sepsis. The failure of neutrophil migration is markedly associated with increased systemic cytokines levels and high mortality during severe sepsis, either in experimental models and patients. Recently, the participation of Toll-like receptors (TLRs) in this failure of neutrophil migration was described. Caspase-1 seems to be important in the activation of TLRs signaling pathways and is also critical to the activation of inflammatory cytokines IL-1 $\beta$ , IL-18 and IL-33 and seems. So, the aim of this study was to evaluate the participation of caspase-1 during severe sepsis. **Methods and Results:** All the experiments were performed according to the ethical guidelines of School of Medicine of Ribeirão Preto, University of São Paulo (process number:129/2008). The caspase-1 deficient mice presented increased resistance to severe sepsis induced by CLP (60% *versus* 0% wild type). However, IL-18 and ST2 (receptor of IL-33) deficient mice did not present reduction on mortality after sepsis (wild type, ST2<sup>-/-</sup> and IL-18<sup>-/-</sup>-deficient mice = 20% of survival). The treatment with antagonist of IL-1 receptor (100mg/kg, sc) 1h before (survival and neutrophil migration) and 6, 12, 24, 36, 48, 60h (survival) after CLP was also unable to modify the survival rate and neutrophil migration of wild type mice underwent to severe sepsis. The reduction on mortality of caspase-1 deficient mice was associated to a decreased systemic levels of TNF- $\alpha$  and IL-6. Despite of the unaltered local levels of cytokines and chemokines, caspase-1 deficient mice underwent to severe sepsis presented an increase of neutrophil migration to peritoneal cavity as compared to wild type (wild type:  $2.5 \times 10^6$ ; Caspase-1<sup>-/-</sup>:  $12 \times 10^6$  neutrophils/cavity), that was supported by an increased rolling and adhesion of leukocytes in these mice. As consequence, a reduced bacterial growth in peritoneal exudates and blood (wild type: Log CFU/ml= 5 and 4.4; caspase-1<sup>-/-</sup>: Log CFU/ml= 3.4 and 3, respectively) was observed in these animals although neutrophils from caspase-1 deficient and wild type mice presented similar killing and cellular viability. **Conclusion:** Altogether, these results demonstrate that in the absence of caspase-1, neutrophil migration to peritoneal cavity is increased and culminates in a reduction of mortality because of the efficient control of the infection. Supported by CNPq, CAPES and FAPESP

#### 04.145

Phototherapy reduces the hyperreactivity and hyporeactivity of trachea smooth muscle segments to cholinergic agonist in a lung inflammation model induced by intestinal reperfusion ischemia in rat. Mafrá de Lima, F<sup>1</sup>, Salgado MAC<sup>2</sup>, Tavares de Lima W<sup>3</sup>, Aimbire F<sup>4</sup> <sup>1</sup>UNIVAP - Laserterapia e Inflamação Pulmonar, <sup>2</sup>FOSJC-UNESP - Morfologia, <sup>3</sup>ICB-USP- Farmacologia, <sup>4</sup>UNICASTELO

**Introduction:** Phototherapy (PhT) is a modulator of acute lung inflammation without evidences of side-effects. Herein we investigated the PhT effect on alterations in reactivity of trachea smooth muscle (TSM) from rats submitted to lung inflammation induced by intestinal reperfusion ischemia (i-I/R). **Methods:** Male Wistar rat weighting between 220-250 g were divided in 5 experimental groups (n=7) being them: control (vehicle), sham (sham-operated), PhT, i-I/R and i-I/R plus PhT (i-I/R + PhT). The TSM segments were isolated and mounted into organ bath apparatus for studies cholinergic reactivity through isometric contraction measurement. The contractile force (tension) was expressed in g/100mg of tissue. For that, concentration-response curves to metacholine (MCh) were performed. The i-I/R group was submitted to ischemia of superior mesenteric artery during 45 minutes (min). After this period, the perfusion was re-established (reperfusion time) during different time intervals which are 30 min, 2 or 4 hours (h). After i-I/R period, the TSM segments were submitted to reactivity studies. The i-I/R + PhT group was irradiated (660 nm) with energy density of 7.5 J/cm<sup>2</sup> during 3 min. The rats were irradiated 15 min after the initial of reperfusion time in all time studied. **Results:** In all time periods studied, the i-I/R presented significant alteration of TSM reactivity to MCh when compared to control group (*control*: 19.44 ± 2.68; *i-I/R 30 min*: 30.92 ± 2.93; *i-I/R 2 h*: 15.46 ± 1.22; *i-I/R 4 h*: 45.19 ± 3.85). Curiously, at the time of 2 h after i-I/R the TSM reactivity to MCh was diminished when compared to respective control group. The cholinergic hyperreactivity 30 min (16.21 ± 1.36) and 4 h (29.54 ± 2.73) after i-I/R was markedly reduced by PhT in comparison to respective groups. Surprisingly, at the time of 2 h after i-I/R, the PhT was efficient in backing the reactivity for control level (*i-I/R*: 15.46 ± 1.22 vs *i-I/R + PhT*: 18.01 ± 1.44). **Discussion:** Independently of TSM contractile response observed after of i-I/R period, the PhT was efficient in backing the TSM reactivity to values lesser. The action mechanism of PhT on alterations in TSM segments reactivity still is poorly investigated. This is the first work showing that PhT is able to reduce either the hyperreactivity as hyperreactivity against to cholinergic agonist in a model of acute lung inflammation induced by i-I/R. Further studies are warranted in this novel area of research aiming to elucidate with much acuity the mechanism of PhT action and its safe use. **Financial Support:** FAPESP (2008/08048-4). **CEP:** A07/CEP/2008



#### 04.146

Acute lung inflammation and endothelial cell damage are decreased after treatment with phototherapy (PhT) in a model of acute lung injury induced by *E. coli* lipopolysaccharide in rat. Aimbire F<sup>1</sup>, Mafrá de Lima F<sup>2</sup> <sup>1</sup>UNICASTELO, <sup>2</sup>UNIVAP - Laserterapia e inflamação pulmonar

**Introduction:** There is no information about phototherapy (PhT) effect on LPS-induced ALI which is characterized by the accumulation of neutrophils into lung and generation of inflammatory mediators; thus, we investigated whether PhT (685nm InGaAlP) attenuate the LPS-induced ALI. **Methods:** Rats were divided into 8 groups ( $n=7$ ). The acute lung injury (ALI) was induced by lipopolysaccharide (LPS) intravenous injection ( $5 \text{ mgkg}^{-1}$ ). In *in vivo* assays, both levels of TNF- $\alpha$  in BAL and in lung tissue were determined by ELISA ( $\text{pgml}^{-1}$ ). The TNF- $\alpha$  level in serum was also measured by ELISA. In *in vitro* assays, the mouse pulmonary arterial endothelial cells (MPAECs) were set up of following manner: The MPAECs treated neither TNF- $\alpha$  nor PhT were considered as control. The MPAECs that received only PhT were considered as laser. The cells bathed with TNF- $\alpha$  but not treated with low level laser were named as TNF- $\alpha$ . By the end, the MPAECs bathed with TNF- $\alpha$  and treated with laser were named as TNF- $\alpha$  + laser. Regarding to *in vivo* PhT, one group of animals receiving a LPS was, in addition, irradiated using an InGaAlP laser. For *in vivo* treatments, all rats were irradiated (685 nm; 35 mW;  $4.5 \text{ J/cm}^2$ ) during 252 seconds on the skin over the upper bronchus at the site of tracheotomy. *In vitro* the MPAECs were incubated with TNF- $\alpha$  during 6 or 24 h, then the cover of the 96-well was removed, and the plates containing MPAECs were irradiated using the same laser parameters as described for the *in vivo* irradiation. **Results:** We found that PhT reduced the accumulation of TNF- $\alpha$  either in BAL (LPS + laser 6h:  $18.83 \pm 1.20$  vs LPS 6h:  $60.80 \pm 1.60$ ), (LPS + laser 24h:  $17.74 \pm 1.20$  vs LPS 24h:  $77.50 \pm 1.60$ ) as lung (LPS + laser 6h:  $0.32 \pm 0.07$  vs LPS 6h:  $1.0 \pm 0.006$ ), (LPS + laser 24h:  $0.80 \pm 0.07$  vs LPS 24 h:  $1.50 \pm 0.06$ ). Otherwise, PhT was efficient neither in reducing of TNF- $\alpha$  concentration in serum nor neutrophils of blood after LPS. In *in vitro* assays, the laser irradiation restored deeply the MPAECs damage induced by 6 or 24 h after TNF- $\alpha$ . With exception of serum assays, all saline groups were significantly different of its respective LPS groups. **Discussion:** The attenuation of the TNF- $\alpha$ -induced pulmonary endothelial monolayer damage by PhT suggests a modulator action of PhT on LPS induced-ALI, through any signaling pathway in endothelial cells of the pulmonary microvasculature that involves TNF- $\alpha$ . Treatment with PhT significantly attenuates the ALI induced by LPS in rats, but it does not lead to a complete inhibition of lung injury. It was suggested that, even if this therapy can be applicable as therapeutic modality for ARDS, its protection may be limited. These results suggest a PhT effect on ALI is partially due to inhibition of TNF- $\alpha$  releasing from neutrophils and lung. **Financial Support:** FAPESP (2007/02596-7). **CEP:** A07/CEP/2008

#### 04.147

Phototherapy (PhT) decreases pulmonary microvascular leakage, neutrophil influx and IL-1 $\beta$  mRNA expression in airway and lung from rat subjected to LPS-induced inflammation. Aimbire F<sup>1</sup>, Mafra de Lima F<sup>2</sup>, Ligeiro de Oliveira AP<sup>3</sup>, Albertini R<sup>1</sup>  
<sup>1</sup>UNICASTELO, <sup>2</sup>UNIVAP - Laserterapia e Inflamação Pulmonar, <sup>3</sup>USP - Farmacologia

**Introduction:** Phototherapy (PhT) is a known anti-inflammatory therapy. Herein we studied the effect of PhT on lung permeability and the IL-1 $\beta$  level in lipopolysaccharide (LPS)-induced pulmonary inflammation. **Methods:** Rats were divided into 12 groups ( $n=7$ ). The acute lung injury (ALI) was induced by LPS intravenous injection (5 mg/kg). Lung permeability was measured by quantifying extravasated albumin concentration in lung homogenate (mg per g lung tissue), inflammatory cells influx was determined by myeloperoxidase activity (OD 460 nm), IL-1 $\beta$  in BAL was determined by ELISA (ng/ml) and IL-1 $\beta$  mRNA expression in trachea was evaluated by RT-PCR. The rats were irradiated (660 nm; 7.5 J/cm<sup>2</sup>; 30 mW) during 20 seconds on the skin over the upper bronchus at the site of tracheotomy after LPS. At the time of 4 h, the rats were challenged with LPS and 1 h later treated with PhT. At the time of 12 h, the rats received LPS and it was irradiated twice, at 1 and 6 h after LPS injection. At the time of 24 h, the rats received LPS and it was irradiated twice, in the first and twelfth hour after LPS. Then, were euthanized 4, 12 and 24 hours after LPS, respectively. **Results:** In all times investigated the LPS increased lung permeability, myeloperoxidase activity and both IL-1 $\beta$  in BAL and IL-1 $\beta$  mRNA expression when compared to respective groups that neither were inflamed nor irradiated (saline). PhT attenuated lung permeability in comparison with group inflamed by LPS but not treated with laser in all periods studied: (LPS + PhT 4h: 30.1  $\pm$  5.3 vs LPS 4h: 40.7  $\pm$  5.2), (LPS + PhT 12h: 30.5  $\pm$  5.3 vs LPS 12h: 48.6  $\pm$  5.2) and (LPS + PhT 24h: 35.2  $\pm$  6.1 vs LPS 24h: 60.1  $\pm$  6.0). In addition, there was reduced myeloperoxidase activity (LPS + PhT 4h: 0.43  $\pm$  0.10 vs LPS 4h: 0.95  $\pm$  0.2), (LPS + PhT 12h: 0.91  $\pm$  0.11 vs LPS 12h: 1.32  $\pm$  0.15), (LPS + PhT 24h: 1.13  $\pm$  0.15 vs LPS 24h: 1.65  $\pm$  0.15) and both IL-1 $\beta$  in BAL (LPS + PhT 4h: 0.43  $\pm$  0.10 vs LPS 4h: 0.95  $\pm$  0.2), (LPS + PhT 12h: 0.91  $\pm$  0.11 vs LPS 12h: 1.32  $\pm$  0.15), (LPS + PhT 24h: 1.13  $\pm$  0.15 vs LPS 24h: 1.65  $\pm$  0.15) and IL-1 $\beta$  mRNA expression in trachea obtained from animals subjected to LPS-induced inflammation. **Discussion:** Unfortunately, we can still not affirm, which signaling pathway that PhT activates in trachea tissue and how much energy reaches the airways. However, the fact is that PhT reduced IL-1 $\beta$  mRNA expression and it we make suggest that this therapy can interact with local production of IL-1 $\beta$ . Taken together, these results suggest that PhT may be a new alternative and co-adjuvant therapy in the treatment of airway diseases related to sepsis, associated to a mechanism involving both the reduction of the synthesis of IL-1 $\beta$  in BAL and IL-1 $\beta$  mRNA expression in trachea from rats systemically inflamed with LPS. **Financial Support:** FAPESP (2007/02596-7). **CEP:** A07/CEP/2008

#### 04.148

Relationship between female sex hormones and the generation of TH17 cells in experimental asthma model. Ligeiro de Oliveira AP<sup>1</sup>, Peron JPS<sup>2</sup>, Golega BA<sup>1</sup>, Rodrigues-Soares C<sup>1</sup>, Oliveira-Filho RM<sup>1</sup>, Rizzo LV<sup>2</sup>, Tavares de Lima W<sup>1</sup> <sup>1</sup>ICB-USP - Farmacologia, <sup>2</sup>ICB-USP - Imunologia

**Objective:** Evaluate the role of female sex hormones over the regulation of the Th17 pathogenic cells in a murine model of asthma. **Methods:** Female BALB/c mice were sensitized to OVA on days 0 and 7 and subsequently challenged on day 14. Mice had their ovaries removed 1 (Ovx-1) or 7 days (Ovx-7) before the first OVA injection on day 0. Pulmonary cellular infiltrate were obtained from bronchoalveolar lavage 24 h following the last antigen challenge. Splenocytes, lymphnodes cells were collected and submitted to flow cytometry analysis of the CD4<sup>+</sup>IL-17<sup>+</sup> (Th17) and CD4<sup>+</sup>IFN-g<sup>+</sup> population. **Results:** Our data points to the fact that the immune response is differently modulated, depending on the presence (Ovx-1) or lack of female sex hormones (Ovx-7) during immunization. This was observed as Ovx-7 group had reduced infiltrate in the lung when compared to its sham-control as well as to Ovx-1 group. Moreover, we observed an increased percentage of Th17 cells in the lymphnodes of Ovx-1 group ( $2.8 \pm 0.2$  %) when compared to its control ( $1.9 \pm 0.1$  %). On the other hand, our Ovx-7 mice ( $0.9 \pm 0.11$  %), showed a reduction of this population in spleen when compared do Sham-7 ( $1.5 \pm 0.1$  %). Concerning IFN-g production, we also observed a reduction of the splenic CD4<sup>+</sup>IFN-g<sup>+</sup> T cells (Sham-7:  $0.46 \pm 0.06$  vs OVx-7:  $0.26 \pm 0.05$  %). **Conclusion:** Our data demonstrates that the allergic immune response in the asthma model is dependent on the presence of female sex hormones during immunization with OVA. We believe that in the lack of hormones (Ovx-7), antigen presentation and/or activation of T cells must be impaired, with a reduced capacity to induce the commitment to the Th17 population which does not occur in the Ovx-1 group, due to the presence of the hormones. Our data prompts us to speculate that female sex hormones are important for the generation of this pathogenic population in the asthma model. **Financial support:** 06/55950-0; 04/14128-0; 07/55631-4. **Ethics committee number:** 78/07/CEEA – ICB/USP.

#### 04.149

Lung myofibroblast activation by IL-13 is under control of metalloproteinase in a three dimensional culture system. Nascimento CVMF, Jurgilas PB, Ferreira TPT, Dalzy DV, Silva PMR, Cordeiro RSB, Martins MA, Perez SAC IOC-FIOCRUZ - Fisiologia e Farmacodinâmica

**Introduction and Aim:** Human asthma is characterized by the accumulation of extracellular matrix components (ECM) which synthesis is mainly controlled by myofibroblast cells. Moreover, ECM remodeling depends on metalloproteinases (MMPs) that have being progressive viewed as regulatory molecules in inflammation. In this perspective, we hypothesized that MMPs are involved in lung myofibroblast activation induced by rmlL-13 in 3D cell culture. **Methods:** Primary myofibroblast cell culture was established after lung dissociation of naïve Balb/c mice. Multicellular spheroids were formed using myofibroblast plated in agarose-coated 96 U-well plates. IL-13-activated myofibroblast was analyzed by measuring collagen, MMP proteolytic activity and eotaxin using Sircol, gelatin zymography and ELISA in the cell free medium on the 4<sup>th</sup> day of culture, respectively. All the procedures involving care and use of laboratory animals were approved by the Animal Ethics Committee of the Fiocruz (CEUA-FIOCRUZ, Prot. 0509/08). **Results:** We demonstrated that IL-13-activated myofibroblast spheroids dose-dependently released collagen in parallel with decreased expression of secreted MMP-9 and MMP-2 activity in the cell free medium, when compared to unstimulated myofibroblast spheroids. Moreover, IL-13 leads to significant induction of C-C chemokine eotaxin release. Pre-treatment with MMP inhibitor ortho-phenanthroline did not impaired spheroid formation but inhibited collagen and eotaxin release. **Discussion:** Our results suggest that collagen and eotaxin release in IL-13-activated myofibroblast spheroids seems to be dependent on metalloproteinase activity. This may indicate that MMPs could be a therapeutic target for the suppression of the lung fibrosis observed in asthma. Financial Support: FAPERJ, CNPq and PDTIS.

#### 04.150

Lung myofibroblast activation by IL-13 is under control of metalloproteinase in a three dimensional culture system. Nascimento CVMF, Dalzy DV, Jurgilas PB, Ferreira TPT, Silva PMR, Cordeiro RSB, Martins MA, Perez SAC <sup>1</sup>IOC-FIOCRUZ - Fisiologia e Farmacodinâmica

**Introduction and Aim:** Human asthma is characterized by the accumulation of extracellular matrix components (ECM) which synthesis is mainly controlled by myofibroblast cells. Moreover, ECM remodeling depends on metalloproteinases (MMPs) that have being progressive viewed as regulatory molecules in inflammation. In this perspective, we hypothesized that MMPs are involved in lung myofibroblast activation induced by rIL-13 in 3D cell culture. **Methods:** Primary myofibroblast cell culture was established after lung dissociation of naïve Balb/c mice. Multicellular spheroids were formed using myofibroblast plated in agarose-coated 96 U-well plates. IL-13-activated myofibroblast was analyzed by measuring collagen, MMP proteolytic activity and eotaxin using Sircol, gelatin zymography and ELISA in the cell free medium on the 4<sup>th</sup> day of culture, respectively. All the procedures involving care and use of laboratory animals were approved by the Animal Ethics Committee of the Fiocruz (CEUA-FIOCRUZ, Prot. 0509/08). **Results:** We demonstrated that IL-13-activated myofibroblast spheroids dose-dependently released collagen in parallel with decreased expression of secreted MMP-9 and MMP-2 activity in the cell free medium, when compared to unstimulated myofibroblast spheroids. Moreover, IL-13 leads to significant induction of C-C chemokine eotaxin release. Pre-treatment with MMP inhibitor ortho-phenanthroline did not impaired spheroid formation but inhibited collagen and eotaxin release. **Discussion:** Our results suggest that collagen and eotaxin release in IL-13-activated myofibroblast spheroids seems to be dependent on metalloproteinase activity. This may indicate that MMPs could be a therapeutic target for the suppression of the lung fibrosis observed in asthma. **Financial Support:** FAPERJ, CNPq and PDTIS.

#### 04.151

Leukotriene controls chemokine production and inflammasome activation in an experimental model of gout. Amaral FA<sup>1</sup>, Costa VV<sup>1</sup>, Coelho FM<sup>2</sup>, Sachs D<sup>3</sup>, Fagundes CT<sup>2</sup>, Teixeira MM<sup>2</sup>, Souza DG<sup>1</sup> <sup>1</sup>UFMG - Microbiologia, <sup>2</sup>UFMG - Bioquímica e Imunologia, <sup>3</sup>FMRP-USP - Farmacologia

**Introduction:** Gout arthritis is characterized by precipitation of uric acid crystals (MSU), especially in the joint, leading to an intense local inflammation. MSU triggers the assembly of the inflammasome, culminating in release of IL-1 $\beta$  and IL-18, which have a crucial role in driving tissue inflammation and nociception. In this model neutrophil influx is relevant for joint hypernociception. In the present study, we have evaluated the role of 5-lipoxygenase (5-LO)-derived leukotriene B<sub>4</sub> (LTB<sub>4</sub>) in driving tissue inflammation, inflammasome-dependent cytokine production and hypernociception. **Methods:** Gout was induced by injecting MSU crystals in the joint of mice (Ethics Committee: 165/2008). At various time points after injection, neutrophil influx, LTB<sub>4</sub> and cytokine production (IL-1 $\beta$ , CXCL1) and hypernociception were evaluated. Intravital microscopy of the knee joint was performed to evaluate leukocyte-endothelial interactions. **Results:** Injection of MSU crystal induced joint inflammation and hypernociception which was maximal at 15h and at 100 $\mu$ g of MSU per joint. Experiments in 5-LO-deficient mice or treatment with a 5-LO inhibitor (MK-886) blocked neutrophil migration and hypernociception. Experiments with BLT (CP-105696) or CystLT (montelukast) antagonists showed the response to be mainly dependent of BLT1 activation. Treatment with MK-886 blocked neutrophil influx and the production of CXCL1. Blockade of the CXCL1 receptor (DF-2162), CXCR2, was also accompanied by inhibition of neutrophil influx and hypernociception. Intravital microscopy showed that both MK-886 and DF-2162 prevented neutrophil adhesion to endothelial cells at 6 h when given before the injection of MSU. However, only DF-2162, but not MK-886, blocked neutrophil-endothelial cell interactions when given just before the intravital procedure, suggesting that prevention of CXCL1 production explains the inhibitory effects of 5-LO inhibition or absence on neutrophil influx. There was inhibition of IL-1 $\beta$  production in 5-LO-deficient mice and after MK886 treatment. In vitro, LTB<sub>4</sub> induced IL-1 $\beta$  production by LPS-stimulated macrophages, demonstrating this lipid is capable of activating the inflammasome. **Discussion:** In a novel model of gout in mice, we show that LTB<sub>4</sub> drives the production of CXCL1, which activates CXCR2 and, consequently, induces the migration of neutrophils. In addition, we show that LTB<sub>4</sub> is a crucial molecule in the assembly of the inflammasome and consequent production of IL-1 $\beta$ . Altogether, our studies provide a novel mechanism by which LTB<sub>4</sub> drives tissue inflammation – by inducing inflammasome assembly, release of inflammasome-dependent cytokines, such as IL-1 $\beta$ , and IL-1 $\beta$ -dependent release of CXCL1. **Financial Support:** CNPq, CAPES, FAPEMIG.

#### 04.152

NK1 receptors are involved in the febrile response induced by lipopolissacharide (LPS) but not by interleukin-1 $\beta$  (IL-1 $\beta$ ). Reis RC<sup>1</sup>, Brito HO<sup>1</sup>, Boller S<sup>1</sup>, Fraga D<sup>2</sup>, Zamprônio AR<sup>1</sup> <sup>1</sup>UFPR - Farmacologia, <sup>2</sup>USP - Farmacologia

**Introduction:** Fever is an essential component of host defense against environmental aggressors such as pathogens. Substance P (SP) is an abundant neuropeptide from nervous fibers, and few data from literature suggest that SP can induce fever, but the mechanisms are unknown. In this study we investigated the effect of a new selective NK1 receptor antagonist, SR140333, in the febrile response induced by LPS and IL-1 $\beta$ . For positive control we also evaluated this antagonist in a protein extravasation model.

**Methods:** Male Wistar rats received SR140333 (0.3, 1.0 or 3.0 mg/kg i.p. or 0.3, 1.0 or 3.0  $\mu$ g, i.c.v.) or the appropriate vehicle and after 30 min different groups were stimulated with LPS (30  $\mu$ g/kg, i.p. or 200 or 400  $\mu$ g, intradermally), IL-1 $\beta$  (3.12 ng, i.c.v.) or SP (500 ng, i.c.v. or 30 pmol, intradermally). In some experiments, captopril (5  $\mu$ g, i.c.v.) was also injected 30 min before SP. Body temperature was measured every 15 min using data loggers implanted into the peritoneal cavity 1 week before. Protein extravasation was evaluated indirectly by measuring the Evans blue extravasation. All the methods were previously approved by Institutional Ethics Committee under protocol nbr. 173. **Results and Discussion:** LPS induced a febrile response that peaked at 4 h after injection ( $\sim$ 1.5  $^{\circ}$ C) and increased protein extravasation (93% and 118%, respectively for 200 and 400  $\mu$ g) while SP increased protein extravasation in 182%. The systemic injection of SR140333 in all doses tested did not modify the body temperature of the animals or the fever and the protein extravasation induced by LPS but reduced 63% protein extravasation induced by SP. However, i.c.v. administration of 1.0 or 3.0  $\mu$ g SR140333, significantly reduced the fever induced by peripheral injection of LPS (33% and 57%, respectively) but, surprisingly, at the higher dose, did not change the IL-1 $\beta$ -induced fever. Also, i.c.v. injection of SP induced a febrile response ( $\sim$ 1.8  $^{\circ}$ C) that peaked at 4 h only after previous administration of captopril and this response was completely blocked by the NK1 receptor antagonist. Altogether these data suggest that SP may be an important central mediator of fever. Also, LPS induces a central (but not peripheral) release of SP to generate fever. However, the central release of SP by LPS does not seem to involve IL-1 $\beta$ . **Financial support:** CNPq, CAPES and Sanofi-Aventis.

#### 04.153

Role of the chemokine CCL5/RANTES on leukocyte-endothelium interactions in an experimental model of herpetic encephalitis. Vilela MC<sup>1</sup>, Mansur DS<sup>2</sup>, Queiroz NL<sup>3</sup>, Rodrigues DH<sup>3</sup>, Amaral DCG<sup>4</sup>, Lima G K<sup>2</sup>, Kroon EG<sup>2</sup>, Campos MA<sup>5</sup>, Teixeira MM<sup>6</sup>, Teixeira AL<sup>7</sup> <sup>1</sup>UFMG - Infectologia e Medicina Tropical, <sup>2</sup>UFMG - Microbiologia, <sup>3</sup>UFMG - Biologia Celular, <sup>4</sup>UFMG - Imunofarmacologia, <sup>5</sup>CPqRR-FIOCRUZ - Imunopatologia, <sup>6</sup>UFMG - Bioquímica e Imunologia, <sup>7</sup>UFMG - Medicina

**Introduction:** The Herpes Simplex Virus-1 (HSV-1) is responsible for several clinical syndromes in humans, including encephalitis. The aim of this study was to evaluate the role of CCL5/RANTES in a severe model of HSV-1 encephalitis using CCR5<sup>-/-</sup> mice.

**Methods:** Wild type C57BL/6 and CCR5<sup>-/-</sup> mice were intracerebrally inoculated with 10<sup>4</sup> PFU of HSV-1 or PBS. Visualization of leukocyte recruitment using intravital microscopy was done at 1 day post-infection (dpi). Brain was removed for chemokine analysis by ELISA and histological studies. Virus titration in cerebral tissue was done by TCID50. All experiments were approved by the Animal Ethics Committee of the Federal University of Minas Gerais (UFMG) from the number 186/06.

**Results:** CCR5<sup>-/-</sup> infected mice showed similar mortality rate, but a significant reduced virus titers in brain at 1dpi when compared to infected wt mice. Intravital microscopy revealed a significant increase in leukocyte adhesion in CCR5<sup>-/-</sup> infected mice at 1 dpi. The infection was also followed by a significant increase in chemokine levels, including CCL2, CCL5, CXCL1 and CXCL9 and in cytokine TNF- $\alpha$  level. CCR5<sup>-/-</sup> mice developed more inflammatory lesions than wt infected mice.

**Discussion:** CCL5/RANTES, a chemokine that binds to CCR1 and CCR5, seems to be relevant in the recruitment of leucocytes in a severe model of HSV-1 encephalitis. Absence of CCL5/RANTES in mice upregulated expression of other inflammatory chemokines in brain of C57BL/6 mice.

**Acknowledgment:** Fapemig and CNPq



#### 04.154

The inflammasome complex is crucial for inflammatory response induced by monosodium urate in An novel model of gouty arthritis in mice. Amaral FA<sup>1</sup>, Costa VV<sup>1</sup>, Rodrigues Coelho F<sup>2</sup>, Ryffel B<sup>3</sup>, Souza DG<sup>1</sup>, Teixeira MM<sup>4</sup> - <sup>1</sup>UFMG - Microbiologia, <sup>2</sup>USP - Farmacologia, <sup>3</sup>CNRS - Immunologie, <sup>4</sup>UFMG - Bioquímica e Imunologia

**Introduction:** The precipitation of uric acid crystals (MSU) in the joint causes gouty arthritis. Once in the articular cavity, MSU induces an intense inflammatory response, characterized by the release of soluble mediators and neutrophilic influx in the joint. *In vitro* studies have shown that MSU activates the inflammasome complex, culminating in release of IL-1 cytokine members, which have crucial role in this context. Our aim was to set up a new animal model of acute gout disease and evaluate the role of the inflammasome and inflammasome-processed mediators involved in the model. **Methods:** Mice used were wild type (WT), NALP3<sup>-/-</sup>, ASC<sup>-/-</sup>, Caspase-1<sup>-/-</sup>, MyD88<sup>-/-</sup>, IL-1R<sup>-/-</sup>, IL-18R<sup>-/-</sup>, and ST2<sup>-/-</sup>. In another group, WT mice were treated with anakinra (IL-1ra). MSU was prepared by precipitation of the crystals after addition of uric acid in borate buffer solution (pH 8.5) and injected (10 µL) into the tibio femoral cavity in mice in different concentrations and time points. Hypernociception was measured by a digital analgesimeter. Samples of the periarticular tissue were collected for cytokine analysis (ELISA) and neutrophil quantification (MPO). A lavage of the joint was made to evaluate the cell infiltration on articular space. **Results:** There was an increase in all inflammatory parameters evaluated in a time and dose response manner after MSU injection compared to vehicle-injected mice. For the subsequent experiments, the dose of 100µg/cavity (15h after injection) was chosen. In mice deficient in inflammasome complex components (NALP3, ASC, Caspase-1), there was a reduced inflammatory response after MSU injection compared to WT. This lower inflammation was also seen in IL-1R<sup>-/-</sup>, MyD88<sup>-/-</sup>, IL-1β<sup>-/-</sup> mice and after the treatment with anakinra. However, inflammation was not different in IL-18R<sup>-/-</sup> and WT mice and only partially decreased in ST2<sup>-/-</sup> mice. **Discussion:** Direct injection of MSU in the knee is an interesting model to evaluate pathophysiological mechanisms leading to inflammation and hypernociception in the context of gout. Furthermore, our results demonstrate that the inflammasome is crucial for MSU-induced inflammation in the joint of mice and show that IL-1β is the most relevant inflammasome-processed cytokine. The effects of the IL-1ra anakinra concur with the idea that blockade of IL-1 may be of therapeutic relevance in the context of gout. **Financial support:** CNPq, FAPEMIG

#### 04.155

Selective blockade of bradykinin-2 receptor activation dampens systemic inflammatory response and prevents disease onset in primary dengue virus infection in mice. Fagundes CT<sup>1</sup>, Costa VV<sup>2</sup>, Silveira KD<sup>3</sup>, Lima CX<sup>2</sup>, Valadão DF<sup>2</sup>, Morcatty TQ<sup>2</sup>, Santos AG<sup>2</sup>, Amaral FA<sup>1</sup>, Souza RS<sup>3</sup>, Cisalpino D<sup>2</sup>, Souza PRS<sup>3</sup>, Pesquero JL<sup>4</sup>, Sousa LP<sup>5</sup>, Teixeira MM<sup>3</sup>, Souza DG<sup>2</sup> <sup>1</sup>ICB-UFMG - Bioquímica e Imunologia/Microbiologia, <sup>2</sup>ICB-UFMG - Microbiologia, <sup>3</sup>ICB-UFMG - Bioquímica e Imunologia, <sup>4</sup>ICB-UFMG - Fisiologia e Biofísica, <sup>5</sup>COLTEC-UFMG - Patologia Clínica

**Introduction:** Dengue is a mosquito-borne infection which has become a major international public health concern. Our group has recently developed murine Dengue virus (DENV) infection models, which resembles the severe dengue human infection. In these models, DENV-infected mice present great hemodynamic alterations, partially due to great endothelial cells and leukocyte activation, leading to an intense inflammatory response. Bradykinin (BK) is a vasoactive peptide that has been implicated in several inflammatory conditions. BK acts on two distinct receptors: B2 and B1 receptors. Here, we evaluated the role played by each BK receptor in primary DENV infection in mice. **Methods:** B2R-deficient (B2R<sup>-/-</sup>), B1R-deficient (B1R<sup>-/-</sup>), B1R/B2R-deficient (B1R/B2R<sup>-/-</sup>), and their respective wild type (WT) control mice were infected with DENV2 (100 PFU, i.p.) or DENV3 (10 PFU, i.p.). After 5 or 7 days of infection, hematocrit and platelets number, neutrophil influx and pro-inflammatory cytokines production in liver and lungs, as well viral loads in liver, were evaluated. For lethality rates after infection, the same infected groups were monitored for 14 days. All procedures have been approved by local ethics committee (protocol 113/09). **Results and discussion:** While WT-infected mice presented great lethality after both DENV2 and DENV3 infection, B2R<sup>-/-</sup> presented great survival. However this protection of lethality was not observed in B1R<sup>-/-</sup>, and B1R/B2R<sup>-/-</sup>. B2R<sup>-/-</sup> mice also showed reduced hematological alterations, as assessed by diminished thrombocytopenia and hemoconcentration, when compared with WT, B1R<sup>-/-</sup> and B1R/B2R<sup>-/-</sup> infected mice. Furthermore, B2R<sup>-/-</sup> mice also presented reduced neutrophil influx to liver and lungs after DENV infection, which can be explained by reduced chemokines (CXCL1, CXCL2 and CCL2) production in these organs after infection. Again, both B1R<sup>-/-</sup> and B1R/B2R<sup>-/-</sup> infected mice did not present marked alteration in the latter parameters when compared to infected WT mice. TNF- $\alpha$  production in liver was also reduced only in B2R<sup>-/-</sup> infected mice. Finally, the viral loads of B2R<sup>-/-</sup> infected mice in liver were markedly reduced after infection. However, B1R<sup>-/-</sup> and B1R/B2R<sup>-/-</sup> infected mice presented DENV titers similar to the titers found in WT infected mice. These data suggest that B2R blockade seems to curb the excessive inflammatory response after DENV infection, leading to reduced disease and diminished lethality. Then, B2R plays an important pro-inflammatory role after DENV primary infection. Despite B1R absence does not result in marked alterations in host response to DENV infection, B1R activation during B2R absence seems to be of great importance, probably due to its important roles in endothelial cells and vascular function. B2R blockade may be an important strategy to avoid the intense inflammatory response unleashed by DENV infection and may represent a new therapeutic approach in severe dengue disease. **Support:** CNPq and FAPEMIG

#### 04.156

ETB receptor activation as a mechanism of modulation of inflammatory pain and neurogenic inflammation in temporomandibular joint of capsaicin-treated rats. Câmara PRS<sup>1</sup>, Martins Porto R<sup>1</sup>, Denadai-Souza A<sup>1</sup>, Ribela MTCP<sup>2</sup> <sup>1</sup>ICB-USP - Farmacologia, <sup>2</sup>IPEN - Biologia Molecular

**Introduction:** Endothelin (ET), a peptide best known for its vascular effects, also evokes pain and hyperalgesia independent from its vascular actions. Several arguments suggest that ET can have nociceptive effects acting directly on its receptors expressed in sensory neurons. The aim of this study was to investigate ET direct effect on pain and plasma extravasation induced by carrageenan in the temporomandibular joint (TMJ). **Methods:** Capsaicin (50 mg/kg) or vehicle (1:1:8; Ethanol:Tween80:NaCl) were administered (sc) to newborn, male Wistar rats. Inflammation was induced 60 days later by a single intra-articular (i.art.) injection of carrageenan (500 µg) into the left TMJ (control group received sterile saline). All experimental protocols were approved by the Animal Care and Use Committee of the University of São Paulo (number. 113, page. 51, book 2). Inflammatory parameters such as plasma extravasation, leukocyte influx and mechanical allodynia (measured as the head-withdrawal force threshold) were evaluated 4 hours after oedematogenic stimuli. ET-1 and ET-3 (0.5 nmol/kg, i.v.), and ETBr antagonist (BQ788; 0.1 mg/kg, i.v.) were administered 3 min before oedematogenic stimuli. ETs and TRPV1 mRNA expression was assessed by RT-PCR. Oedema formation was evaluated by measurement of extravascular accumulation of injected <sup>125</sup>I-human serum albumin in the TMJ soft tissues of anaesthetized rats. **Results:** Capsaicin neonatal treatment significantly (p<0.001) reduced the oedema formation, leukocyte influx and mechanical allodynia in TMJ when compared to control group, while ETBr antagonism increased all parameters. ET-1 agonist treatment reduced both the plasma extravasation and myeloperoxidase activity. Capsular RNA for ET-1 was significantly (p<0.001) augmented in the TMJ of rats treated with capsaicin when compared to controls. **Discussion:** Our results suggest, for the first time, that ET-1 via ETBr activation reduces plasma extravasation, leukocyte influx and inflammatory pain in the TMJ in capsaicin-treated rats. Câmara, PRS was supported by CNPq (Post-Doctoral fellow - 150507/2007-0)

#### 04.157

Inhibition of nitric oxide synthase affects antioxidant enzyme activities in allergic mice lungs. Augusto AC<sup>1</sup>, Benetti LR<sup>2</sup>, Pelaquini EH<sup>2</sup>, Nogueira JS<sup>2</sup>, Marques MG<sup>2</sup>, Santos KL<sup>2</sup>, Lopes CO<sup>2</sup>, Gurgueira SA<sup>1</sup>, Ferreira HHA<sup>2</sup> <sup>1</sup>USF - Oxidative Stress, <sup>2</sup>USF - Inflammation

**Introduction:** A variety of inflammatory mediators result in the typical pathophysiologic changes in asthma. An imbalance between reactive oxygen species (ROS) production and antioxidant enzyme activities, as manganese-superoxide dismutase (MnSOD), glutathione peroxidase (GPx) and catalase (CAT), is another factor that contributes to the chronic inflammation process in asthma. Otherwise, increased levels of nitric oxide (NO) are found in asthma (Comhair & Erzurum, *Am. J. Physiol. Lung Cell Mol. Physiol.* 283:L246, 2002). **Objective:** Since nitric oxide (NO) reacts with superoxide anion ( $O_2^{\cdot-}$ ) to form cytotoxic compounds, we investigated if the inhibition of NO synthesis affects antioxidant enzyme activities in allergic mice lungs. **Materials and Methods:** All experiments were approved by the animal ethics committee of USF (protocol 002.11.08). Balb/c mice were s.c. sensitized at day 0 and 7 with ovalbumin (OA). Intranasal OA challenge was performed 1 week after. Control non-challenged mice received only intranasal saline. Two hours before and 4 and 12 hours after the OA challenge, mice were i.p. injected with non-selective inhibitor, L-NAME, or with the iNOS specific inhibitor 1400W. The control (non-treated) group received only saline via i.p.. At 48 hours after OVA-challenge, the mice were sacrificed and the lungs were removed, flash frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ . 150 mg of lung tissue was homogenized in 1 ml of PBS containing protease inhibitors and then centrifuged. The supernatant was used to analyze the oxidative stress by the activities of aconitase, a tricarboxylic acid (TCA) cycle enzyme easily inactivated by  $O_2^{\cdot-}$ , and by fumarase, another TCA cycle enzyme that is not easily inactivated by  $O_2^{\cdot-}$ . The antioxidant enzyme activities were also evaluated in study. **Results:** We observed in control allergic mice a 26% decrease on lung aconitase activity, compared to non-challenged mice. Fumarase activity was not affected. In this group, a reduction on antioxidant enzyme activities was also detected (GPx 18.7%; MnSOD 11.8% and CAT 10.7%). Treatment of allergic mice with L-NAME resulted in a decrease on lung aconitase and CAT (41.4 and 56.5%, respectively), whereas GPx activity was increased in 25.6% compared to control group. No significant increase was detected on MnSOD. Similar results were observed in 1400W-treated mice that showed reduction on aconitase and CAT (52.2 and 26%, respectively) as well as increased GPx activity (41.1%). A discrete alteration (15.5%) was observed on MnSOD activity. No significant variation was detected on fumarase activity in lungs from L-NAME or 1400W-treated mice. **Conclusions:** Treatments with NO synthesis inhibitors produced an increase mainly on GPx activity that could be seen as a positive lungs response to neutralize ROS. However, decrease on aconitase activity indicated an increase of oxidative stress in airways of treated allergic mice. These results suggest that inhibition of NO synthesis did not protect lungs against oxidative stress. The decrease on CAT activity produced by these treatments needs additional studies. **Financial support:** FAPESP and CNPq

#### 04.158

Participation of P2X7 purinergic receptors in the hemorrhagic cystitis induced with cyclophosphamide in mice. Martins JP<sup>1</sup>, Santos Jr AA<sup>2</sup>, Boeira VT<sup>2</sup>, Coutinho R<sup>4</sup>, Battastini AMO<sup>5</sup>, Santos DS<sup>3</sup>, Morrone FB<sup>3</sup>, Campos MM<sup>6</sup> <sup>1</sup>PUCRS - Medicina, <sup>2</sup>INCT-PUCRS - Biologia Molecular e Funcional, <sup>3</sup>PUCRS - Farmácia, <sup>4</sup>IBCCF-UFRJ, <sup>5</sup>UFRGS - Bioquímica, <sup>6</sup>PUCRS - Odontologia

**Introduction:** Extracellular nucleotides are important signaling molecules that mediate many biological effects, through the purinergic receptor activation (Ralevic et al., *Pharmacol. Rev.*, 50, 413, 1998). ATP is generated in response to cellular damage, and the P2X7 receptors have an essential role in the onset and maintenance of pathological changes (Chesselli et al., *Pain*, 114, 386, 2005). The hemorrhagic cystitis (HC) is a well known adverse effect of therapy with cyclophosphamide (CYP) used in patients in the treatment of many solid tumors (Mesquita et al., *Rev. Bras. Reumatol*, 47, 396, 2007). These urotoxic effects are attributed to the toxic metabolic of the CYP, named acrolein, which can be partially prevented by 2-mercaptoetanosulfonato de sodio (Mesna) (Katz et al., *J Cancer Res. Clin. Oncol.*, 121, 128, 1995). The present study aimed to determine the role of P2X7 receptors in the model of hemorrhagic cystitis induced by CYP in mice. **Methods:** Male Swiss mice (N= 5; 25-30 g) were used. HC was induced by a single administration of CYP (300 mg/kg, i.p.). Immediately after, mice were housed in individual plastic cages to observe the spontaneous behavior for 4 h, for 2 min every half-hour. Three behavioral parameters were considered: (i) general activity (walking, rearing, climbing, grooming etc.); (ii) immobility time; and (iii) indicatives of visceral pain behavior ('crises'). In addition, the spontaneous behavior of mice was also scored according to the following scale: 0 = normal; 1 = piloerection; 2 = strong piloerection; 3 = labored breathing; 4 = abdomen licking; and 5 = abdomen stretching and contractions (Olivar et al., *Eur. J. Pain.*, 3, 141, 1999). We have also performed the gross examination of bladders at 6 h, in order to determine the presence of edema and hemorrhage. The wet weight of bladders (g per 100 g of body weight) was also registered at this time-point (Gray et al., *J. Urol.*, 136, 497, 1986). Mice were treated with the selective P2X7 receptor antagonist A438079 (100 mmol/kg) (McGaraughty et al., *Neurosci.*, 146, 1817, 2007), given 30 min before and 4 h after the CYP. Control animals received saline at the same intervals of time. All the experimental procedures were approved by the Local Ethics Committee (08/00074, CEUA, PUCRS). **Results:** This preliminary set of results shows that pretreatment with the selective P2X7 receptor antagonist A438079 inhibited the nociceptive behavior score induced by CYP ( $33 \pm 15$  %). In addition, the administration of A438079 produced a reduction of both edema and hemorrhage indexes ( $33 \pm 17$  %) in the gross evaluation. Of note, A438079 treatment markedly reduced the wet weight of bladders ( $53 \pm 10$  %). **Discussion:** In the recent years, the interest in the therapeutical potential of purinergic receptors has dramatically increased (Burnstock et al., *Pharmacol. Rev.*, 58, 58, 2006). Our study revealed the importance of P2X7 receptors in the HC induced by CYP. It is tempting to suggest that pharmacological inhibition of these receptors might represent a new therapeutical alternative for this pathological condition. **Financial support:** CNPq, PROBOLSAS-PUCRS.

#### 04.159

Heparin inhibits the myotoxic effect of *Apis mellifera* crude venom. Gaban GA, Fonseca TF, El-Kik CZ, Fernandes FFA, Melo PA UFRJ - Farmacologia Básica e Clínica

In many countries, including Brazil, bees are cultivated to get products like honey, beeswax and *propolis*. In Brazil, during the 50's, there was the hybridization of *Apis mellifera* (African) with European species forming an extremely aggressive new species which attacks animals and beekeepers being able to cause serious systemic reactions or even death <sup>(1, 2)</sup>. Recent data indicate the occurrence of more than 600 cases of accidents with bees in a year in the State of Rio de Janeiro, which can take even greater proportions if we consider the cases occurring throughout Brazil. This study aims to assess the ability of heparin (a polyanion) to antagonize the activity of of *Apis mellifera* venom with *in vitro* and *in vivo* approaches. Using the modified method of Marinette (1985) we evaluated *in vitro* the phospholipase activity using as substrate the suspension of chicken egg yolk. Initially it was done to examine the effect of venom in increasing concentrations (0.1 to 10 µg/mL). Next we antagonized the venom with increasing concentrations of heparin (0.01 to 0.5 mg/mL). The myotoxicity *in vivo* was examined by the evaluation of edema and activity of creatine-kinase (CK) in plasma. We used adult mice (20-25 g) divided into groups of 5 animals. The activity of CK in plasma was measured 2 hours after perimuscular (pm) injection in increasing doses of venom (0.1 to 1.0 mg/kg) in the hind limb muscle *extensor digitorum longus* <sup>(3)</sup>. Then we tested the effect of increasing doses of heparin (3 to 30 mg/kg), pre-incubated for 30 minutes with the bee venom. The edema was observed 15, 30, 45, 60 and 90 minutes after the intramuscular injection of venom pre-incubated with heparin using a paquimeter. In the phospholipase activity heparin showed a concentration-dependent inhibition of the bee venom (1µg/ml). For myotoxicity *in vivo* heparin showed significant inhibitory effect of the venom (3 mg/kg), and for edema it was observed inhibition of the venom preincubated with heparin 1,0 and 3,0 mg/kg. Our results show that heparin, a polyanion, protects from many significant actions of bee venom, probably due to its interaction with positive charges of toxins present in the venom. **Financial Support:** CAPES, CNPq, PRONEX and FAPERJ. **Bibliografia:** 1. Barravieira, B. Ed. EPUC, p. 33; 1994; 2. Bücherl, W. Ed. Syntex, p. 89; 1979; 3. Melo, PA. *Braz. J. Med. Biol. Res.* v.21, p. 545;1988

#### 04.160

O fator de ativação plaquetário (PAF) desempenha importante papel na malária cerebral experimental. Queiroz NL<sup>1</sup>, Miranda AS<sup>2</sup>, Rodrigues DH<sup>3</sup>, Vilela MC<sup>3</sup>, Amaral DCG<sup>3</sup>, Camargos ERS<sup>4</sup>, Carvalho LJM<sup>5</sup>, Teixeira MM<sup>6</sup>, Teixeira AL<sup>7</sup> <sup>1</sup>UFMG - Parasitologia, <sup>2</sup>UFMG - Infectologia e Medicina Tropical, <sup>3</sup>ICB-UFMG, <sup>4</sup>UFMG - Morfologia, <sup>5</sup>FIOCRUZ - Imunologia, <sup>6</sup>UFMG - Bioquímica e Imunologia, <sup>7</sup>UFMG - Medicina

**Introdução:** A malária cerebral (MC) é uma manifestação grave da infecção por *Plasmodium falciparum*. No modelo murino de MC por *Plasmodium berghei* ANKA (PbA), a MC é o resultado de uma resposta inflamatória exacerbada do hospedeiro. Adicionalmente, o fator de ativação plaquetário (PAF) é um mediador inflamatório capaz de desencadear processos inflamatórios, como alteração na permeabilidade vascular e ativação de leucócitos, com graves consequências para o hospedeiro. **Objetivos:** Investigar o papel do receptor do fator de ativação plaquetário (PAFR) na infecção por PbA. **Material e Métodos:** Camundongos C57Bl/6 WT e PAFR<sup>-/-</sup> foram infectados i.p. com inoculo de 10<sup>6</sup> hemácias parasitadas. A microscopia intravital foi utilizada para analisar os eventos de rolamento e adesão de leucócitos na microcirculação cerebral. O tecido cerebral foi avaliado por técnicas histopatológicas (H&E e cresil violeta) com o objetivo de determinar alterações morfológicas e dano neuronal. O seqüestro de monócitos/macrófagos para o tecido cerebral foi quantificado por ensaio espectrofotométrico da atividade da enzima N-acetil-b-D-glicosaminidase (NAG). A concentração de TNF- $\alpha$  e das quimiocinas CXCL1, CXCL9, CCL2, CCL3 e CCL5 foi dosada em homogenato de tecido cerebral através de ELISA. Adicionalmente, o tratamento de animais C57Bl/6 com a droga antagonista de PAFR (UK-74505) foi realizado diariamente a partir do terceiro dia de infecção. Este trabalho foi aprovado pelo Comitê de Ética animal (CETEA) da UFMG através do parecer 193/06. **Resultados:** Camundongos PAFR<sup>-/-</sup> apresentaram uma maior sobrevida à infecção e um padrão de variação de massa corporal distinto dos animais WT. No quinto dia de infecção, a análise histopatológica demonstrou infiltrado inflamatório perivascular, hemorragia e dano neuronal mais intensos em camundongos C57Bl/6 WT quando comparados com animais PAFR<sup>-/-</sup>. Nesta mesma data, animais infectados apresentaram aumento no número de leucócitos rolando e aderidos no endotélio cerebral em comparação com animais controle. Entretanto, apenas o rolamento apresentou diferença significativa entre os grupos WT e PAFR<sup>-/-</sup> infectados, sendo maior neste último. No quinto dia de infecção, com exceção da citocina TNF- $\alpha$ , os valores de NAG e da concentração das quimiocinas não apresentaram diferença entre os grupos WT e PAFR<sup>-/-</sup>. A estratégia de tratamento (UK-74505) utilizada foi capaz de mimetizar os dados de sobrevida, variação de massa corporal e parasitemia encontrado nos animais PAFR<sup>-/-</sup>. **Discussão:** Os resultados sugerem o papel do PAFR no desenvolvimento do processo inflamatório que acarreta na MC e o bloqueio deste receptor com UK-74505 é capaz de prevenir o desenvolvimento da MC. **Suporte financeiro:** CAPES, CNPq, FAPEMIG.

#### 04.161

Influência dos hormônios sexuais femininos (HSF) sobre a produção/liberação de citocinas no pulmão em modelo murino de asma aguda. Accetturi BG<sup>1</sup>, Ligeiro de Oliveira AP<sup>1</sup>, Rodrigues-Soares C<sup>1</sup>, Domingos HV<sup>1</sup>, Oliveira-Filho RM<sup>1</sup>, Lima C<sup>2</sup>, Tavares de Lima W<sup>1</sup> <sup>1</sup>ICB-USP - Farmacologia, <sup>2</sup>Instituto Butantan - Imunologia

**Introdução:** Em estudos anteriores mostramos que a sensibilização antigênica 7 dias após a ovariectomia (OVx) reduz a inflamação pulmonar em modelo agudo de asma, sugerindo que o curso da inflamação alérgica pulmonar (IAP) depende dos níveis circulantes de HSF no momento da sensibilização antigênica. No presente estudo, apresentamos dados acerca do papel dos hormônios sexuais femininos na modulação dos mecanismos envolvidos com o desencadeamento da inflamação alérgica pulmonar. Portanto, avaliamos a modulação dos hormônios sexuais femininos sobre os níveis de IL-1 $\beta$ , TNF- $\alpha$ , IL-4, IL-5 e IL-13 e IL-10 em explante pulmonar. **Material e métodos:** Foram utilizados camundongos Balb/C fêmeas ovariectomizadas (OVx) ou falsamente operadas (sham), sensibilizados e desafiados com OVA. Os animais foram eutanasiados com dose excessiva de cetamina e xilazina e o pulmão retirado para quantificação de citocinas pelo método de explante pulmonar. As citocinas IL-1b, IL-4, IL-5, IL-10, IL-13 e TNF-a foram quantificadas por ELISA, através de Kits Duo Set (R & D System<sup>®</sup>). Os ensaios foram conduzidos seguindo as especificações do fabricante. **Resultados:** Nossos dados mostraram aumento significativo da liberação de IL-1b, IL-4, IL-5, IL-13 e TNF- $\alpha$  no explante pulmonar de animais Sham OVx alérgico (com e sem estímulo de LPS ou OVA) em relação aos seus respectivos grupos basais. No grupo OVx alérgico (com e sem estímulo de LPS ou OVA) observamos redução significativa dos níveis destas citocinas em relação ao grupo Sham OVx. Com relação a IL-10, verificamos aumento significativo dessa citocina nos animais OVx quando comparado ao grupo Sham OVx. **Conclusão:** Os dados obtidos sugerem que os HSF podem interferir com a produção/liberação de citocinas no pulmão, podendo assim modular o desenvolvimento da inflamação alérgica pulmonar. **Apoio financeiro:** FAPESP (05/02271-5, 2007 / 52220-3), CNPQ. **Comissão de ética:** 121/06/CEEA



#### 04.162

Influência dos hormônios sexuais femininos sobre a inflamação alérgica pulmonar em modelo murino de asma crônica. Rodrigues-Soares C<sup>1</sup>, Martins IO<sup>1</sup>, Vieira RP<sup>2</sup>, Golega BA<sup>1</sup>, Ligeiro de Oliveira AP<sup>1</sup>, Oliveira-Filho RM<sup>1</sup>, Tavares de Lima W<sup>1</sup> <sup>1</sup>ICB-USP - Farmacologia, <sup>2</sup>USP - Patologia

**Objetivos:** A asma é doença inflamatória pulmonar crônica caracterizada por obstrução das vias aéreas, hiperreatividade e migração de células para o pulmão, tais como neutrófilos, eosinófilos, macrófagos e linfócitos. A presença de muco, colágeno e fibrose são características do processo inflamatório crônico e estão presentes no remodelamento das vias aéreas na asma. Trabalhos anteriores do nosso grupo sugerem a existência de um mecanismo de interferência dos hormônios sexuais femininos com o sistema imune dependente do período de tempo existente entre a remoção dos ovários e a sensibilização dos animais com o antígeno. Nestes estudos os hormônios sexuais femininos são potenciais determinantes da gravidade da asma. Assim, neste estudo avaliamos o papel dos hormônios sexuais femininos na inflamação pulmonar em modelo experimental de asma crônica. **Métodos e resultados:** Fêmeas (C57Bl/6) submetidas a ovariectomia (OVx), confirmada pelo peso uterino e esfregaço vaginal, foram sensibilizadas com OVA (10 mg OVA/10 mg de alumínio, i.p) após 7 dias da cirurgia. Como controles foram utilizadas fêmeas falso-operadas (Sham-OVx). Decorridos 21 dias os animais foram desafiados por 15 min com solução de OVA 1%, durante 3 dias consecutivos. Este procedimento foi repetido por 3 semanas com 4 dias de intervalo entre um ciclo e outro de desafio com OVA. Decorridos 47 dias os animais foram desafiados e o experimento conduzido após 24h. Foram realizados lavados broncoalveolar (LBA), leucograma, lavado femural (LF), análise histológica de amostras do pulmão e determinação das concentrações de interleucinas (IL) 4,5,10 e 13. **Resultados:** Os dados obtidos indicam que o grupo OVx teve redução do número total de células no LBA em comparação com o grupo Sham-OVx (Sham:  $71,25 \pm 7,3$  vs OVx:  $4,6 \pm 0,6 \times 10^4$ ). Por outro lado verificamos aumento no grupo OVx da celularidade total do sangue (Sham:  $365 \pm 59,2$  vs OVx:  $580 \pm 38,7 \text{ mm}^3$ ) e do LF (Sham:  $54,2 \pm 4,0$  vs OVx:  $124 \pm 17,5 \times 10^6$ ). Observamos também redução na produção de muco (Sham:  $40,33 \pm 0,8819$  vs OVx:  $26,47 \pm 0,9171$ ) e colágeno (Sham:  $4,775 \pm 0,6810$  vs OVx:  $2,634 \pm 0,6506$ ) no grupo OVx em relação ao grupo Sham-OVx. Em relação às concentrações de citocinas, observou-se que no grupo OVx houve redução na produção de IL-5 em relação ao grupo Sham-OVx (Sham:  $2,265 \pm 0,7571$  vs OVx:  $1,515 \pm 0,2899$ ), todavia não foram encontradas diferenças significantes entre os grupos em relação as IL-4, 10,13. **Conclusão:** A presença dos hormônios sexuais femininos influencia o curso da inflamação alérgica pulmonar crônica de forma a modular positivamente a celularidade pulmonar e a produção de muco e colágeno nas vias aéreas após o desafio crônico com o antígeno. **Apoio Financeiro:** CNPq, FAPESP.

#### 04.163

Influence of female sex hormones on the remodeling and airway responsiveness in a murine model of asthma. Martins IO<sup>1</sup>, Rodrigues-Soares C<sup>1</sup>, Ligeiro de Oliveira AP<sup>1</sup>, Vieira RP<sup>2</sup>, Tavares de Lima W<sup>1</sup> <sup>1</sup>ICB-USP - Farmacologia, <sup>2</sup>USP - Farmacologia, <sup>2</sup>USP - Patologia

**Introduction:** Asthma is a chronic lung inflammatory disease characterized by airway tissue injury and inflammatory cell influx. The chronic inflammation modifies bronchial structure, causing remodeling and increased responsiveness. Remodeling is recognized by fibrosis of sub-epithelial basement membrane, hypertrophy of bronchial glands, goblet cell hyperplasia and thickening of airway epithelium. Remodeling has been recognized as one of the most important factors related to bronchial increased responsiveness and impairment of lungs function. Compelling evidence shows that female sex hormones (HSF) exert a controversial role in women during menses and post-menopausal period. Experimental and clinical evidences show that HSF attenuate/maximize inflammatory process in acute allergic lung inflammation. In this study we investigated role of HSF in airway remodeling and tracheal responsiveness in a murine model of chronic asthma. **Material and Methods:** Female C57B/6 mice were subjected to ovaries removal (OVx) and 7 days later they were OVA- sensitized by subcutaneous route. The animals were boosted after 1 week (OVA 1mg/ml SC). After seven days, OVA-challenge (aerosol, 15 min, OVA 1%) three times a week during 03 weeks was performed (OVx-allergic mice). Elapsed 72h of the last OVA-challenge the mice were euthanized and the maximal contractile response (Emax) of tracheal rings to methacholine (MCh) was performed *in vitro* using an organ bath system. Histological evaluations (*picrusirius red* and PAS staining) to identify the collagen deposition and mucus production on bronchial compartment were respectively carried out (Image Pro-Plus" software). Control group consisted of Sham OVx-allergic mice. **Results:** OVx-allergic mice significantly reduced tracheal responsiveness to MCh (Emax: 3.65 g) in comparison to Sham-OVx allergic mice (Emax: 1.6 g) Bronchial mucus and collagen productions were also reduced after chronic antigen exposure of OVx mice compared with the significant increased observe in Sham OVx- allergic mice. **Conclusions:** Removal of ovaries affected the magnitude of lung inflammatory response after chronic exposure to antigen suggesting that sex hormones could mediate the airway responsiveness and remodeling in asthmatic women. Financial support: FAPESP, CNPq. Ethical number protocol: 101 CEEA ICB USP

#### 04.164

Activation of peroxisome proliferator-activated receptor- $\gamma$  improve the survival in severe sepsis by inhibition of serum levels of alpha-1-acid glycoprotein. Mestriner FLAC<sup>1</sup>, Spiller F<sup>1</sup>, Sonogo F<sup>1</sup>, Czaikoski PG<sup>1</sup>, Vieira SM<sup>2</sup>, Dal-Secco D<sup>3</sup>, Cunha FQ<sup>1</sup>  
<sup>1</sup>FMRP-USP - Pharmacology, <sup>2</sup>COPE-INPA, <sup>3</sup>UFSC - Pharmacology

Peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor superfamily, have a newly recognized role in several inflammatory conditions. PPAR $\gamma$  activation was showed to attenuate the systemic inflammatory response during experimental endotoxic shock and, as a consequence, improved the survival rate. However, the role of PPAR $\gamma$  activation for neutrophil migration and, consequently, for sepsis outcome was not fully explored. Therefore, our objective was evaluated the effects of PPAR $\gamma$  activation on neutrophil migration to infectious focus and for the systemic inflammatory response during severe sepsis induced by cecal ligation and puncture. These experimental protocols were approved (089/2006) by CETEA – FMRP – USP. Our results showed that the pretreatments of mice subjected to severe sepsis with rosiglitazone (an agonist of PPAR $\gamma$ ), improved the neutrophil migration into the infection foci. As a result, the infection was controlled, as showed by reduction of CFU content in the infection focus and blood, and the neutrophil sequestration to lung was prevented. Accordingly, the pre-treatment with rosiglitazone improved the survival rate from 0% to ~40% at 8 days after surgery. Attractively, latter therapeutic treatment of the severe septic mice with this PPAR $\gamma$  agonist (3, 6, 12 h after surgery and each 12 h until 5 days) also improved the survival rate from 0% to ~40%. Moreover, rosiglitazone-pretreatment prevented the serum rise of the acute phase protein alpha-1-acid glycoprotein (AGP) during severe sepsis. AGP was described to increase the mortality in sepsis by their inhibitory action under neutrophil migration. Our results suggest that pharmacological activation of PPAR $\gamma$  during human severe sepsis could decrease the high mortality rates observed on this pathological condition.

#### 04.165

Modulation of GATA-3 expression is involved in resistance to steroid therapy in a murine model of allergic airway inflammation. Serra MF, Pão CRR, Anjos-Valotta EA dos, Jurgilas PB, Couto GC, Cotias AC, Pires ALA, Cordeiro RSB, Silva PMR, Martins MA FIOCRUZ - Fisiologia e Farmacodinâmica

**Introduction:** Antigen inhalation in asthmatic results in an early response, accompanied by airway hyperresponsiveness (AHR) and inflammatory cell infiltration into the lungs that is mediated via expression of Th2 cytokines including IL-4, IL-5 and IL-13, which are regulated predominantly by transcription factor GATA-3. It was recently reported that corticosteroids have a potent inhibitory effect on GATA-3 by interfering with its phosphorylation and nuclear translocation, which are pivotal steps in the mode of action of this transcription factor. Although glucocorticoids (GCs) are highly effective anti-inflammatory agents, a small subset of patients shows persistent tissue inflammation and AHR despite treatment with high doses of GC. Since in previous studies we have demonstrated that the number of allergen provocations distinguishes the sensitiveness to dexamethasone in the A/J strain, we have investigated here the effectiveness of the GC therapy on Th2 cytokines and GATA-3 expression 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 0 and 14 by a mixture of Al(OH)<sub>3</sub> and ovalbumin (OVA), and challenged for 2 or 4 consecutive days starting at day 19 post-sensitization. The animals were subjected to treatment with dexamethasone (DEX, 3 mg/kg, oral), or vehicle, 1 h before each provocation. Invasive and non-invasive barometric plethysmography were employed for measurement of airway hyperresponsiveness (AHR). Peribronchial eosinophil infiltration and subepithelial airway fibrosis were analyzed 24 h post-challenge, by histomorphometry in formalin-fixed and paraffin-embedded lung sections stained with Hematoxylin and Eosin (H & E) or Trichrome Gomori, respectively. The chemokine eotaxin (CCL-11), IL-4, IL-5 and IL-13 were quantified in the lung tissue by ELISA as markers of the inflammatory status. Western blotting was used to investigate the expression of GATA-3. (License number - CEUA 0085-02). **Results:** We found that the two-provocation regimen was active in causing AHR, eosinophilic inflammation and fibrogenesis, all of which being clearly sensitive to DEX. As expected, these changes were intensified in those animals subjected to four allergen provocations. DEX remained active in preventing the eosinophil accumulation of BAL fluid samples but failed to alter tissue eosinophilia. Similarly, AHR, increased generation of IL-4, IL-13, eotaxin and fibrogenesis also appeared sensitive to DEX in case of two but not four OVA provocations. We also demonstrated that DEX inhibited GATA-3 expression in the lung tissue following 2 but not 4 consecutive OVA provocations. **Conclusion:** 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. Since, in parallel, the expression of GATA-3 in the lung also appeared increased and less sensitive to DEX, it is not unlikely that an overexpression of GATA-3 may contribute to the state of GC refractoriness seen in this model. **Financial support:** FAPERJ, CNPq and PRONEX

#### 04.166

Protein disulfide isomerase regulates phagocyte NADPH oxidase activation. Possible interaction with p47<sup>phox</sup>. Paes AMA<sup>1</sup>, Verissimo-Filho S<sup>2</sup>, Janiszewski M<sup>2</sup>, Laurindo FRM<sup>3</sup>, Lopes LR<sup>2</sup> <sup>1</sup>DCF-UFMA, <sup>2</sup>ICB-USP, <sup>3</sup>InCor-FMUSP

**Introduction:** The phagocytic NADPH oxidase catalyses the production of superoxide (O<sub>2</sub><sup>•</sup>) at the expense of NADPH. Activation of the catalytic complex is dependent on the assembly of the cytosolic subunits p47<sup>phox</sup>, p67<sup>phox</sup>, p40<sup>phox</sup> and Rac2 with the membrane-bound cytochrome b<sub>558</sub>, constituted by p22<sup>phox</sup> and gp91<sup>phox</sup>. We have previously shown that non-phagocytic NADPH oxidase is regulated by the redox chaperone protein disulfide isomerase (PDI; Laurindo *et al.*, *Antioxid Redox Signal*, 10:1101, 2008). Since neutrophil NADPH oxidase is a better characterized system, we sought to investigate the role of PDI in O<sub>2</sub><sup>•</sup> generation during the oxidative burst.

**Methods:** A semi-recombinant cell-free system composed by GST-bound recombinant proteins p47<sup>phox</sup>, p67<sup>phox</sup> and Rac2 incubated with neutrophil membranes was used to assess O<sub>2</sub><sup>•</sup> generation assayed by SOD-inhibitable cytochrome c reduction. NADPH oxidase activation in the presence of PDI antagonists, bacitracin (1mM) or scrRNase (100mg/mL), recombinant PDI (50 - 200 nM), as well as, PDI-mimetic peptides (1mM) containing the CxxC active site sequence were investigated. To further assess PDI/NADPH oxidase interaction resting or PMA-activated (100 ng/ml, 12 min.) neutrophils were submitted to subcellular fractionation followed by isolation of cytosol, membranes, specific granules and azurophilic granules. These fractions were used to characterize PDI translocation to the membrane upon stimulus and to investigate the interaction with NADPH oxidase subunits. All protocols here applied have been approved by the Human Research Ethics Committee (689/CEP).

**RESULTS AND DISCUSSION:** The addition of the PDI inhibitor scrambled RNase (100 µg/ml) and bacitracin (1mM) decreased by 75% (from 6.40 ± 0.55 to 1.65 ± 0.29 nmol O<sub>2</sub><sup>•</sup>/min/10<sup>7</sup> cell eq) or completely abolished NADPH oxidase activity, respectively. In order to investigate if the redox status of PDI could modulate superoxide generation, PDI was previously oxidized [PDI-S<sub>2</sub>] or reduced [PDI-(SH)<sub>2</sub>] by treatment with H<sub>2</sub>O<sub>2</sub> (0,5mM) or DTT (1mM), respectively. PDI-S<sub>2</sub> (100 nM) improved the rate of superoxide production, (from 6.03 ± 0.27 to 7.97 ± 0.28 nmol O<sub>2</sub><sup>•</sup>/min/10<sup>7</sup> cell eq, p<0,05). PDI-(SH)<sub>2</sub> had the opposite effect and decreased O<sub>2</sub><sup>•</sup> generation (to 4.37 ± 0.57 nmol O<sub>2</sub><sup>•</sup>/min/10<sup>7</sup> cell eq., p<0.01). The addition of peptides (1mM) containing PDI's redox CxxC sequence inhibited superoxide generation by 70 %. The same effect was not observed with the addition of the scrambled or mutant peptide, where the cysteines were substituted by alanines. Western blotting analyses showed that PMA activation induced PDI translocation to the membrane, as well as, increased PDI association to the cytoskeleton. PMA also induced co-immunoprecipitation of p47<sup>phox</sup> and p67<sup>phox</sup> with PDI. GST pull-down assays showed that upon activation, p47<sup>phox</sup> associates to the reduced form of PDI. Assessment of p47<sup>phox</sup> and PDI redox state *in vivo* showed that PDI and p47<sup>phox</sup> redox status shifts upon PMA activation. Taken together, these results strongly suggest a role for PDI redox state in oxidase modulation, possibly via interaction with p47<sup>phox</sup>. Therefore, PDI could act as a novel redox sensitive regulatory protein of neutrophil NADPH oxidase. **SUPPORT:** FAPESP, CNPq, CAPES

#### 04.167

Efeitos de uma metaloproteinase isolada de veneno de serpente na produção de mediadores inflamatórios envolvidos na inflamação articular: participação de sinoviócitos do tipo B. Fernandes CM<sup>1</sup>, Leiguez Jr E<sup>1</sup>, Rocha FA<sup>2</sup>, Gutiérrez JM<sup>3</sup>, Teixeira CFP<sup>1</sup> <sup>1</sup>Instituto Butantan - Farmacologia, <sup>2</sup>FM-UFC - Medicina Clínica, <sup>3</sup>ICP-UCR

**Introdução:** As metaloproteinases de venenos de serpentes apresentam homologia estrutural e funcional com as de mamíferos (MMPs), cujos níveis estão elevados em doenças inflamatórias, como a artrite reumatóide. Recentemente, demonstramos que a metaloproteinase BaP1, isolada do veneno de *Bothrops asper*, desencadeia eventos inflamatórios articulares e dor, com a liberação de importantes mediadores inflamatórios, como a prostaglandina E<sub>2</sub> (PGE<sub>2</sub>) e o fator de necrose tumoral (TNF- $\alpha$ ). Estes dados indicaram que, além de estarem associadas à destruição da cartilagem, as MMPs desempenham papel importante no desencadeamento de inflamação articular. Durante processos inflamatórios articulares, os fibroblastos sinoviais (sinoviócitos do tipo B) são as principais células envolvidas na produção e liberação de mediadores inflamatórios. Desse modo, os objetivos deste estudo foram avaliar a capacidade da BaP1 induzir, *in vivo*, a liberação das interleucinas (IL) -1, -6 e -18 e caracterizar a ação direta da BaP1 sobre sinoviócitos do tipo B, quanto à: 1) liberação de IL-1, -6 e -18, PGE<sub>2</sub> e o TNF- $\alpha$  e 2) expressão protéica das ciclooxigenases-1 e -2 (COX-1 e -2). **Métodos:** Para os estudos *in vivo* (Autorização do Comitê de Ética Animal, Instituto Butantan nº 576/09), ratos Wistar machos foram injetados por via intra-articular (i.art.) com BaP1 ou albumina sérica bovina (BSA - controle) (5mg/articulação). Entre 30 minutos e 48 horas, avaliou-se a liberação das IL-1, -6 e -18, por ensaio imunoenzimático específico (EIA). Para os estudos *in vitro*, os sinoviócitos do tipo B foram isolados das membranas sinoviais articulares de ratos e incubados com a BaP1 (12,5 mg/mL) ou meio de cultura RPMI (controle) e após determinados períodos de tempo (30 minutos à 24 horas) avaliou-se a liberação das IL-1, -6 e -18 e da PGE<sub>2</sub>, por EIA, do TNF- $\alpha$  por ensaio de citotoxicidade sobre células L929 e a expressão protéica das COX -1 e -2, por *Western blotting*. **Resultados:** A BaP1 induziu a liberação de IL-1 e -6, após 1 e 3 horas e de IL-18, após 12, 24 e 48 horas de sua injeção i.art.. A incubação da BaP1 com os sinoviócitos resultou na liberação de IL-1, após 12 e 24 horas de incubação, de IL-6, em todos os períodos de tempo estudados, de IL-18, após 1 e 24 horas, de PGE<sub>2</sub>, após 1 e 6 horas e de TNF- $\alpha$  entre 1 e 6 de incubação. Além disso, a BaP1 foi capaz de induzir a expressão protéica de COX-2 (entre 1 e 6 horas), mas não de COX-1 por essas células. **Discussão:** Os resultados demonstram que a BaP1 induz a liberação de interleucinas pró-inflamatórias, na cavidade articular de ratos e exerce um efeito estimulatório direto sobre os sinoviócitos, resultando na liberação desses mediadores e de PGE<sub>2</sub> e TNF- $\alpha$ . A expressão de COX-2 deve constituir uma etapa importante do mecanismo de ação da BaP1, para a produção da PGE<sub>2</sub>. Ainda, os sinoviócitos devem contribuir para o desencadeamento de eventos inflamatórios, induzidos pela BaP1, na articulação dos animais. Em conjunto, esses dados sugerem que os sinoviócitos devem ser células alvos da ação de metaloproteinases, durante processos inflamatórios articulares. **Apoio financeiro:** FAPESP 06/50723-5; 08/58988-3

#### 04.168

The dual role of heme oxygenase-1 in severe polymicrobial sepsis. Freitas A<sup>1</sup>, Alves-Filho JC<sup>1</sup>, Nascimento DCB<sup>2</sup>, Sonogo F<sup>1</sup>, Spiller F<sup>1</sup>, Dal-Secco D<sup>1</sup>, Suavinha MM<sup>1</sup>, Cunha FQ<sup>1</sup> <sup>1</sup>FMRP-USP - Farmacologia, <sup>2</sup>FMRP-USP - Imunologia e Bioquímica Básica

**Introduction:** During severe (S) sepsis occurs a marked failure of neutrophil migration into infectious focus, which is associated with dissemination of infection resulting in high mortality. Recently, we showed that heme oxygenase (HO) products, carbon monoxide and biliverdin, down regulate neutrophil recruitment by reducing the neutrophil/endothelium rolling and adhesion in a non-infectious inflammatory model. The present study aimed to investigate the role of HO-1 pathway in severe polymicrobial sepsis induced by cecal ligation and puncture (CLP). **Methods:** This study was approved by the ethical committee of School of Medicine of Ribeirão Preto, USP, license number: 085/2007. To evaluate the role of HO-1 in S-CLP, we used two experimental protocols: Balb/c mice were subjected to pretreatment (30 min before S-CLP) or pretreatment (30 min before S-CLP) followed by posttreatment protocol (6 h after S-CLP) with a specific HO-1 inhibitor, zinc protoporphyrin IX (ZnPPIX, 30 mg/kg, s.c). Mice were killed 6h after CLP and HO-1 expression in mesentery and in blood neutrophils were determined. In another set of experiment, mice were sacrificed 6 h and 12 h after CLP and intraperitoneal neutrophil migration, bacteremia, lung neutrophil sequestration, cytokines and mean arterial pressure (MAP) were determined. The survival rate of animals was also evaluated. **Results:** It was observed a significant increase in HO-1 expression in mesentery and in blood neutrophils after S-CLP. The ZnPPIX-pretreatment prevents the failure of neutrophil endothelium rolling, adhesion and migration as compared with mice pretreated with vehicle and subjected to S-CLP. As consequence, these mice presented reduced bacteremia, low levels of seric TNF-alpha and lung neutrophil sequestration, reduced liver, kidney and cardiac injury and improve the MAP, resulting in increase of survival rate. Surprisingly, the continuous inhibition of HO-1 did not avoid the mortality. Moreover, these mice presented neutrophil migration failure, high levels of seric TNF-alpha, raised lung neutrophil sequestration and liver and kidney dysfunction. **Conclusion:** These data suggested that HO-1 pathway has a dual effect during severe sepsis: On the one hand, the HO-1 inhibition in the early stagy of sepsis prevents the neutrophil migration impairment. On the other hand, the continuous HO-1 inhibition promotes the failure of neutrophil migration. Therefore, the data provides further insight into the physiopathology of sepsis. **Acknowledgements:** We thank Giuliana Bertozi, Fabíola Leslie Mestriner, Ana Kátia dos Santos and Diva Amabile Montanha de Sousa for technical assistance. **Supported by:** FAPESP

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Efeitos da serinoproteinase PA-BJ, isolada do veneno da serpente *Bothrops jararaca*, em células endoteliais *in vitro*: na participação das ciclooxigenases-1 e -2 na liberação de prostaciclina. Lima SA<sup>1</sup>, Matsubara MH<sup>1</sup>, Moreira V<sup>1</sup>, Serrano SMT<sup>2</sup>, Teixeira CFP<sup>1</sup> - <sup>1</sup>Instituto Butantan - Farmacologia, <sup>2</sup>Instituto Butantan - Toxinologia

**Introdução:** O envenenamento causado pelas serpentes da família Viperidae é caracterizado por graves distúrbios da coagulação sanguínea, com comprometimento do endotélio. Dentre os componentes desses venenos, as serinoproteinases são as principais enzimas envolvidas nas alterações da hemostasia, que ocorrem no envenenamento. No entanto, os efeitos das serinoproteinases no endotélio não foram esclarecidos até o presente. Do veneno da serpente *Bothrops jararaca*, foi isolada a serinoproteinase PA-BJ, reconhecida como enzima trombina-símile. Esta enzima promove a liberação de prostaciclina (PGI<sub>2</sub>) por células endoteliais (CEs) e induz agregação plaquetária por meio dos receptores PAR-1 e -4. Deste modo, os objetivos deste estudo foram avaliar os efeitos da PA-BJ sobre células endoteliais, em cultura, quanto à: a) expressão protéica de ciclooxigenase-1 e -2 (COX-1 e -2) e b) participação das COX-1 e -2 na liberação de prostaciclina. **Métodos:** Células endoteliais murinas, da linhagem tEnd, foram cultivadas em meio de cultura RPMI-1640 com 10% soro fetal bovino e semeadas em microplacas de 96 poços, para a formação de monocamadas, em incubadora com atmosfera controlada (5% CO<sub>2</sub>/37°C). Após atingirem confluência, as CEs foram incubadas com PA-BJ (1 µM) ou trombina (1 U/mL) (controle positivo) ou meio de cultura RPMI (controle negativo) por 24 horas. Decorrido este período de tempo, a expressão protéica das COX-1 e -2 foi avaliada, nas CEs, por *Western blotting*. A participação dessas enzimas, na produção de PGI<sub>2</sub> foi avaliada após a pré-incubação das células com a indometacina (10 µM) ou valeril salicilato (250 µM) ou etoricoxibe (10µM), 1 hora antes da adição da PA-BJ ou trombina ou RPMI. Após 24 horas, a produção de PGI<sub>2</sub> foi avaliada nos sobrenadantes das CEs por ensaio imunoenzimático específico (EIA). **Resultados:** A PA-BJ, na concentração e período avaliados, não foi capaz de alterar o padrão de expressão protéica das COX-1 e -2 em relação ao controle. Por outro lado, a trombina (controle positivo) aumentou a expressão protéica de ambas isoformas das COXs, de modo significativo, em relação ao controle. A pré-incubação das células endoteliais com o inibidor não seletivo das COXs (indometacina) ou com o inibidor seletivo da COX-1 (valeril salicilato) ou com o inibidor seletivo da COX-2 (etoricoxibe), diminuiu significativamente a liberação de PGI<sub>2</sub> induzida pela PA-BJ ou pela trombina, em relação ao controle. **Discussão:** Em conjunto, os resultados obtidos demonstram que a atividade das COX-1 e -2 devem representar um dos mecanismos prováveis do efeito da PA-BJ, na produção de PGI<sub>2</sub>, em CEs, uma vez que não houve aumento na expressão protéica das ciclooxigenases. **Apoio financeiro: FAPESP**



#### 04.170

Liberação local de mediadores quimiotáticos e aumento de leucócitos circulantes, induzidos pelo veneno de *Bothrops insularis* (VBi), em camundongos. Souto P, Moreira V, Sampaio MC, Teixeira CFP Instituto Butantan - Farmacologia

**Introdução:** A serpente *Bothrops insularis*, conhecida como jararaca-ilhoa, apresenta características genéticas e comportamentais diferenciadas, em relação às espécies continentais botrópicas. Estudos anteriores demonstraram que o veneno dessa serpente induz influxo leucocitário para o local de sua injeção, caracterizado pelo acúmulo de polimorfonucleares (PMN) entre 12 e 24 horas e de mononucleares (MN) ao longo de todos os tempos estudados. No entanto, os mediadores quimiotáticos envolvidos nesse evento ainda não foram elucidados. Além disso, uma vez que a migração de leucócitos para o foco inflamatório é precedida pelo aumento dessas células na circulação sanguínea, os objetivos deste estudo foram investigar se o VBi altera o número de leucócitos no sangue periférico dos animais e avaliar os efeitos do VBi na liberação de tromboxana B<sub>2</sub> (TXB<sub>2</sub>), de leucotrieno B<sub>4</sub> (LTB<sub>4</sub>) e da quimiocina CCL2 na cavidade peritoneal de camundongos. **Métodos:** Todos os protocolos foram aprovados pela Comissão de Ética no Uso de Animais do Instituto Butantan (463/08). Camundongos Swiss machos (18-20 g) receberam injeção intraperitoneal (i.p.) de VBi (0,05 µg/g) ou de salina apirogênica (controle). Após 30min, 1, 3, 6, 12, 24 ou 48h dessas injeções, o exsudato peritoneal foi coletado, centrifugado e o sobrenadante foi utilizado para a determinação dos níveis de TXB<sub>2</sub>, de LTB<sub>4</sub> e de CCL2, por ensaio imunoenzimático (EIA). O número total e diferencial dos leucócitos circulantes foi avaliado 24h antes (basal) ou 1, 3 ou 6h após a injeção i.p. do VBi ou de salina apirogênica. O número total de leucócitos foi determinado após a diluição de uma alíquota de sangue, coletada da extremidade caudal dos animais, em líquido do Turk (1:20, v/v) e contado em câmara de Neubauer, sob microscopia de luz. O número de leucócitos mononucleares (MN) e polimorfonucleares (PMN) foi determinado a partir de extensões sanguíneas, coradas com HEMA<sup>3</sup>, com base em critério de morfologia convencional, sob microscopia de luz. **Resultados:** O VBi causou aumento significativo da concentração de TXB<sub>2</sub> (2924,9±335,6 pg/mL) e de LTB<sub>4</sub> (2668,5±566,3 pg/mL) na 6<sup>a</sup>h após a sua injeção, em comparação aos controles (1785,2±209,8 e 555,8±247,8 pg/mL, respectivamente). A injeção do VBi causou um aumento significativo da concentração de CCL2 (9206,4±236,6 pg/mL), ao longo de todo o período de tempo estudado, em relação aos controles (3311,5±207,4 pg/mL). Além disso, o VBi induziu um aumento significativo do número de leucócitos totais (146±16,4 x10<sup>5</sup> Leuc./mL) de MN (102,5±11,05 x10<sup>5</sup> MN/mL) e de PMN (43,5±7 x10<sup>5</sup> PMN/mL) na circulação dos animais, na 3<sup>a</sup>h após a sua injeção, em comparação aos controles (64,6±5,4, 54,25±5 e 10,4±0,6 x10<sup>5</sup> células/mL, respectivamente). **Discussão:** Os dados obtidos demonstram que o VBi causou aumento da liberação de TXB<sub>2</sub>, LTB<sub>4</sub> e CCL2, sugerindo a participação desses mediadores quimiotáticos no recrutamento leucocitário para a cavidade peritoneal dos animais, observado anteriormente. Além disso, o aumento do número de leucócitos circulantes, causado pela injeção do VBi, parece contribuir para o recrutamento de leucócitos para o foco inflamatório. **Apoio Financeiro:** FAPESP, CNPq

#### 04.171

A MT-III, uma fosfolipase A<sub>2</sub> secretada, isolada do veneno da serpente *Bothrops asper*, induz a formação de corpúsculos lipídicos dependente da PI3K, p38MAPK E ERK1/2 e a expressão protéica da adrp, em macrófagos. Leiguez Jr E<sup>1</sup>, Zuliani JP<sup>2</sup>, Fernandes CM<sup>1</sup>, Sampaio MC<sup>1</sup>, Gutiérrez JM<sup>3</sup>, Teixeira CFP<sup>1</sup> <sup>1</sup>Instituto Butantan - Farmacologia, <sup>2</sup>IPEPATRO - Bioquímica, <sup>3</sup>ICP-UCR

**Introdução:** A miotoxina-III (MT-III), uma fosfolipase A<sub>2</sub> (FLA<sub>2</sub>), isolada do veneno da serpente *Bothrops asper*, induz reação inflamatória local marcante e o aumento do número de corpúsculos lipídicos (CLs), em macrófagos isolados. Essas inclusões lipídicas são formadas por lipídeos neutros, envoltos por uma única membrana de fosfolipídeos e por proteínas específicas, como a proteína relacionada à diferenciação de adipócitos (*adipose differentiation-related protein* - ADRP). Esta proteína é considerada um marcador de CLs e está relacionada ao acúmulo de lipídeos e à diferenciação de macrófagos em células espumosas. A formação de CLs constitui um processo rápido e regulado por vias de sinalização específicas. Diante disso, os objetivos desse trabalho foram avaliar a participação das proteínas PI3K, PTK, p38MAPK e ERK 1/2 na formação de CLs, induzida pela MT-III e a capacidade desta fosfolipase A<sub>2</sub> induzir o aumento da expressão da ADRP. **Materiais e Métodos:** (Comitê de Ética em Animais do Instituto Butantan, Protocolo: 540/08). Macrófagos elicitados foram obtidos da cavidade peritoneal de camundongos Swiss machos (18-20 g), após 96 horas da injeção de tioglicolato a 3 %. Essas células foram incubadas com a MT-III (6,3 µg/mL) ou meio de cultura RPMI (controle), por 1, 3 e 6 horas e foi mensurada a expressão protéica da ADRP por *Western blotting*. Para avaliação da participação das proteínas sinalizadoras, envolvidas na formação de CLs, os macrófagos foram pré-tratados com inibidores específicos: SB202190 (5 µM), inibidor da p38MAPK e PD98059 (25 µM), inibidor da ERK 1/2, LY294002 (1µM) e Wortmanina (5nM), inibidores da PI3K, Herbimicina (10µM), inibidor da PTK, e estimulados pela MT-III (6,3 µg/mL), por 1 hora. A formação dos CLs foi mensurada por marcação com tetróxido de ósmio a 1 % e contagem sob microscópio de contraste de fase. **Resultados:** A MT-III induziu um aumento expressão protéica da ADRP (500 %), significativo em relação ao controle, após 6 horas de sua incubação com os macrófagos. O pré-tratamento dos macrófagos com o inibidores da p38 ou ERK 1/2 ou PI3Ks reduziu (51,5, 45 e 71 %, respectivamente) a formação de CLs, induzida pela MT-III, em relação ao controle. Por outro lado, o pré-tratamento das células com o inibidor da PTK, não afetou a formação dos CLs, estimulada pela MT-III. **Discussão:** Em conjunto, esses dados demonstram a capacidade de uma FLA<sub>2</sub> secretada induzir o aumento da expressão protéica da ADRP, em macrófagos. Ainda, a formação de CLs, induzida pela MT-III, é dependente das proteínas PI3K, p38 e ERK 1/2, mas não da PTK. Esses dados sugerem um papel relevante dos corpúsculos lipídicos na ação inflamatória da FLA<sub>2</sub> MT-III. Além disso, esses dados abrem novas perspectivas sobre o papel de FLA<sub>2</sub> secretadas em patologias relacionadas ao desequilíbrio lipídico. **Apoio Financeiro:** FAPESP (06/58334-8); INCTOX, CNPq

#### 04.172

Aumento do número de neutrófilos circulantes e liberação local de mediadores quimiotáticos induzidos pelo veneno da serpente *Bothrops moojeni* (VBm), em camundongos. Sampaio MC<sup>1</sup>, Fernandes CM<sup>2</sup>, Leiguez Jr E<sup>2</sup>, Souto P<sup>2</sup>, Frare EO<sup>2</sup>, Teixeira CFP<sup>1</sup> <sup>1</sup>ICB-USP - Imunologia, <sup>2</sup>Instituto Butantan Farmacologia

**Introdução:** O veneno da serpente *Bothrops moojeni* (VBm) induz uma reação local intensa, com edema grave, hemorragia e mionecrose em humanos e em animais de experimentação. Estudos anteriores demonstraram que o VBm causa um importante influxo de leucócitos polimorfo e mononucleares para o local de sua injeção contudo, os mediadores quimiotáticos liberados por esse veneno ainda não foram investigados. Além disso, determinados estímulos inflamatórios induzem aumento de leucócitos circulantes. Desse modo, o objetivo deste estudo foi avaliar a capacidade do VBm liberar mediadores quimiotáticos, como a tromboxana A<sub>2</sub> (TXA<sub>2</sub>), do leucotrieno B<sub>4</sub> (LTB<sub>4</sub>) e da quimiocina CCL2, na cavidade peritoneal de camundongos, bem como o número de leucócitos circulantes no sangue periférico. **Métodos:** Todos os protocolos foram aprovados pela Comissão de Ética no Uso de Animais do Instituto Butantan (461/08). Camundongos Swiss machos (18-20 g) receberam injeção intraperitoneal (i.p.) de VBm (0,25 mg/g) ou de salina apirogênica (controle). O lavado da cavidade peritoneal dos animais foi coletado após 10 e 30 minutos, 1, 3, 6, 24 ou 48 horas dessas injeções. O exsudato foi centrifugado e o sobrenadante foi utilizado para a determinação dos níveis de TXB<sub>2</sub>, de LTB<sub>4</sub> e de CCL2, por ensaio imunoenzimático (EIA). O número total e diferencial dos leucócitos circulantes foi avaliado 1, 3 ou 6 horas após a injeção i.p. do VBm ou de salina apirogênica. O número total de leucócitos foi determinado após a diluição de uma alíquota de sangue, coletada da extremidade caudal dos animais, em líquido do Turk (1:20, v/v) e contado em câmara de Neubauer, sob microscopia de luz. Os leucócitos circulantes foram classificados e tipados a partir de extensões sanguíneas, coradas por Giemsa, com base em critério de morfologia convencional, sob microscopia de luz. **Resultados:** O VBm causou aumento significativo da concentração de TXB<sub>2</sub>, aos 10 e 30 minutos (média de 2596 ± 141 pg/mL), com máximo efeito em 1 hora (3953 ± 228 pg/mL), se comparado ao controle (1012 ± 76 pg/mL). A injeção do VBm induziu um aumento dos níveis de LTB<sub>4</sub>, na 6ª hora após a sua injeção (2368 ± 353 pg/mL), se comparado ao controle (548 ± 56 pg/mL). Os níveis de CCL2 aumentaram significativamente a partir da 1ª hora (2142 ± 117 pg/mL), com efeito máximo na 3ª hora (5924 ± 911 pg/mL) após a injeção do VBm, se comparados aos controles (média de 603 ± 83 pg/mL). Além disso, o VBm causou o aumento significativo do número de neutrófilos, na circulação periférica dos animais, na 3ª hora (27,2 ± 3,5 x10<sup>5</sup> células/mL) após a sua injeção, em comparação aos controles (6,9 ± 0,3 x10<sup>5</sup> células/mL). **Discussão:** Em conjunto, os resultados obtidos demonstram que o VBm causou a liberação da TXB<sub>2</sub>, do LTB<sub>4</sub> e de CCL2. Estes resultados sugerem a participação desses mediadores no recrutamento leucocitário induzido pelo VBm. Ainda, o aumento do número de neutrófilos circulantes, causado pela injeção do VBm, deve refletir no número dessas células, recrutadas para o foco inflamatório. **Apoio Financeiro:** FAPESP (06/58333-1), CNPq

#### 04.173

Dexametasona reverte a inflamação e a dismotilidade gástrica associada à mucosite intestinal por 5-fluorouracil em ratos. Lucetti LT<sup>1</sup>, Oliveira GJ<sup>1</sup>, Medeiros J-VR<sup>1</sup>, Barbosa ALR<sup>1</sup>, Brito GAC<sup>2</sup>, Ribeiro RA<sup>1</sup>, Soares PMG<sup>2</sup>, Souza MHLP<sup>1</sup> - <sup>1</sup>UFC - Fisiologia e Farmacologia, <sup>2</sup>UFC - Morfologia

**Introdução:** A mucosite induzida por antineoplásicos é um fator limitante na terapia anticâncer. Mucosite é um termo clínico que descreve uma síndrome caracterizada por ulceração da mucosa de todo o trato digestivo (Sonis, Oral, Oncol., v.34, p.39, 1998). Assim, a mucosite intestinal resulta em eventos inflamatórios, que levam as alterações de permeabilidade e trânsito intestinal, com a participação de citocinas pró-inflamatórias. A dexametasona (DEX) é um importante inibidor da síntese de citocinas (LIMA, Eur J Oral Sci, v.113(3), p.210, 2005). **Objetivos:** Investigar o papel da DEX nos aspectos inflamatórios e funcionais da mucosite intestinal induzida por 5-FU em ratos. **Métodos:** Protocolo foi aprovado pelo comitê de ética (protocolo 62/07). Ratos Wistar machos (200 – 250g) foram tratados com 5-FU (150 mg/Kg, i.p. dose única) ou com solução salina (controle). Outro grupo recebeu 5-FU + DEX (2,5mg/Kg, s.c.) meia hora após 5-FU até o dia do sacrifício, diariamente. No 3º dia após o 5-FU ou 5-FU + DEX, os animais foram sacrificados, amostras de duodeno (D), foram retiradas para avaliação histopatológica por escores, mensuração da atividade de mieloperoxidase por espectrofotometria, para dosagem de citocinas (pg/300µl) por ELISA e para imunohistoquímica de TNF- $\alpha$ , IL-1 $\beta$  e iNOS. Para o experimento de esvaziamento gástrico os animais receberam, por gavagem, 1,5 ml da refeição teste que consiste de um marcador não absorvível (0,75mg/ml de vermelho de fenol em 5% de glicose). Decorridos 20min, os animais foram sacrificados e a retenção gástrica foi medida por espectrofotometria a 540nm. **Resultados:** 5-FU induziu importantes alterações histopatológicas com a presença de vilos encurtados com células vacuolizadas, necrose das criptas, intenso infiltrado inflamatório, vacuolização e edema, o que foi revertido após o tratamento com DEX (valores: C= 0 (0-0), 5-FU= 2 (2-3), 5-FU + DEX= 0 (0-1). O tratamento com DEX também foi capaz de promover uma menor imunomarcagem para as citocinas (TNF- $\alpha$ , IL-1 $\beta$  e iNOS) quando comparadas aos animais que receberam 5-FU. Com relação à dosagem de TNF- $\alpha$ , IL-1 $\beta$  o tratamento com DEX foi capaz de reverter o aumento na concentração dessas citocinas no duodeno de animais com mucosite por 5-FU (valores: TNF- $\alpha$  (C= 153,70 $\pm$ 80,90, 5-FU= 585,10 $\pm$ 99,27, 5-FU + DEX= 167,40  $\pm$  28,32) e IL-1 $\beta$  (C= 361,90 $\pm$ 46,11, 5-FU= 1494,00 $\pm$ 163,60, 5-FU + DEX= 1030,00 $\pm$ 101,90). O tratamento com DEX melhorou a retenção gástrica observada nos animais que receberam 5-FU (valores: C= 43,48 $\pm$ 2,64%, 5-FU= 63,21 $\pm$ 1,79%, 5-FU + DEX= 53,82 $\pm$ 1,26%). **Conclusão:** Dexametasona reverteu os achados inflamatórios e funcionais da mucosite intestinal experimental causada por 5-FU em ratos. **Apoio Financeiro:** CNPq

Inflamação associada à mucosite intestinal por 5-fluorouracil em ratos – papel da pentoxifilina. Lucetti LT, Oliveira GJ, Barbosa ALR, Medeiros J-VR, Brito GAC, Ribeiro RA, Soares PMG, Souza MHL UFC - Fisiologia e Farmacologia

**Introdução:** Mucosite é um termo clínico que descreve uma síndrome caracterizada por ulceração da mucosa de todo o trato digestivo (Sonis, Oral, Oncol., v.34, p.39, 1998), sendo um freqüente efeito colateral associado ao uso de 5-fluorouracil (5-FU). Pentoxifilina (PTX) é um importante inibidor da síntese de citocinas, além de apresentar efeito protetor sobre a mucosite oral por 5-FU em hamster (LIMA, Eur. J. Oral. Sci., 113: 210; 2005). Assim, a mucosite intestinal resulta em eventos inflamatórios, com a participação de citocinas pró-inflamatórias. **Objetivos:** Investigar o papel da PTX nos aspectos inflamatórios da mucosite intestinal induzida por 5-FU em ratos. **Métodos:** Protocolo foi aprovado pelo comitê de ética (protocolo 62/07). Ratos Wistar machos (200 – 250g) foram tratados com 5-FU (150 mg/Kg, i.p. dose única) ou com solução salina (controle). Outros grupos receberam 5-FU + PTX (90mg/Kg, diariamente, i.p.) meia hora após 5-FU até o dia do sacrifício. No 3º dia após o 5-FU ou 5-FU + PTX, os animais foram sacrificados, amostras de duodeno (D), foram retiradas para avaliação histopatológica por escores, para mensuração da atividade de mieloperoxidase por espectrofotometria, para dosagem de citocinas (pg/300µl) por ELISA e para imunohistoquímica de TNF- $\alpha$ , IL-1 $\beta$  e iNOS. **Resultados:** PTX foi capaz de reverter significativamente às alterações histopatológicas como a presença de vilos encurtados com células vacuolizadas, necrose das criptas, intenso infiltrado inflamatório, vacuolização e edemas encontrados na mucosite intestinal por 5-FU (valores: C= 0 (0-0), 5-FU= 2 (2-3), 5-FU + PTX= 1 (0-1)). O tratamento com PTX também foi capaz de promover uma menor imunomarcagem para as citocinas (TNF- $\alpha$ , IL-1 $\beta$  e iNOS) quando comparadas aos animais que receberam 5-FU. O tratamento com PTX atenuou o aumento na concentração TNF- $\alpha$ , IL-1 $\beta$  no duodeno de animais com mucosite por 5-FU (valores: TNF- $\alpha$  (C= 153,70 $\pm$ 80,90, 5-FU= 585,10 $\pm$ 99,27, 5-FU + PTX= 212,50 $\pm$  37,14) e IL-1 $\beta$  (C= 361,9 $\pm$ 46,11, 5-FU= 1494 $\pm$ 163,60, 5-FU + PTX= 873,4 $\pm$ 97,38)). **Conclusão:** Os achados inflamatórios da mucosite intestinal experimental causada por 5-FU em ratos foram prevenidos após o tratamento com pentoxifilina. **Apoio Financeiro:** CNPq