04. Inflammation

04.001 Evaluation of involvement of B_1 and B_2 kinin receptors in systemic inflammation induced by Periodontitis. Prestes AP, Machado WM, Olchanheski Junior LR, Fernandes D UEPG – Pharmaceutical Sciences

Introduction: Inflammatory processes are associated with increased plasma levels of bradykinin (BK) and its metabolites Lys-des-Arg^9-BK (DABK) and des-Arg^{10}-Lys-BK. The action of kinins is mediated by activation of B_1 and B_2 receptors. (Campos MM. Brit J Pharmacol. 114:1005 p., 1995). The BK acts trough the constitutive B_2 receptor stimulation. In contrast, the B_1 receptor which are expressed only in tissues that have suffered trauma or infection are activated by metabolites of BK. Studies suggest that the pathophysiological action of B_1 receptors correlates with chronic inflammation, such as in periodontitis, rheumatoid arthritis and osteomyelitis. (Imamura T. J. Clin. Invest. 94: 361 p., 1994). **Objectives:** The purpose of this study was to investigate the participation of B_1 and B_2 receptors in systemic inflammatory process promoted by periodontitis in rats. **Methods:** Experiments were conducted on non-fasted male Wistar rats (200-300g) housed at 22 ± 2°C with a 12h : 12h light-dark cycle. A ligature was placed around rat mandibular and maxilar molars to induce periodontitis. A simulated procedure was performed in the sham group. After 14 days, the animals received intraplantar injection of 50μL of saline (control paw) and 50μL of saline containing BK (3 nmol/paw) or DABK (30 nmol/paw) in the contralateral paw (paw test). Edema was measured using plethysmometer from the intervals of 10, 20, 30, 60 and 120 minutes (min), and expressed in ml difference between the test and control paws. The results are presented as mean ± standard error of the mean. The two-way ANOVA followed by post hoc Bonferroni test was used for statistical evaluation between experimental groups with $p < 0.05$ considered significant. All procedures were approved by the ethics committe. Protocol number UEPG - 23080.034301/2009-36. **Results:** The values obtained for the paw edema induced by BK (n = 8) in time intervals of 10, 20, 30, 60 and 120 min were respectively 0.30 ± 0.03 ml, 0.41 ± 0.04 ml, 0.35 ± 0.05 ml, 0.29 ± 0.03 ml and 0.15 ± 0.03 ml for periodontitis animals and 0.22 ± 0.04 mL, 0.27 ± 0,05 ml, 0.25 ± 0.05 ml, 0.18 ± 0.02 ml and 0.16 ± 0.03 ml for sham operated animals. The values obtained for the paw edema induced by DABK (n = 7) time intervals of 10, 20, 30, 60 and 120 min were respectively 0.27 ± 0.04 mL, 0.43 ± 0.04 ml, 0.26 ± 0.05 ml, 0.29 ml ± 0.06 ml and 0.07 ± 0.03 ml for the animals periodontitis and 0.19 ± 0.06 ml, 0.23 ± 0.05 ml, 0.16 ± 0.04 ml, 0.11 ml ± 0.04 ml and 0.07 ± 0.03 ml for animals sham operated. Significant statistical differences between the experimental groups were observed in the time of 20 min for B_2 agonist and in the time 20 and 60 min for B_1 agonist. **Conclusion:** The results show that B_1 receptors are effectively expressed in systemic inflammation induced by periodontitis in rats. **Financial Support:** CNPq and Fundação Araucária.

04.002 The effect of mirtenol in models of acute inflammation carrageenan-induced in rats.

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Introduction: Myrtenol is a monoterpene of pleasant smell, it is employed in the cosmetics industry, and it is part of the chemical composition of essential oils from aromatic species that presents anti-inflammatory activity. This work aims to test, for the first time, the anti-inflammatory potential of myrtenol in models of paw edema and air pouch carrageenan-induced in Wistar rats. **Methods:** Wistar rats were treated with vehicle (0.9% saline, 10 mL/kg, p.o.), Myrtenol (12.5, 25, 50 mg/kg, p.o.) or indomethacin (10 mg/kg, p.o.) 60 min before injection of carrageenan (1%, 0.1 mL, i.pl.) in the sub-plantar region of the animal right hindpaw. After 1, 2, 3, 4 and 5h from the carrageenan administration, the paw diameter was determined using a plethysmometer (Insight ®). The edema was expressed as the difference, in milliliters (mL), between the final and initial volume of the paw. In the model of pouch air carrageenan-induced, the animals were anesthetized (ketamine and xylazine 50 and 5 mg/kg, i.m., respectively), trichotomized and were injected with 20 mL of sterile air administered in the intra-scapular area of the back and maintained by re-inflation with 10 mL of air 3 days later. On the sixth day the animals were treated p.o. with vehicle, myrtenol (12.5, 25 and 50 mg/kg) or dexamethasone (0.5 mg/kg). After one hour was administered 0.1 mL of carrageenan (1%) into the pouch and four hours later the animals were euthanized (sodium pentobarbital 100 mg/kg, i.p.); the contents of the air pouch were removed using Pasteur pipette after injection of 10 mL of PBS. The exudate cells were separated and the total number of leukocytes was counted (mm^3). The content was collected, transported to Falcon tubes, diluted (1:20) with Turk solution and performed the counting of total leukocytes in a Neubauer chamber. Ethics Committee of Animal Experimentation - CEEA/PI N°. 008/12. **Results and Discussion:** Myrtenol (25 mg/kg) significantly inhibited ($p<0.01$) of increase in paw edema by Cg in the 5 hours of observation, with an inhibition peak in the 5th hour (0.48 ± 00.4), when compared the vehicle (1.39 ± 0.11). The group treated with myrtenol (50 mg/kg) showed significant inhibition of the edema only at the 2nd hour of observation (0.62 ± 0.11) ($p<0.001$). Similarly to myrtenol (25 mg/kg), indomethacin (10 mg/kg) was able to reduce the edema observed at all times ($p<0.001$). Moreover, the treatment of the animals with myrtenol (25 and 50 mg/kg) and dexamethasone (0.5 mg/kg) reduced significantly ($p<0.01$ and $p<0.001$, respectively) the leukocyte migration (2890.0 ± 793.9, 3860.0 ± 387.7 and 1200.0 ± 153.3, respectively) to the air pouch four hours after Cg injection into the cavity when compared to saline group (5875.0 ± 589.0). These results indicate that myrtenol possesses anti-inflammatory activity by interfering with inflammatory mediators involved in acute inflammation induced by carrageenan. Studies are in progress in the attempt to clarify the action mechanism of this monoterpene in the inflammatory process. **Financial support:** UFPI/CAPES. Bhatia, S.P., McGinty, D., Letizia, C.S., Api, A.M. Food Chemic. Toxicol. 46, 237, 2008. Judzentiene, A.; Mockute, D. Chemistry. 14, 103, 2003. Muller, A.A.; Reiter, S.A.; Heider, K.G.; Wagner, Planta Med, 65, 7, 2001. Winter, C.A, Risley, E.A, Nuss, G.W. Proc Soc Exp Biol Med. 111, 544, 1962.

04.003 Chronic administration of methylmalonate induces neuroinflammation in young rats.

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Introduction: The methylmalonic acidemia is an inborn error of metabolism characterized by methylmalonic acid (MMA) accumulation in body fluids and tissues, causing neurological dysfunction, mitochondrial failure and oxidative stress (2,5,7,8). Although neurological evidence demonstrate that infection and/or inflammation mediators facilitate metabolic crises in patients (6), the involvement of neuroinflammatory processes in the neuropathology of this organic acidemia is not yet established. Therefore, the objective of the present work was to verify if the experimental model of MMA chronic injection in young rats alters the inflammation markers in cerebral cortex. **Methods:** Newborn Wistar rats were subcutaneously injected with MMA from the 5th to the 28th day of life, twice a day, ranged from 0.72 to 1.67 μmol/gram of body weight as a function of animal age (4). Three days after the last injection, the animals were euthanized, the blood was collected and the cerebral cortex was removed and immediately frozen. The total number of neutrophils in blood was determined through the differential count by the method of May-Grünwald-Giemsa and the total number of leukocytes was determined in Neubauer chamber after the dilution. The content of interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) was determined in cerebral cortex and blood serum by enzyme-linked immunosorbent assay kit. The determination of inducible nitric oxide synthase (iNOS) and 3-nitrotyrosine (3-NT) levels were carried out by Western blot analysis and by high performance liquid chromatography/ultraviolet detection, respectively. Data were analyzed by unpaired *t* test and the value of t is presented if $P<0.05$. **Results:** In the blood there was an increase in the mononuclear cells, reduction in the number of polymorphonuclear cells (t=5.505); an increase in the number of other types of blood leukocytes and a reduction in the neutrophils number in MMA-treated group (t=2.612). Furthermore, an increase in IL-1β (t=12.20 and 3.199) and TNF-α (t=14.61 and 5.357) levels in blood serum and cerebral cortex were observed. Concomitantly, MMA increased the expression of iNOS (t=2.402) and 3-NT levels (t=2.718) in the cerebral cortex of rats. **Discussion**: The overall results indicate that chronic administration of MMA increased pro-inflammatory markers in the cerebral cortex and reduced immune system defenses in blood. This leads to speculate that, through mechanisms not yet elucidated, the neuroinflammatory processes during critical periods of development may contribute to the progression of cognitive impairment in patients with methylmalonic acidemia as well as in other neurodegenerative diseases (1,3). **References**: 1. Aronica and Crino. Epilepsia 52-Suppl 3:26 (2011); 2. Chandler et al. Faseb J 23:1252 (2009); 3. Chung et al. BMB rep 43:225 (2010); 4. Dutra et al. Braz J Med Biol Res 24:595 (1991); 5. Fernandes et al. Cell Mol Neurobiol 31:775 (2011); 6. Horster and Hoffmann. Pediatr Nephrol 19:1071 (2004); 7. Manoli and Venditti. GeneReviews (1993); 8. Oberholzer et al. Arch Dis Child 42:492 (1967); Work supported by CNPq and CAPES. The authors gratefully acknowledge the kind help of BioEx and LabNeuro.

04.004 Role of hydrogen sulfide on apoptotic proteins expressions in allergic mice lungs.

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Introduction: Oxidative stress plays an important role in the pathogenesis of airway allergic diseases. In allergic asthma, activated inflammatory cells produce large amounts of reactive oxygen species (ROS) that may contribute to tissue injury. ROS were also involved in apoptotic pathway which includes enhanced ROS production by eosinophils. Apoptosis pathways can be initiated by diverse stimuli but all of them converge into common mechanisms involving cleavage of specific aspartic acids (caspases), as caspases 3, 6, 7, 8 and 9. Activation of these caspases results in activation of executioner caspases, such as caspase-3, that go ahead into cell apoptosis. Caspase-3 has been shown to be an important effector caspase in eosinophils following mitochondrial activation involving the Bcl-2 family protein Bax (Park and Bochner (Allergy Asthma Immunol Res. 2:87, 2010). Our previous study showed that the pre-treatment of OVA-sensitized mice with hydrogen sulfide (H_2S)-donor, NaHS, resulted in significant reduction of eosinophil migration to the lungs and increase in antioxidant enzymes as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) activities (Benetti et al Eur J Pharmacol. 698: 463, 2013. We, herein, investigated the effect of H_2S on the expression of apoptotic proteins caspase-3 and Bax in allergic mice lungs. **Methods:** All animal care and experimental procedures were approved by the local animal ethics committee (San Francisco University, Brazil; license number 001.03.12). BALB/c mice were subcutaneously sensitized with ovalbumin (OVA) and, 7 days later, were intranasally challenged with OVA twice-daily for 2 consecutive days. Half of the challenged mice were treated with NaHS or with the irreversible inhibitor of CSE enzyme, propargylglicine (PAG), 30 min before the OVA challenge. All the mice were killed 48 h after the first challenge; the lungs were removed and homogenized in buffer phosphate containing a protease inhibitor cocktail. Caspase-3 and Bax protein expressions were assessed by Western blotting.All data are expressed as mean ± S.E.M. of 6-7 independent experiments and were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test for multiple comparisons. Statistical significance was established at P<0.05. **Results:** The treatment of allergic mice with NaHS produced significant reduction (P<0.02) of caspase-3 expression [Caspase 3/b actin (relative density) = allergic non-treated control: 0.87 ± 0,03; NaHS-treated: 0.67 ± 0.08]. This effect was significantly reverted (P<0.005 compared to NaHS) by treatment of allergic mice with PAG (Caspase 3/b actin (relative density) = 1,35 ± 0.15). The expression of protein Bax was modified neither by NaHS nor by PAG treatments [Caspase 3/b actin (relative density) = allergic non-treated control: 1.1 ± 0.09; NaHS-treated: 1.3 ± 0.3; PAG-treated: 1.05 ± 0,2]. **Discussion**: Our results suggest that the H_2S could prevent allergic lungs damage through their antiapoptotic effect. The decrease in caspase-3 expression can be a consequence of ROS level decrease by antioxidant enzymes activities. These actions were produced by NaHS-treatment in the lung of allergic mice. **Financial Support**: Fapesp and CNPq

04.005 Anti-inflammatory activity of a new pyrazole derivative – LQFM 021. Florentino IF[1], Galdino PM[2], Oliveira LP[2], Sousa LV[2], Menegatti R[1], Costa EA[2] – [1]FF-UFG, [2]ICB-UFG

Introduction: The pyrazolone compounds have in their structure a pyrazoline ring, which is heterocyclic ring of five members with two adjacent nitrogen atoms and three carbons. These derivatives may have anti-inflammatory, analgesic and antipyretic activities. The Antipyrine was the first pyrazolone derivative used in the treatment of pain and inflammation, after research led to the discovery of aminopyrine and dipyrone. Research seeks the development of new analgesic agents with better pharmacological activity and less adverse effects. The aim of this work was to evaluate the antinoceptive and anti-inflammatory effects of the pyrazole derivative LQFM-021 synthesized in the Laboratory of Medicinal Pharmaceutical/FF/UFG. **Methods:** Male *Swiss* albino mice weighing approximately 30 g were used in this study; the experimental protocol was approved by the Research Ethics Committee of UFG (number 017/13). Pharmacological methods: acetic acid-induced abdominal writhing, formalin-induced pain, carrageenan-induced paw edema and -pleurisy tests. **Results and Discussion:** In the abdominal writhing test, the treatments (v.o.) with LQFM 021 (17; 75 and 300 mg/kg p.o.) reduced the number of writhing by 47.6; 51.8 and 64.1%, respectively. In the first phase of the formalin test, the treatments with LQFM 021 (15; 30 and 60 mg/kg p.o.) or morphine (10 mg/kg s.c.) reduced the licking time (s) by 33.8; 35.3; 43.0 and 99.5%, respectively, in the second phase, the same doses of LQFM 021 reduced the licking time (s) by 43.9;58.4 and 52.4%, respectively, and morphine (10 mg/kg s.c.) and indomethacin (10 mg/kg p.o.) reduced in 57.3 and 99.8%, respectively. In the paw edema test, the treatments with LQFM 021 (30 and 60 mg/kg p.o.) or indomethacin (10 mg/kg p.o.) began to reduced the edema starting from the 2nd hour by 25.3; 33.7 and 33.3%, respectively, at 3rd hour by 25.5; 33.0 and 36.0%, respectively, and at 4th hour by 30.4; 29.0 and 34.9%, respectively. In the pleurisy tests the treatment with LQFM 021 (30 mg/kg p.o.) reduced the leukocyte migration into pleural cavity by 38.1%, with reduction of polymorphonuclear recruitment by 32.8%, increased lymphocyte and macrophages by 43.4 and 65.5%, respectively. In pleurisy test was also observed a reduction of the Evan's blue concentration in the pleural exudates in 30.9% and decrease myeloperoxidase activity by 43.0%. In conclusion the results presented in this study revealed that the pyrazole derivative LQFM-021 exhibited antinociceptive activity in animal models of acute nociception. This pyrazole derivate has analgesic activity that involving central mechanisms and anti-inflammatory activity. The LQFM 021 was able to suppress inflammation induced by carrageenan, confirming anti-inflammatory activity that likely involves reduction in leukocyte migration, myeloperoxidase activity, exudation into pleural cavity and inhibition of edema formation. **Sources of research support:** FUNAPE/UFG, CNPq, FAPEG and CAPES.

04.006 Inosine effects on inflamed skin: **Purine P1(A_{2A}) receptor as a target**. Oliveira BDV[1], Lapa FR[2], Otuki MF[1], Santos ARS[2], Cabrini DA[1] [1]UFPR – Farmacologia, [2]UFSC – Farmacologia

Introduction: Inosine, adenosine first metabolite, was almost forgotten over 20 years, but in the late 90's with the discovery of its cytoprotective and anti-inflammatory effect and cloning of P1 adenosine receptors, attention turned again to inosine as a pharmacologic target. Our study aimed to verify if inosine exerted its effect on inflamed skin throughout a purine receptor (Burnstock *et al.*, 2012; Shafy *et al.*, 2012). **Methods:** Acute and chronic inflammation were produced by croton oil single and multiple topical application in mice ear, respectively. Thickness of ear was measured to ascertain oedema formation. P1 receptors antagonists were applied (ip) to verify inosine activation of these receptors on skin. Tissue samples were collected to enzymatic, immunoenzymatic and histological analysis. All experiments were approved by the Institutional Ethics Committee (approval protocol number 392/2009). **Results:** Inosine reduced acute oedema in 57 ± 12 % (1 mg/ear), and myeloperoxidase (MPO) activity in 84 ± 6% (0.6 mg/ear). The pretreatment with ZM241385 (A2a antagonist) and Caffeine (unspecific antagonist) reverted inosine effect 79.6 ± 7.7 % and 77.4 ± 10 %, respectively. Both antagonists also reverted inosine reduction effect on cell migration with inhibition of 89.0 ± 5.1 % and 86.0 ± 6.2 % respectively. The DPCPX (A1) and alloxazine (A3) receptors antagonists did not exerted effect on inosine oedema and cell migration reduction. Cytokines TNF-α and IL-1β were reduced by inosine treatment in 100% and 65.0 ± 11 %, respectively. Inosine also reduced chronic oedema in 64.0 ± 4% (0.6 mg/ear), MPO and N-acetyl-β-d-glucosaminidase (NAG) in 57.0 ± 8% and 34.0 ± 7% respectively (p>0,05%). **Discution:** Our data includes to the literature the topical anti-inflammatory action of inosine, showing reduction of inflammatory parameters on skin. This anti-inflammatory effect, of inosine on skin, is probably related to A2a receptor activation, although in chronic inflammation this action may be related to energy restoration and amelioration of wound healing and angiogenesis. Thus, these results encourage the research about inosine on chronic skin disorders. **Support:** CAPES, CNPq

04.007 New strain of Proteus sp. potentiates LTC_4 expression in lung inflammatory response induced by LPS. Ferreira RR[1,2], Tambellini VY[1], Silva RC[3], dos Santos LA[2], Balbino AM[2], Vasconcellos SP[4], Fernandes L[2], Landgraf MA[2,5], Landgraf RG[2] [1]ICB-USP – Biotério Central, [2]Unifesp-Diadema – Inflamação e Farmacologia Vascular, [3]Unifesp – Medicina Translacional, [4]Unifesp-Diadema – Ciências Biológicas, [5]ICB-USP – Farmacologia

Introduction: *P. mirabilis* is a gram negative bacillus belonging to the family Enterobacteriaceae, described as an etiologic agent in various infections. **Objectives**: Evaluate the pulmonary inflammatory response in mice infected with different strains of *Proteus* sp.. **Methods:** Groups of 6-8 male C57Bl/6 mice (20-25g) were infected with a suspension containing 10^{-1} CFU of *P. mirabilis* ATCC 25933, NCDC 2059-70 *(*40 µl, i.n.) or with 10^{-1} CFU of *Proteus* sp. *(*40 µl, i.n.) isolated from the lungs of mice in routine screening of the Control Laboratory of the Health Central Animal ICB/USP (CAM strain). Twenty-four hours after infection, mice were given LPS (*E. coli* – Sigma, i.n.). After a further period of 24 hours, mice were euthanized with overdose of anesthetic (150 mg/kg) and the bronchoalveolar lavage fluid was collected to evaluate total and differential cellular infiltration in lung. A fragment of lung tissue was removed, fixed and stained for subsequent histological analysis and other fragment is prepared to lipid mediators quantification (CEP 1666/09). **Results:** The administration of LPS increased 48x the total cells in bronchoalveolar lavage fluid, when compared to control mice. Similar result was obtained in mice infected with ATCC (50x). The *influx* of inflammatory *cells* into bronchoalveolar lavage fluid was higher in mice infected with ATCC (60%) plus LPS than the group infected with only ATCC. The CAM strain induced significant cell infiltration (89x), in bronchoalveolar lavage fluid, when compared to animal control; the administration of LPS in mice infected with the CAM strain increased cellular infiltration in 101%, when compared to mice that were infected with CAM strain. Histological analysis of cell infiltrate in lung tissue showed that both infection with ATCC (168%) and CAM strain (225%) induced increased cell influx into the lung, when compared to control group. After LPS administration, mice infected with the CAM strain showed increased lung inflammatory infiltrate (25%) when compared with the ATCC strain. The administration of LPS increased 94% PGE_2 production when compared to control group and this increase has not been altered in any of the experimental groups. LTB_4 production was no different between the groups, however levels of LTC_4 were increased (405%) only in groups infected with the CAM strain **Discussion/Conclusion**: Administration of LPS induced a higher lung inflammation response in mice infected with *P. mirabilis* CAM strain than in mice infected with *P. mirabilis* ATCC strain and this phenomenon is modulate by leukotriene expression. **Acknowledgements:** FAPESP (2010/01404-0, 2012/51104-8) and CNPq.

04.008 Investigation of topical photodynamic effect of cationic porphyrin. Carrenho LZB[1], Vandresen CC[1], Dallagnol JCC[1], Gonçalves AG[1], Noseda MD[2], Noseda MED[2], Ducatti D[2], Orsato A[2], Cabrini DA[3], Barreira SMW[1], Otuki MF[3] [1]UFPR – Ciências Farmacêuticas, [2]UFPR – Bioquímica, [3]UFPR – Farmacologia

Introduction: Photodynamic therapy (PDT) is a treatment modality that uses light in appropriated wavelength, oxygen and a photosensitizer in order to produce reactive oxygen species, inducing cell death. It is indicated for treatment of skin inflammatory hyperproliferative diseases such as skin cancer and psoriasis, once this treatment is capable to reduce cell viability by inhibiting epithelial cells proliferation. In this study we evaluated the possible photosensitizer property of cationic porphyrin (20-phenyl-5,10,15-tri (*N*-methylpyridinium-4-yl) porphyrin triiodide). **Methods:** Female Swiss mice (20-30g) were used. Cationic porphyrin activity was valued in the animal models of 12-Otetradecanoilforbol acetato (TPA) edema. Thus, the increase of the ear thickness (µm) was measured with a digital micrometer (Great MT-045B) before and 6 h after application of TPA (2.5 µg /ear), respectively. TPA, as well as cationic porphyrin (0.01 to 0.3 mg/ear) or dexamethasone (0.05 mg/ear), as a positive control, were dissolved in 20 µL of acetone and applied on the inner right ear. To irradiation studies, after treatment, animal were exposed to a white light from a compatible fiber optic probe (400-800 nm) attached to a 250 W quartz/halogen lamp (LumaCare®, USA, model LC122) at a fluence rate of 100 mW cm^{-2}during 20 minutes. All animal procedures were approved by the Institutional Ethics Committee (n531). **Results:** The combination of cationic porphyrin with irradiation and dexamethasone inhibited inflammatory parameters in TPA-induced edema models in a dose-dependent manner with DI_{50}=0.001248 (0.0006338-0.39) mg/ear and maximum inhibition of 87,9 ± 3,4% (0,1 mg/ear) as well as MPO activity with DI_{50}= 0.019 (0.0004350-0.9189) mg/ear and inhibition of 94.6 ± 3,3% (0.3 mg/ear). Dexamethasone used as a positive control inhibited 79.2 ± 5.4% (0.05 mg/ear) of edema and inflammatory cell infiltration (MPO). Mice control group (TPA and irradiation) showed no change in edema or in MPO activity produced by the phlogistic agent. The same was observed in a group of animals that received only porphyrin treatment alone without light irradiation. **Discussion:** These results suggest that cationic porphyrin with irradiation is a potential topical anti-inflammatory agent. Since it inverted skin inflammation induced for TPA, it should be considered as a new potential tool for the treatment of skin inflammatory diseases. Further investigation is necessary to elucidate the mechanism and support efficacy and security of this cationic porphyrin. **Support:** CAPES, CNPq and Fundação Araucária.

04.009 Standardization of animal model for screening of wound healing substances. Souza BB, Magalhães JF, Castro AB, Raimundo JM, Bonavita AG UFRJ

Introduction: Skin wound healing is a dynamic and complex process very highly orchestrated that is activated whenever there is a break in continuity of the skin. Generally it is divided in 3 phases: i) inflammation; ii) granulation tissue formation and re-epithelization; and iii) wound contraction and remodeling. This process is deficient in some diseases as diabetes mellitus. So the development of new and potent healing substances is necessary. However the actual methods described to evaluate wound healing do not show any standardization and uses a great number of animals to test only a few substances. So in this present work we are standardizing an animal model of wound healing that uses fewer animals and able to screening more healing substances. **Methods:** Female Wistar rats weighing 250-300g were anesthetized by intraperitoneal injection of ketamine (100mg/kg) and xylazine (20mg/kg) and the dorsal region was depilated and sterilized with a 70% alcohol solution. Then four fragments of the skin were surgically removed. As control model of wound healing we performed the most described method where only one fragment of $1cm^2$ is removed. The wound healing was evaluated by photos taken of the dorsal area for a period of 14 days and the images were analyzed using ImageJ software and represented as percentage of open wound.All exepriments were approved by Animal Care Ethical Comission of our institution under protocol MACAEE003. **Results and discussion:** The kinetic of wound healing in our four wounds model shows that all wounds completely heal in the same time (about 21 days) and in a similar way. These findings were equivalent with the data obtained with the one wound model. Our results suggest that the 4 wounds model is effective tool for screening of wound healing substances since is possible to test more samples in the same animal and using fewer animals for that.

04.010 Preventive and therapeutic anti-TNF-α therapy with pentoxifylline decreases arthritis and the associated periodontal co-morbidity in mice. Queiroz-Junior CM[1], Bessoni RLC[1], Costa VV[2], Souza DG[2], Teixeira MM[3], Silva TA[1] [1]UFMG – Oral Pathology, [2]UFMG – Microbiology, [3]UFMG – Biochemistry and Immunology

Introduction: The association between rheumatoid arthritis (RA) and periodontal disease has long been studied and some reports suggest that treating RA may improve the associated PD, and vice versa. This study aimed to evaluate the effects of an anti-tumor necrosis factor (TNF)-alpha therapy with pentoxifylline (PTX) in an experimental model of RA-associated PD. **Methods:** Male C57BL/6 mice were subjected to chronic antigen-induced arthritis (AIA) and daily treated with PTX (50 mg/kg, i.p.) using preventive (Pre-PTX) or therapeutic (The-PTX) strategies. Fourteen days after the antigen challenge, mice were euthanized and knee joints, maxillae and serum were collected for microscopic and/or immunoenzimatic analysis. ANOVA followed by Newman-Keuls post-test was used for statistical analysis. This study was approved by the institutional ethics committee (165/09). **Results:** AIA triggered significant leukocyte recruitment to the synovial cavity (C: 56253 ± 18748; AIA: 300000 ± 30619 cells/ml; $p<0.05$), tissue damage and proteoglycan loss (C: 92; AIA: 39 % joint proteoglycan; $p<0.05$) in the knee joint. Pre-PTX and The-PTX regimens decreased these signs of joint inflammation (Pre-PTX: 131253 ± 47184; The-PTX: 75003 ± 30617 cells/ml; Pre-PTX: 68; The-PTX: 66 % joint proteoglycan; $p<0.05$ *versus* AIA). The increased levels of TNF-α and IL-17 in periarticular tissues of AIA mice were also reduced by both PTX treatments (TNF-α: C: 214 ± 67; AIA: 454 ± 64 pg/100 mg tissue; $p<0.05$; Pre-PTX: 280 ± 33; The-PTX: 173 ± 44 pg/100 mg tissue; $p<0.05$ *versus* AIA; IL-17: C: 746 ± 232; AIA: 1770 ± 178 pg/100 mg tissue; $p<0.05$; Pre-PTX: 644 ± 62; The-PTX: 372 ± 96 pg/100 mg tissue; $p<0.05$ *versus* AIA). Serum levels of C-reactive protein, which were augmented after AIA, were reduced by the PTX regimens. Concomitantly to AIA, mice presented alveolar bone loss (C: 0.31 ± 0.01; AIA: 0.38 ± 0.02 mm^2; $p<0.05$), and recruitment of osteoclasts (C: 0.4 ± 0.06; AIA: 1.8 ± 0.3 TRAP+ cells/field; $p<0.05$) to periodontal tissues. Pre-PTX and The-PTX prevented and treated these signs of PD (Pre-PTX: 0.25 ± 0.02; The-PTX: 0.25 ± 0.01 cm^2; $p<0.05$ *versus* AIA; Pre-PTX: 0.08 ± 0.08; The-PTX: 0.08 ± 0.08 TRAP+ cells/field; $p<0.05$*versus* AIA). PTX treatment also decreased TNF-α (C: 118 ± 61; AIA: 752 ± 66 pg/100 mg tissue; $p<0.05$; Pre-PTX: 494 ± 35; The-PTX: 408 ± 26 pg/100 mg tissue; $p<0.05$ *versus* AIA) and increased IL-10 (C: 569 ± 87; AIA: 254 ± 151; Pre-PTX: 919 ± 162; $p<0.05$ *versus* AIA; The-PTX: 652 ± 68 pg/100 mg tissue;) expression in the maxillae of AIA mice, although it did not affect the expression of IFN-γ and IL-17. **Conclusions:** The current study shows the anti-inflammatory and bone protective effects of preventive and therapeutic PTX treatments, which decreased the joint damage triggered by AIA and the associated periodontal co-morbidity. **Support:** FAPEMIG, CAPES, CNPq

04.011 Tumor necrosis factor-alpha reduces adenosine diphosphate-induced platelet aggregation. Bonfitto PHL, Marcondes S, Antunes E FCM-Unicamp

Introduction: Platelets are the key cells for homeostasis maintenance, in the formation of pathological thrombus and, in the last few years, there has been growing evidence indicating their important role in inflammation. Tumor necrosis factor alpha (TNF-alpha) is an essential mediator in the pathogenesis of many inflammatory and cardiovascular disorders. It is produced by different cells including macrophages, T-cells, neutrophils, endothelial cells and cardiomyocytes. TNF-a induces iNOS expression and stimulates cyclooxygenase 2 (COX-2) and NADPH oxidase activity. In addition, TNF-a favors thrombus formation by increasing tissue factor expression and decreasing tissue plasminogen activator expression. The biological effects of TNF-a are mediated by binding on two different receptors—TNFR1 (CD120a, p55) and TNFR2 (CD120b, p75). Platelets express both types of TNF-a receptors, but there are just a few works showing the effects of TNF-a on these cells. TNF-a increases collagen-induced platelet aggregation and significant CD40L expression on platelet membrane in patients with heart failure. Therefore, in this study, we decided to investigate the effects of TNF-a in rat platelets stimulated by adenosine diphosphate (ADP). **Methods:** The present study was approved by the Human Ethics Committee of the State University of Campinas (UNICAMP) protocol number 2947-1. Blood from abdominal aorta of male Wistar rats (250-320g) was collected in ACD-C (9:1 v/v). Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min. The platelets were washed using citrated buffer (pH 6.0) and the number was adjusted to $1.2x10^{8}$ plat/ml. CaCl2 (1mM) was added to the platelet suspension. Platelet aggregation was measured in a two channel aggregometer (Chronolog Lumi-Aggregometer model 560-Ca) at 37°C with stirring (1000 rpm). Platelets were incubated with crescent concentrations of TNF-a (1 to 3000 pg/ml) for 5 to 30 min before addition of ADP (10 μM). The statistical significance between groups was determined by using ANOVA followed by the Tukey test. **Results:** Addition of ADP (10 μM) to washed platelet suspension induced a significant aggregation (62 ± 5%). ADP(10 μM)-induced aggregation was significantly reduced by pre-incubation of platelets with TNF-a 3000 pg/ml for 15 min and 30 min (inhibition of 58 ± 9% and 65 ± 7% compared to platelets incubated with saline, respectively). However, platelet aggregation was not modified by this high concentration of TNF-a after incubation for 5 min. TNF-a incubated with platelets for 30 min concentration–dependently reduced ADP-induced aggregation (18 ± 7%, 38 ± 4% and 62 ± 5% reduction at TNF-a concentration of 1, 10 and 100 pg/ml compared to platelets incubated with saline, respectively. N=5-8 animals). Similarly, the incubation of platelets with TNF-a for 15 min caused a dose-dependent inhibition of platelet aggregation (6 ± 2%, 31 ± 6% and 42 ± 2% reduction at TNF-a concentration of 1, 10 and 100 pg/ml compared to platelets incubated with saline, respectively. N=5). **Conclusion:** TNF-a time- and concentration–dependently reduces ADP-induced aggregation. Maximal platelet aggregation inhibition was reached at 30 min of incubation with 100 pg/ml of TNF-a. These results are the opposite to those described in the literature, probably because of the differences in the experimental conditions (humans with heart failure versus healthy rats) and the platelet agonists used (collagen versus ADP). The mechanisms involved in the inhibitory effect of TNF-a on platelet aggregation are under current investigation. **Supported by:** CAPES

04.012 Endothelial cell P2Y1 receptor purinergic signaling during chronic inflammation.

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Introduction and Aim: Schistosomiasis is an intravascular parasitic disease related to chronic inflammation. Previous data showed a host endothelial cell dysfunction (Silva *et al.*, 1998 *Comp. Biochem. Physiol.*, 120: 417, Oliveira et al., 2011, Plos One, 6:e23547) and alteration of purinergic P2X7 receptor, which is involved in inflammasome activation (Oliveira et al., 2013, Purinergic Signal. 9:81). The objective of this work was to evaluate the influence of chronic inflammation upon endothelial P2Y1 receptor (P2Y1R) signaling and the activity of the ectonucleotidase (ENTPD). **Methods:** All protocols were approved by the ethics committee of the UFRJ (DFBCICB011). Male Swiss mice (control and infected with *Schistosoma mansoni* cercariae, 75 days-old) were anesthetized and euthanized. The mesenteric endothelial cell culture and the isolation of mononuclear cells through Ficoll-Paque Plus gradient were performed as previous described (Oliveira *et al.*, Plos One 6:e23547, 2011). **Cell adhesion assays**: Confluent endothelial cells were incubated for 4h with vehicle (basal), the selective agonist of the P2Y1R 2MeSATP (30-60 μM) or MRS2179 (300 nM, antagonist) plus 2MeSATP. Next, 1E4 mononuclear cells/well were added and incubated for 30 min. After this period the non-adherent mononuclear cells were removed and four fields per well were randomly chosen and imaged (Olympus IX71 microscope, 400X). **Ectonucleotidase activity**: Confluent endothelial cells were incubated with 50 μM ATP and ^{32}P-ATP for 5 min. The radioactivity was determined by liquid scintillation counter. **P2Y1R Western blot**: 10 μg of proteins were loaded on a 10% SDS-PAGE gel, transferred to PVDF membrane and sequentially incubated with the monoclonal antibody anti-P2Y1R (1:1000; 2h) and secundary antibody anti-rabbit (1:500; 1h, Santa Cruz Biotechnology). The detection was performed by ECL. **Results and Discussion:** Mesenteric inflammation occurs during schistosomiasis (Turner et al., PLoS Pathogen, 8:e1003063, 2012). Data showed that the endothelial ectonucleotidase activity is increased during chronic inflammation, promoting a higher ATP hydrolysis (3.64 ± 0.74 pmol Pi/μg/min, P < 0.05) than in controls (1.66 ± 0.17 pmol Pi/μg/min, n = 8-11). RT-PCR data (n = 4) showed that there is an increased expression of ENTPD-2 in the inflamed group (P < 0.05), an enzyme with high affinity for ATP, but with a much lower affinity for ADP. The other enzyme (ENTPD-1) that is able to hydrolyze ATP and ADP equally is not expressed in these cells. Next, we investigated the influence of P2Y1R on leukocyte adhesion to endothelial cell. In the control group, the agonist 2MeSATP induced a concentration-dependent cell adhesion that was blocked by MRS2179 (one way ANOVA and Newman Keuls test, n =21-33, P < 0.01). However, in the inflamed group, there was a higher basal adhesion value as compared to control (P < 0.01), close to the maximal effect in the control group. Pre-treatment with MRS2179 reduced the values suggesting an autocrine endothelial cell activation due to the formation of extracellular ADP. The expression of P2Y1R was similar in both groups either by Western blot (densitometric data: 0.95 ± 0.04 and 0.96 ± 0.1 a.u, n = 5) or RT-PCR assays (n = 4). Leukocyte adhesion is a hallmark of inflammation and precedes diapedese. All together, present data suggest that during inflammation there is an increased formation of extracellular ADP and activation of endothelial P2Y1R that leads to an autocrine activation. These data might contribute to the mesenteric inflammation observed during schistosomiasis. **Support**: CNPq

04.013 Anti-inflammatory effect of methanolic extract and guanidine alkaloid N-1, N-2, N-3-triisopentenylguanidine from alchornea glandulosa in mice. Iwamoto RD[1], Formagio Neto F[1], Formagio ASN[2], Sarragiotto MH[3], Vieira MC[2], Kassuya CAL[1] [1]UFGD – Health Science, [2]UFGD – Agricultural Science, [3]UEM – Chemistry

Introduction: *Alchornea glandulosa* is popularly known as "amor seco", "tanheiro-de-folha-redonda", "tanheiro" or "canela-raposa" and is found in the southern and southwestern regions of Brazil. This plant has been used in folk medicine for the treatment of inflammatory diseases but there are no scientific publications to support these effects and thereby this work aimed to evaluate the anti-inflammatory properties of methanolic extract from leaves of *A. glandulosa* (MEAG) as well as the isolated compound guanidine alkaloid *N*-1, *N*-2, *N*-3-triisopentenylguanidine (AG) in mice. **Methods:** All experimental procedures were approved by the ethics committee for research on laboratory animals of the UFGD (Nbr. 005/2012). Experiments were conducted in male *Swiss* mice (20-25g, n=7) from UFGD. Anti-inflammatory effects of MEAG and AG-1 were studied in carrageenan-induced paw oedema (groups: MEAG 100 or 300 mg/kg p.o, AG-1 5 or 30 mg/kg p.o., and dexamethasone 1 mg/kg sc.) followed by myeloperoxidase (MPO) activity analysis (mD.O), croton oil-induced ear oedema (MEAG 1 mg/ear, vehicle, or dexamethasone topically administered in the right ear 15 min prior to the application of croton oil) and carrageenan-induced leukocyte migration in pleurisy (groups Vehicle, MEAG 100 or 300 mg/kg p.o, AG-1 5 or 30 mg/kg p.o, dexamethasone 1 mg/kg sc.), all performed in mice. All data are presented as the mean ± S.E.M. The difference between the groups was evaluated by one-way ANOVA followed by Student Newman-Keuls test. Significant values with $p<0.05$. **Results and Discussion:** MEAG (100 and 300 mg/kg) and AG-1 (5 and 30 mg/kg) significantly inhibited paw oedema formation by 76 ± 5 %, 79 ± 3% 65 ± 3% and 36 ± 6%, respectively, and decreased significantly the increase in carrageenan-induced MPO activity. 5 and 30 mg/kg of AG-1 inhibited MPO activity by approximately 82 ± 8% and 44 ± 5 %, respectively. For dexamethasone was 46 ± 4%. MEAG (100 and 300 mg/Kg) and AG-1 (5 and 30 mg/kg) decreased cell migration with maximum inhibitons of 64 ± 9%, 68 ± 16, 61 ± 5% and 85 ± 5%, respectively. MEAG inhibited oedema by 24 ± 2% and 17 ± 2%, after 4 and 6 h after croton oil, respectively.**Conclusion:** Methanolic extract of *A. glandulosa* exhibited oral and topical anti-inflammatory activities and these properties may be due, at least in part, to the presence of bioactive constituents such as the guanidine alkaloid *N*-1, *N*-2, *N*-3-triisopentenylguanidine. **Keywords:** *Alchornea glandulosa,* inflammation, mice. **References:** Conegero LdS, Ide RM, Nazari AS, Sarragiotto MH, Dias Filho BP, Nakamura CV, et al. Constituintes químicos de Alchornea glandulosa (Euphorbiaceae). Química Nova. 2003;26:825-7. Kassuya CA, Cremoneze A, Barros LF, Simas AS, Lapa Fda R, Mello-Silva R, et al. Antipyretic and anti-inflammatory properties of the ethanolic extract, dichloromethane fraction and costunolide from Magnolia ovata (Magnoliaceae). J Ethnopharmacol. 2009 Jul 30;124(3):369-76. Urrea-Bulla A, Suarez MM, Moreno-Murillo B. Biological activity of phenolic compounds from Alchornea glandulosa. Fitoterapia. 2004 Jun;75(3-4):392-4. **Acknowledgements:** The authors are grateful to FUNDECT, UFGD and CAPES for providing a research grant and fellowships.

04.014 Nitroxyl donor reduces septic arthritis inflammation in mice. Staurengo-Ferrari L[1], Miyazawa KWR[1], Domiciano TP[1], Ribeiro FAP[1], Pelayo JS[2], Miranda K[3], Casagrande R[4], Verri Junior WA[1] [1]UEL – Patologia, [2]UEL – Microbiologia, [3]University of Arizona, [4]UEL – Ciências Farmacêuticas

Introduction: *S.aureus*–induced septic arthritis remains a serious clinical problem, which induces permanent joint dysfunction in 40% of patients. Nitric oxide is an important microbicidal agent in host defense, but also regulates inflammation and pain. The uncharged form of NO($NO^{\bullet}$) is considered responsible for biological activities of NO. However, $NO^{\bullet}$ and the negatively charged form of NO(nitroxyl[(NO^{-}]) are relatively independent molecules since they present similar relevance, but may present different functions. Herein, we investigated the antinociceptive and antiinflamatory effects of the NO^{-} donor,Angeli's salt(AS), in a joint model of septic arthritis. Furthermore, we addressed its direct microbicidal effect *in vitro.* **Methods:** A suspension of *S. aureus* ATCC 6538 was prepared in PBS. The suspension was inoculated into the knee joints of Swiss mice, weighing 20g(n=6). The dose used was $1x10^{7}$CFU/joint. The negative control received 10 uL of PBS. The mice were treated daily with AS(3 mg/kg,sc,150 µL) or vehicle(NaOH 10mM) after intra-articular(i.a.) injection of *S.aureus* suspension. The mechanical hyperalgesia and edema were evaluated at every other day until 28th day after inoculation with an electronic pressure meter and caliper, respectively. Mice were sacrificed at 7, 14, 21 and 28th day after inoculation of *S.aureus* and the knee joint was harvested for determination of leukocyte recruitment, viable bacteria, cytokine production(IL-1β, TNFα, IFNγ, IL-17, IL-33) and proteoglycan levels. The microbicidal effect was determined by minimal inhibitory concentration. Statistical differences were considered to be significant at $p<0.05$ analyzed by one-way ANOVA and Tukey's test. Animal care and handling procedures were approved by the Ethics Committee of UEL(protocol n.33358).**Results and Discussion:** The mechanical hyperalgesia and edema were up to 40% and 64% lower in the group treated with AS compared to positive control group treated with vehicle, respectively at the point of highest difference during the 28 days. The group treated with AS presented significant reduction compared to positive control group treated with vehicle on days 7, 14, 21 and 28 after i.a. injection of *S. aureus* regarding leukocyte migration(83, 67, 89 and 85% for total leukocytes; 85, 70, 82 and 91% for Nø, and 73, 67, 86 and 88% for mononuclear cells respectively),viable bacteria recovery(92, 76, 67 and 77% respectively),cytokine production(IL-1β: 82, 44, 35 and 45% respectively; TNFα: 93, 68, 86 and 54% respectively; IFNγ: 82, 75, 65 and 62% respectively; IL-17: 94, 51, 76, 84% respectively; and IL-33: 47, 30, 71 and 98% respectively) and prevention of proteoglycan content(78, 60, 38, 96% respectively).Moreover, NO^{-} at the concentration of 10 uM inhibited up to 82% the growth of *S.aureus in vitro* compared to control antibiotic(ciprofloxacin 40µg). These results demonstrate that the mechanisms activated by NO^{-} are related to reduction of infection and inflammation indicating its possible usefulness as therapeutic molecule in septic arthritis. **Financial support:** CAPES, Decit/SCTIE/MS intermediated by CNPq and support of SETI/Fundação Araucária.

04.015 Role of glucocorticoid receptors on the transcription of genes related to LPS(*iv*)-induced effect on rat pineal gland. Fernandes PACM, Tamura EK, da Silveira Cruz-Machado S, Marçola M, Carvalho-Sousa CE, Muxel SM, Markus RP IB-USP – Fisiologia

Introduction: Pineal gland is an important player of the defense system in mammals (Markus *et al*., Neuroimmunomodulation, 14, 126, 2007), capable to functionally respond to several immunological signals produced during inflammatory processes. The production of melatonin by this gland is modulated by intracellular glucocorticoid receptor (GR, Ferreira*et al*., J Pineal Res, 38, 182, 2005) and nuclear factor kB (NFkB) activation by pathogen and/or damage associated molecular patterns, such as, the tumor necrosis factor (TNF, Fernandes *et al*., J Pineal Res, 41, 344, 2006) and lipopolysaccharides (LPS, da Silveira Cruz-Machado *et al*., J Pineal Res, 49, 183, 2010). LPS (iv) induces a transient inhibition of pineal gland melatonin synthesis (Tamura *et al*., PLoS One, 12 - e13958, 2010). In this study, we evaluated an array of genes involved in the integration of LPS, TNF and GR activation in pineal glands of rats injected with LPS. **Methods:** Male Wistar rats (250-350g, 12h/12h light/dark cycle) were injected with vehicle or RU-486, an antagonist of GR, (10 mg/kg, ip, two consecutive days one hour before darkness, ZT11) two hours before the challenge with LPS (0.5 mg/kg, iv) or saline. Animals were killed two hours after LPS injection (ZT18). Pineal glands were collected for the determination of mRNA content (pool of two or three glands) of immune-related genes by real time RT-PCR (rat TLR PCR-ARRAY, Qiagen). Data are expressed as mean ± sem and represent fold of increase over nighttime naïve animals. Animal Ethics Committees protocol: 122/2011 (CEUA IBUSP). **Results:** LPS injection significantly increase the transcription of *Ccl2*, *Csf2*, *Csf3*, *Cxcl10*, *Chuck*, *Clec4e*, *Cd14*, *Cd80*, *Cebpb*, *Fos*, *Infb1*, *Il1a*, *Il1b*, *Il6*, *Il6ra*, *Irf1*, *Nfkbia*, *Nfkb2*, *Ptgs2 (COX2)*, *Tnf*, *Tlr2*, *Tnfr1* and *Traf6* genes. The treatment with RU486 inhibits the LPS-induced transcription of the genes *Cebpb* (4.25 ± 1.10 *vs* 12.41 ± 2.18, n=3), *Clec4e* (4.37 ± 1.07 *vs* 15.33 ± 2.20, n=3), *Nfkbia* (0.847 ± 0.34 *vs* 2.67 ± 0,.8, n=3), *Ptgs2 (COX2)* (5.96 ± 1.67 vs 15.09 ± 1.45, n=3), *Tnf* (22.50 ± 3.05 *vs* 49.64 ± 9.31, n=3) and *Traf6* (1.86 ± 0.12 *vs* 4.12 ± 0.57, n=3). **Discussion:** The data show the relevance of endogenous glucocorticoid on the control of pineal function under the challenge of a molecular pattern associated to Gram-negative bacteria. In conclusion, together with enhancing the transcription of some pro-inflammatory genes (Tnfr1 Traf6, Cebpb, Tnf and Ptgs2), pineal GR-activation also leads the transcription of Nfkbia, which encodes the protein that sequestrate NFkB dimmers in the cytoplasm Financial support: FAPESP (Project: 2010/52687-1).

04.016 Inhibitory effects of staphylococcal enterotoxin type A (SEA) and B (SEB) on mice bone marrow eosinophil adhesion *in vitro*. Ferreira-Duarte AP[1], Torres ASP[1], de Souza IA[1], Mello GC[2], Antunes E[2], Squebola-Cola DM[2] [1]FMJ – Biology and Physiology, [2]FCM-Unicamp

Background: Clinical evidences have shown a strong correlation between SEA and SEB on bronchial asthma exacerbation. We have described that SEA and SEB mice airways exposition aggravate the pulmonary allergic inflammation by exacerbation of lung eosinophils (EO) infiltration and increased bone marrow (BM) eosinopoeisis. In the present study we evaluated the influence of mice pulmonary SEA or SEB exposition on BM EO *in vitro* adhesion induced by eotaxin. Effect of the incubation of BM EO from naïve animals with SEA and SEB on *in vitro* adhesion induced by eotaxin and RANTES was also evaluated. **Method:** BALB/C mice femurs were removed after killing, flushing with 2.5 mL of Iscove´s medium and submitted to granulocyte isolation protocol. The supernatants were collected, centrifuged (500 g for 10 min at 4°C), and the cell pellets resuspended to 4×10^6 cells/ml. Adhesion assays were carried out in 96-well plates pre-coated with recombinant mouse VCAM-1 and ICAM-1 (2.5 µg/ml) for 30 min in the presence of eotaxin or RANTES. The EO adhesion was calculated by measuring EO peroxidase activity on adherent cells. **Results:** Mice airways exposition to SEA for 48 h reduced the adhesive response of BM EO in ICAM-1 coated plates (eotaxin/untreated mice BM EO: 12.7 ± 2.5; eotaxin/SEA treated mice BM EO: 8.0 ± 0.1 OD/EOS$\times10^6$ cells). Similar results were observed with BM EO from SEB-treated mice for 16 h (eotaxin/untreated mice BM EO: 15.7 ± 2.7; eotaxin/SEB treated mice BM EO: 9.5 ± 1.2 OD/EOS$\times10^6$ cells). BM EO from naïve mice incubated *in vitro* with SEA or SEB for 2 h also exhibited reduced adhesive response when stimulated by eotaxin or RANTES (eotaxin/VCAM-1: 20.5 ± 2.5; SEA: 12.3 ± 1.0; SEB: 13.2 ± 1.2; RANTES/VCAM-1: 10.7 ± 0.8; SEA: 2.6 ± 0.8; SEB: 5.2 ± 0.6; eotaxin/ICAM-1: 14.0 ± 1.3; SEA: 10.2 ± 0.9; SEB: 10.7 ± 1.5; RANTES/ICAM-1:11.9 ± 1.1; SEA: 6.6 ± 0.5; SEB: 6.1 ± 1.0 OD/EOS$\times$ 10^6 cells). **Conclusion:** The inhibitory effect of SEA and SEB on BM EO *in vitro* adhesion suggests a role of these toxins on downregulation of BM EO surface adhesion molecules which contribute to clarify the mechanisms involved on the association between *Staphylococcus aureus* infections and allergic respiratory disease. **Financial Support:** Fundação de Amparo a Pesquisa do Estado de São Paulo (2009/16522-0; 2012/05561-8).

04.017 Dipyrone and its active metabolites produce antipyretic effect by acting at central nervous system. Malvar DC, Vaz LLV, Assis DCR, Melo MCC, Aguiar FA, Clososki GC, Souza GEP
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Introduction: 4-methylaminoantipyrine (4-MAA) and 4-aminoantipyrine (4-AA) are the antipyretic active metabolites of dipyrone. We observed that fifteen minutes after intraperitoneal administration of dipyrone these metabolites are found in high concentrations in the cerebrospinal fluid and hypothalamus (Aguiar et al., *Bioanalysis*, 2013, submitted article). In this study we evaluated if the antipyretic effect of dipyrone and its active metabolites is related to an action at central nervous system (CNS). **Methods:** Body temperature (Tb, ^oC) from male Wistar rats (180-200g, n=6-8) was measured every 15 min for up 6 h by radiotelemetry (Martins et al., *J Appl Physiol*. 113: 1456–1465, 2012). The animals were treated intracerebroventricularly (i.c.v.) route with saline, dipyrone (1000-2000µg), 4-AA (350-1000µg) or 4-MAA (120-750µg) 30 min before or 2 h after intraperitoneal injection of saline or LPS (50µg kg^{-1}). Ethical Commission protocol nº 200/2008 – CETEA/FMRP-USP. **Results.** I.c.v. post-treatment, but not pre-treatment, with dipyrone, 4-MAA or 4-AA produce a transient but significant and dose-related reduction of LPS-induced fever (Table). The highest dose of 4-MAA did not change the Tb from control animals treated with saline. However, the highest dose of 4-AA (1000 µg) increased the Tb while high dose of dipyrone (2000 µg) induced Tb and motor activity increase in control animals (not shown). I.c.v. administration of intermediary dose of dipyrone (1500 µg) or 4-AA (750 µg) did not change the Tb from control animals.

Table: Antipyretic effect of post-treatment with dipyrone (1500µg), 4-MAA (750µg) e 4-AA (750µg) injected i.c.v. on LPS-induced fever.

Time (hours)#	Saline/LPS	Dipyrone 1500µg/LPS	Saline/LPS	4-MAA 750µg/LPS	Saline/LPS	4-AA 750µg/LPS
2.00	1.15 ± 0.12	1.07 ± 0.05	1.31 ± 0.07	1.23 ± 0.15	1.24 ± 0.05	1.30 ± 0.13
2.25	1.58 ± 0.09	1.03 ± 0.09***	1.68 ± 0.09	1.04 ± 0.08***	1.64 ± 0.05	1.07 ± 0.12*
2.50	1.62 ± 0.08	1.20 ± 0.08*	1.88 ± 0.10	1.13 ± 0.07***	1.76 ± 0.04	1.20 ± 0.09*
2.75	1.73 ± 0.05	1.47 ± 0.08	1.83 ± 0.08	1.20 ± 0.06***	1.83 ± 0.08	1.30 ± 0.13*
3.00	1.70 ± 0.07	1.55 ± 0.07	1.79 ± 0.04	1.30 ± 0.09*	1.61 ± 0.04	1.40 ± 0.14
3.25	1.65 ± 0.09	1.58 ± 0.08	1.63 ± 0.04	1.47 ± 0.07	1.59 ± 0.05	1.43 ± 0.18

Time after LPS injection.
Data are reported as mean ± standard error of the mean (SEM) of variation of Tb.
Differences from control group were considered significant when: *$p<0.05$; ***$p<0.001$.

Discussion: These results suggest that dipyrone metabolites induce antipyretic effect by acting at CNS. The transient and low antipyretic effect of dipyrone and its metabolites may be explained by a fast redistribution of these active metabolites reducing its brain concentration. However, we cannot rule out the possibility of a peripheral antipyretic effect involved in the mechanism of action of 4-MAA or 4-AA. **Financial support:** FAPESP (2008/09443-4), CNPq (303877/2010-3).

04.018 Immunotoxin IL13-PE attenuates silica-induced lung fibrosis by a mechanism independent of epithelial cell damage. Ferreira TPT[1], Arantes ACS[1], Nascimento CVF[1], Olsen PC[1], Guimarães FV[1], Puri R[2], Hogaboam C[3], Martins MA[1], Silva PMR[1] [1]IOC-Fiocruz – Inflamação, [2]FDA-NIH – Biologics Evaluation and Research, [3]UMich – Pathology

Introduction: Silicosis is a chronic lung disease characterized by granulomatous inflammation and fibronodular response. Therapeutic treatment of silica-stimulated mice with the immunotoxin IL13-PE suppressed the fibrotic response and granuloma formation (Ferreira et al., FESBE meeting, 2011). Since epithelial cells are considered as important targets in context of fibrosis (secretion of cytokines, growth factors and cell attraction), in this study we investigated the potential cytotoxic effect of IL13-PE on these cells by means of *in* vitro and *in vivo* systems. **Methods**: Male Swiss-Webster mice (18-20 g) were intranasally (i.n.) instilled with silica particles (10 mg/50 µL) and IL13-PE was administered i.n. (200 ng/ day), every other day, starting on day 21 up to day 27 post silica provocation. After 24 h, animals were killed and lung samples were prepared for further analyses. A human lung carcinoma epithelial cell line A 549 (ATCC) was used and activation/apoptosis were evaluated. All experimental procedures were approved by the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (license L034/09). **Results**: We showed that A549 cells constitutively produce IL-8 and under conditions of silica stimulation (300 µg/mL), the levels increased from 351.3 ± 37.7 pg/mL to 4687.6 ± 691.4 pg/mL (mean ± SEM; n=4, $p< 0.05$). Treatment with 200 and 400 ng/mL of IL13-PE, 1 h prior to stimulation, decreased IL-8 generation in a concentration-dependent manner (values were 760.7 ± 99.4 pg/mL and 363.8 ± 55.0 pg/mL, respectively). No significant difference in cell viability between cells of the control group and those treated with IL13-PE alone as attested by MTT assay. However, under conditions of exposure to silica, a reduction of approximately 40% in cell viability was noted. Incubation with IL13-PE, 1 h prior to silica, produced a concentration-dependent decrease in cell viability as compared with silica alone. In order to compare to what happened *in vivo*, by means of TUNEL assay, we observed that treatment with IL13-PE did not alter airways epithelial cell viability, suggesting that these cells do not seem to contribute to the suppressive effect of the immunotoxin. **Conclusion**: Altogether, our findings reinforce the ability of IL13-PE to accelerate the resolution of fibrosis in silicotic mice and provide evidence that this effect is independent of the IL13-PE cytotoxic activity on lung epithelial cells. Our data also show that although cell lines are convenient and easier to use, as they are not physiological, one must have in mind that they may exhibit different behavior when compared to primary cells. **Financial Support:** FIOCRUZ, CNPq, FAPERJ (Brazil), Global REACH Michigan University (USA).

04.019 Zymosan injected into rat air pouches induces fever dependent and independent on prostaglandins. Marquiafável FS, Melo MCC, Souza GEP FCFRP-USP – Física e Química

Introduction: Previously, we observed that prostaglandins are involved in the fever induced by intra-articular injection of zymosan in rats (Kanashiro et al., Am J Physiol Regul Integr Comp Physiol 296:1631, 2009). Moreover, celecoxib inhibits the fever induced by intra air pouch injection of zymosan in rats (Marquiafável et al., SBFTE 2012). So, the aim of this study was an additional investigation about the mediators involved in the fever induced by zymosan injected into the air pouches in rats. **Methods:** 1^{th}day: under deep anesthesia (xylazine $20mg.kg^{-1}$+ ketamine $58mg.kg^{-1}$, ip) 20ml of sterile air was injected subcutaneously in the back and a transmitter was implanted into peritoneal cavity of male Wistar rats (180g). 3^{th}day: 10ml of sterile air was injected into the air pouches (ap). 6th day: the animals received vehicle (saline), dexamethasone (0.5 $mg.kg^{-1}$, sc), indomethacin (2, $5mg.kg^{-1}$, ip), paracetamol (acetaminophen; $225mg.kg^{-1}$, po) 30 min before ap injection of zymosan ($1mg.ml^{-1}$, 1ml) or saline (control group). The variation in body temperature reported in Celsius degree (D^oC), was measured every 15 min for up to 6h by radiotelemetry (Ethical Commission Protocol n° 12.1.322.53.2 – CEUA/RP-USP). **Results:** Zymosan induced a dose-dependent febrile response that began at 2.5h, peaked at 3.5h and remained for up 6 hours. Dexamethasone and paracetamol inhibited the zymosan air pouch-induced fever ($1mg.ml^{-1}$). However, indometacin reduced only the initial (2.45h) phase of this response.

Effect of dexamethasone, indomethacin and paracetamol on fever induced by zymosan injected into air pouch (iap) in rats.

	Dexamethasone (sc)			**Indomethacin (ip)**				**Paracetamol (po)**			
Treatment ($mg.kg^{-1}$)**	**saline**	**saline**	**0.5**	**saline**	**saline**	**2**	**5**	**saline**	**225**	**saline**	**225**
Zymosan ($mg.ml^{-1}$ iap)	**-**	**1**	**1**	**-**	**1**	**1**	**1**	**-**	**-**	**1**	**1**
Δ°C*(2.45h)	*-0.15 ± 0.16*	*0.67 ± 0.09*	*0.08 ± 0.07*	*0.07 ± 0.14*	*1.08 ± 0.11*	*0.66 ± 0.08*	*0.45 ± 0.09*	*0.05 ± 0.09*	*0.01 ± 0.18*	*1.63 ± 0.14*	*0.13 ± 0.14*
Δ°C*(3.5h)	*-0.75 ± 0.19*	*1.27 ± 0.24*	*0.32 ± 0.07*	*0.09 ± 0.07*	*1.14 ± 0.1*	*1.17 ± 0.09*	*0.89 ± 0.08*	*0.03 ± 0.09*	*-0.07 ± 0.15*	*1.8 ± 0.15*	*0.46 ± 0.2*

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

Discussion: Since dexamethasone a steroidal anti-inflammatory drug fully blocks the zymosan-induced fever it could be suggested that besides prostaglandins others mediators, such as cytokines, are involved in this response. Indomethacin was ineffective in the later phase (after 3.5h) but dose-dependently inhibited the initial phase of fever to zymosan suggesting a strong involvement of prostaglandins in starting this response. Paracetamol a COX-2 inhibitor (Engström Ruud L., Neuropharmacol. 71:124, 2013; Hins B., The FASEB J. 22: 383, 2008) also abolished this fever suggesting the involvement of others mediators besides PGs since paracetamol blocks fever induced by ET-1, CRF, $PGF_{2\alpha}$, MIP-1 a and *Tityus serrulatus* scorpion venom which independ on prostaglandin synthesis (Moraes et al., present congress). **Financial Support**: CNPq - CAPES.

04.020 Antinociceptive properties of ethanolic extracts of plant species present in Restinga of Jurubatiba National Park. Mello RJ, Carmo PL, Bonavita AG, Muzitano MF, Leal ICR, Guimarães DO, Konno TUP, Raimundo JM UFRJ

Introduction: The Restinga of Jurubatiba National Park (PARNA Jurubatiba), northern Rio de Janeiro state, protects a region rich in habitats and flora and can be considered a reserve of bioactive natural products. Thus, the purpose of this study was to evaluate the pharmacological potential of some plant species found at PARNA Jurubatiba – *Humiria balsamifera* (Humiriaceae), *Mandevilla moricandiana* (Apocynaceae), *Ocotea notata* (Lauraceae), *Passiflora mucronata* (Passifloraceae), *Peplonia asteria* (Asclepidaceae),*Stachytarpheta schottiana* (Verbenaceae), *Tapirira guianensis* (Anacardiaceae) and *Vitex polygama* (Lamiaceae) - by using classical animal models of chemical nociception. **Methods:** The antinociceptive and antiinflammatory activities of ethanolic extracts of leaves of the 8 species were investigated using the formalin and writhing tests. Experiments were performed on male Swiss mice (18-22 g), which were randomly separated into groups of 6 animals. Extracts were solubilised in DMSO and given intraperitonially (i.p.; 5-20 mg/kg) 15 min prior to testing. For the writhing test, acetic acid (0.8%, v/v, 10ml/kg) was injected i.p. and the abdominal constrictions were counted over a period of 10 min. For the formalin test, animals received 20 ml of a 2.5% formalin solution injected intraplantarly in the right hindpaw. The time spent licking the injected paw was recorded during the neurogenic (0-5 min) and inflammatory (15-30 min) phases. All protocols were approved by the Animal Care and Use Committee (CCS/UFRJ) under license number Macaé 02. **Results:** Ethanolic extracts of *H. balsamifera*, *O. notata*, *S.schottiana* and *T. guianensis*, at 10 mg/kg, had no effect on acetic acid-induced visceral pain. At 5 and 10 mg/kg, *P. mucronata* extract significantly reduced the number of writhings from 30.5 ± 4.3 to 13.3 ± 7.9 and 3.0 ± 1.8, respectively (P <0.05). *M. moricandiana* (5 mg/kg) and *V. polygama* (10 mg/kg) extracts were also able to reduce the number of writhings to 3.8 ± 1.8 and 15.6 ± 5.4 (P<0.05). Moreover, the extracts of *P. mucronata*, *M. moricandiana*, *P. asteria* and *V. polygama*significantly inhibited the inflammatory phase of the formalin test at the dose of 10 mg/kg. Reaction time was reduced from 493.4 ± 18.7 s to 257.0 ± 52.7 , 274.0 ± 21.5, 404.6 ± 28.9 and 171.8 ± 28.5 s, respectively (P <0.05). **Discussion:** Our results indicate that the ethanolic extracts of *P. mucronata*, *M. moricandiana*, *P. asteria* and *V. polygama* have antinociceptive activity. *H. balsamifera*, *O. notata*, *S.schottiana* and *T. Guianensis* extracts may be tested at higher doses. **Financial support:** FAPERJ, UFRJ.

04.021 Polyinosinic: polycytidylic acid as a model of febrile response in rats. Bastos-Pereira AL[1], Fraga D[2], Simm B[3], Ott D[3], Roth J[3], Zampronio AR[1] [1]UFPR – Pharmacology, [2]UFMS – Pharmacology, [3]JLU-UniGuiessen – Veterinary Sciences

Introduction: This study investigated peripheral and central mediators involved in Polyinosinic:Polycytidylic acid(PIC)-induced fever and a possible direct action of this Pathogen-Associated Molecular Pattern (PAMP) in fever-related brain regions. **Methods:** Procedures were approved by the UFPR Ethical Committee in Animal Use (#621). Intraperitoneal (ip) data loggers were implanted in male Wistar rats for body temperature measurement. A intracerebroventricular (icv) cannula was also implanted (when necessary). Non-selective cyclooxygenase (COX) inhibitor Indomethacin (2 $mg.kg^{-1}$, ip), selective COX-2 inhibitor Celecoxib (5 $mg.kg^{-1}$, orally), Endothelin (ET) receptor B antagonist BQ788 (3 ρmol, icv) and the selective NK1 receptor antagonist SR140333B (3 µg, icv) were injected 30 min before PIC administration (300 $µg.kg^{-1}$, ip). Control groups received the appropriate vehicle. Tumor necrosis factor(TNF)-α, Interleukin(IL)-1β, IL-6 and prostaglandin(PG)E_2 from plasma and/or cerebrospinal fluid (CSF) were accessed by ELISA or EIA. Primary microcultures of the rat median pre-optic nucleus (MNPO) were established from excised brain tissue of rat pups and after 5 days, cells were loaded with fura-2-AM for intracellular Ca^{2+} measurements. PIC was administered *in bolus,* in a concentration of $100ug.mL^{-1}$. Phenotypic identification of cells was confirmed by immunocytochemistry. **Results:** PIC induced a febrile response of up to 1.2°C that started at 2h, peaked at 3h and returned to normal around 4h. This PAMP also induced a small increase in TNF-a plasma levels: a sample with 241.5 $ρg.mL^{-1}$ and other three samples below detection, compared to vehicle group (all samples below detection). Increased levels of IL-1β were also observed in the plasma of PIC-treated rats (5.6 ± 5 for the Vehicle; 207.3 ± 43.4 $ρg.mL^{-1}$ for the PIC group). No significant increase was seen on plasma IL-6 levels of PIC-treated group (and 62.8 ± 8.7 for the Vehicle; 101.2 ± 25.9 $ρg.mL^{-1}$ for the PIC group). Treatment with Indomethacin or Celecoxib abolished the febrile response induced by PIC. It also led to an increase in the levels of PGE_2 in the CSF which were completely blocked by indomethacin (9.6 ± 5.35 for Vehicle; 391.3 ± 103.8 for PIC and 249.7 ± 17.1 $ρg.mL^{-1}$for Indomethacin plus PIC groups). The icv administration of BQ788 and SR140333B did not reduce the febrile response induced by PIC. *In vitro* studies showed that *in bolus* application of PIC increased Ca^{+2} signaling. Of all MNPO cells investigated, about 4% from neurons and astrocytes, and 18% of microglia cells responded to stimulation with PIC, with mean average-ratio [340/380 nm] values of about 0.2 in all cell types. **Discussion:** The results suggest that PIC induces a febrile response that is mediated by cytokines like TNF-a and IL-1β, which in turn generated PG as central mediators. On the other hand, ET-1 (via ET_B receptors) and substance P are not involved in this febrile response. Moreover, PIC can directly induce a response on cells located on a brain-barrier protected region (MNPO), although weaker if compared to more permeable areas (Ott *et al,* Neurosci Lett 530, 64, 2012). These observations about the mechanisms by which this PAMP induces fever can help in the determination of a valid model of viral febrile response. **Acknowledgements:** REUNI, CNPq, Araucária Foundation and CAPES.

04.022 Effect of CB1 and ETA receptors blockage in the survival rate and body temperature after cecal ligation and puncture (CLP) in rats. Leite MCG[1], Brito HO[1], Bastos-Pereira AL[1], Fraga D[2], Zampronio AR[1] [1]UFPR – Farmacologia, [2]UFMS

Introduction: Previous studies showed that endothelin-1 reduced the frequency, but not the amplitude, of spontaneous post-synaptic excitatory currents in vasopressinergic (AVP) magnocellular cells of rats through activation of ET_A receptors suggesting a pre-synaptic effect (Zampronio et al., J. Neurosci. 30:16855,2010). CB_1 receptor antagonist AM251 abolished this effect suggesting the involvement of endogenous cannabinoids acting retrogradely. In this study, we initiated the evaluation if this mechanism, identified in brain slices, could be important during sepsis. **Methods:** Sepsis was induced in male Wistar rats (180-200g) by CLP (Figueiredo et al., Med. Microbiol. Immunol., 201:219, 2012). A survival curve was performed using 1, 3 and 9 punctures by a 16G needle. Sham-operated animals (control) had their cecum exposed but not ligated or punctured. In subsequent experiments, animals were treated with CB_1 receptor antagonist rimonabant (Rim, 10 or 20 mg/kg, by oral route) 4 h after CLP or ET_A receptor antagonist BQ123 (100pM, i.c.v.), 2 h and 4 h, 4 h and 8 h or 8h after CLP or with ET_B receptor antagonist BQ788 (100pM, i.c.v), 4 h and 8 h after CLP and survival rate was analyzed for 7 days. For body temperature (T_b) measurement, 5 days prior the CLP, dataloggers were implanted in the peritoneal cavity and T_b was measured at 30 min intervals, 2 h before CLP up to 24 h. All procedures were approved by the institution's Ethical Committee for Animal Use (# 629). **Results:** Sham-operated animals had a survival rate of 100% in all experiments. Animals showed a survival rate of 84, 26 and 0% with 1, 3 and 9 punctures, respectively. Three punctures were selected for subsequent experiments. Animals treated with Rim 4 h after CLP showed a survival rate of 73% at both doses, significantly different from CLP group (34% survival rate). All groups had an increase in T_b after surgery that peaked at 4 h (Sham: 1.75 ± 0.4; CLP: 1.74 ± 0.4 °C). At this time point, CLP animals were treated with Rim or Saline. Six hours after CLP, Sham-operated animals and CLP/Rim animals had significantly lower T_b (0.56 ± 0.2 and 0.35 ± 0.4 °C, respectively) than control CLP animals (1.64 ± 0.4 °C). The same pattern was observed after 8 h and after this point T_b was similar in all groups. The treatment of the animals with BQ123 2 h and 4 h, or 8 h after CLP did not significantly improved the survival rate (44% and 20% for BQ123-treated group respectively compared to 58% and 20% after CLP). However, treatment of animals with BQ123 4 h and 8 h after CLP significantly improved the survival rate of the animals (71% for BQ123-treated group compared to 14 % for CLP group). ET_B receptor antagonist, BQ788, given at the same protocol did not improved survival rate (40% for BQ788-treated group compared to 22% for CLP group). **Discussion:** The blockage of central CB_1 and ET_A receptors, 4 h and 4 and 8 h respectively, after CLP improved the mortality rate of animals after CLP. The treatment of the animals with rimonabant 4 h after the CLP also reduces T_b levels to values similar to the Sham-operated animals. Further investigation if increases in AVP blood levels occur during these protocols will be carried out. **Financial support:** CNPq and REUNI.

04.023 TNFR1, but not TNFR2, is crucial to the development and progression of systemic inflammation and organ damage during experimental sepsis. Melo PH, Nascimento CBD, Ferreira RG, Scortegagna GT, Borges VF, Cunha FQ, Alves-Filho JC FMRP-USP – Basic and Applied Immunology

Introduction: Sepsis is a systemic inflammatory response syndrome resulting from an infectious process that leads to a high mortality rate (Angus and Wax: Crit Care Med, 2001). The Tumor Necrosis Factor (TNF) is a pleiotropic cytokine involved in several processes underlying the inflammatory response. TNF induces responses through activation of two different cognate receptors: TNFR1 and TNFR2 (Faustman and Davis: Nat rev, 2010). TNF expression rises dramatically during the acute phase of sepsis, playing a role in the progression of systemic inflammatory responses and organ damage (Tracey and Cerami, Annu Rev Med. 1994). Thus, our aim was to address the roles of TNFR1 and TNFR2 in sepsis development. **Methods:** We evaluated the participation of TNF receptors in the pathophysiological events observed during acute phase of sepsis (6 to 72 hours after sepsis). $TNFR1^{-/-}$, $TNFR1/2^{-/-}$ and WT mice developed severe sepsis induced by cecal ligation and puncture (CLP) and received basic support (hydration and antibiotics). All mean of the results were analyzed by one-way analysis of variance and to Tukey post test was used (compare all pairs of columns) with 95% confidence intervals ($p < 0.05$). **Results:** Initially we observed that TNF receptor deficient mice had increased survival rates and reduced clinical scores after the induction of sepsis, although no difference was found between $TNFR1^{-/-}$ and $TNFR1/2^{-/-}$ mice (75% of survival for both vs. 40% of survival in WT mice). We found that both types of deficient mice had more efficient control of the bacterial growth in local and systemic sites during sepsis when compared to WT mice ($p<0,05$). However, no difference in local and systemic bacterial load was observed between $TNFR1^{-/-}$ and $TNFR1/2^{-/-}$ septic mice ($p>0,05$). Subsequently, we evaluated lung neutrophil sequestration using a myeloperoxidase assay (MPO), $TNFR1^{-/-}$ ($1.7 \pm 0.1 \times 10^3$ cells/mg of lung) and $TNFR1/2^{-/-}$ ($1.5 \pm 0.6 \times 10^3$ cells/mg of lung) septic mice had lower number of neutrophils in lung compared to WT septic mice ($9.1 \pm 1.2 \times 10^3$cells/mg of lung). Moreover, $TNFR1^{-/-}$ and $TNFR1/2^{-/-}$ septic mice had lower levels of IL-1β (3.3 ± 1.6 and 4.9 ± 2.4 pg/ml serum, respectively) and IL-6 (0.3 ± 0.1 and 0.2 ± 0.03 pg/ml serum, respectively) compared to WT septic mice (IL-1β: 16.1 ± 1.6 and IL-6: 920.0 ± 82.5 pg/ml serum), although no difference was found between $TNFR1^{-/-}$ and $TNFR1/2^{-/-}$ septic mice in the systemic inflammatory response. CK-MB and AST enzyme activity, BUN and endocan levels were also measured to estimate the heart and liver injury, kidney function and kidney/pulmonary endothelium damage, respectively. $TNFR1^{-/-}$ and $TNFR1/2^{-/-}$ septic mice had lower activity of CK-MB (502.7 ± 84.6 and 492.3 ± 204.5, respectively), lower activity of AST (41.3 ± 2.2 and 48.4 ± 2.8, respectively), lower levels of BUN (6.1 ± 3.8 and 6.6 ± 5.2 mg/dl, respectively) and endocan (153.4 ± 13.7 and 118.3 ± 14.0 pg/ml, respectively) compared to WT septic mice (CK-MB: 1851.0 ± 514.2, AST: 99.0 ± 7.5, BUN: 22.6 ± 7.4 and endocan: 445.8 ± 137.6 pg/ml). Thus, TNF receptor deficient septic mice had reduced organ damage compared to WT septic mice, although no difference was found between $TNFR1^{-/-}$ and $TNFR1/2^{-/-}$ septic mice. **Conclusion:** Altogether, these data suggest that TNFR1 receptor activation during sepsis culminates in impaired infection control, leading to increased systemic inflammation and organ damage. Moreover, TNFR2 does not seem to be crucial for eliciting progression of inflammation during sepsis. **Financial Support**: CNPq, FAEPA and FAPESP. **Animal Ethics Committees:** n° 152/2011 (CETEA –Ribeirão Preto Medical School, University of São Paulo)

04.024 Pulmonary Fibroblasts express CXCR4 and produce CCL3, CXCL2, LTB4 and LTC4 after CXCL12 stimulation. Danilucci TM, Oliveira SHP FOA-Unesp-Araçatuba – Basic Sciences

Introduction and aim: CXCL12/CXCR4 axis plays a critical role in the recruitment of inflammatory leukocytes in the lung. The aim of this study is to investigate whether pulmonary fibroblasts express CXCR4 and produce CCL3, CXCL2, LTB4 and LTC4 after CXCL12 stimulation and evaluate the mechanism involved in the process. **Methods and Results:** Animal ethics committee: FOA-0969-2012. Fibroblasts were isolated from lungs of Balb/c male. To confirm the purity and characteristic of fibroblast cultures, pulmonary fibroblasts were seeded for immunofluorescence and histological analysis. After fibroblasts were stimulated with CXCL12 (1, 10, 50 and 100 ng/mL). After 1, 6 and 24 h supernatant was collected. The level of CCL3, CXCL2, LTB4 and LTC4 in the supernatant was analyzed by ELISA. CXCL12 (100ng/mL) induced CCL3 (1635 ± 40,8 *vs* control group 553 ± 95,2 pg/mL), CXCL2 (9001 ± 505 *vs* 2430 ± 224 pg/mL) , LTB4 (94 ± 6,7 *vs* 47,14 ± 12,4 pg/mL) and LTC4 (18,4 ± 1,1 *vs* 7 ± 1,5 pg/mL) production at 24 hours after stimulation.To investigate whether CCL3, CXCL2, LTB4 and LTC4 are dependent on CCL3 and CXCL2 production, pulmonary fibroblasts were pretreated with anti-CCL3 and anti-CXCL2 antibodies. CCL3 production is not dependent on CXCL2; but CXCL2 production is dependent on CCL3 production. LTB4 production can be partially down-regulated by CXCL2 and CCL3 production and LTC4 production is dependent on CCL3 and CXCL2 production. We evaluated the CXCR4 expression in pulmonary fibroblasts by RT-PCR. It was observed that pulmonary fibroblast constitutively expressed CXCR4 (0,7 ± 0) and CXCL12 stimulation up-regulated its expression (1,1 ± 0); measure unit: CXCR4/β-actin. Therefore, we investigate the possible involvement of CXCL12/CXCR4 axis-induced CCL3, CXCL2, LTB4 and LTC4 production. For that CXCL12-stimulated pulmonary fibroblasts were pretreated with anti-CCL3 anti-CXCL2 antibodies or MK886, a 5-lipoxygenase inhibitor, and CXCR4 expression was evaluated. Constitutive CXCR4 expression was inhibited by anti-CCL3 (0,9 ± 0) antibody and/or MK886 (0,3 ± 0) and CXCR4 mRNA-induced was inhibited by anti-CXCL2 (0 ± 0) compared to control group (0,8 ± 0); measure unit: CXCR4/β-actin. These data indicate the involvement of CCL3, CXCL2 and lipids mediator production in the CXCR4-CXCL12 axis activation. Pulmonary fibroblasts were pretreated with MK886, dexamethasone (Dexa) and/or loratadine (Lor) for 30 minutes followed by stimulation with CXCL12 (100 ng/mL) for 24 hours. CCL3 was inhibited by Dexa (261 ± 11) and Dexa plus Lor (85 ± 10.2) compared to CXCL12 group (507 ± 48). CXCL2 was also inhibited by Dexa (668 ± 32) and Dexa plus Lor (200 ± 61) compared to CXCL12 group (1353 ± 111). LTB4 and LTC4 were inhibited respectively by MK886 (26 ± 2,1; 9,4 ± 0,4), Lor (33 ± 3,7; 96,6 ± 1), Dexa (26,2 ± 3.2; 8 ± 2) and Dexa plus Lor (29,6 ± 2; 4,8 ± 1,2) compared to CXCL12 group (81.4 ± 4.5; 20.5 ± 0.6); measure unit: pg/mL. We have identified p38, MEK1/2, PI-3K and JNK intracellular signaling pathways playing a role in CCL3, CXCL2 and LTB4 production after CXCL12/CXCR4 axis activation. **Conclusion**: This study has explored, for the first time, the mechanism through which CXCL12/CXCR4-stimulate pulmonary fibroblast production of CCL3, CXCL2, LTB4 and LTC4 mediated by different signaling transduction pathways depending on the inflammatory mediator produced. **Financial support**: CAPES and FAPESP (2011/06070-5).

04.025 Preventive treatment with dexamethasone changes the progression and increase neuroinflammation in Experimental Autoimmune Encephalomyelitis. Santos NB, Lopes DCF, Novaes LS, Duque EA, Wiezel G, Munhoz CD USP – Farmacologia

Introduction Multiple sclerosis (MS) is a neurodegenerative autoimmune disease characterized by demyelination in the central nervous system (CNS). Several studies showed that the cells infiltrate (mainly macrophages and CD4+ cells) are intimately involved in pathological processes of MS. Moreover, the participation of glial cells (microglia and astrocytes) appears to contribute decisively to the worsening of MS, since these cell types are the main mediators of neuroinflammatory response. Primary treatments used in MS are the glucocorticoids (GCs), such as methylprednisolone and dexamethasone, which are generally used in the acute phase, due to its immunosuppressive and anti-inflammatory properties. However, there is growing literature suggesting that GCs are not always anti-inflammatory and in some experimental models and clinical aspects may enhance inflammation, such as activation of the transcription factor NFkB induced by LPS (lipopolysaccharide of Gram-negative bacteria). **Methods** Here, we have used male mice of C57BL/6 with or without Acute Experimental Encephalomyelitis (EAE) and treated with Dexamethasone (DEX 1 mg/kg) or saline for 7 days before immunization. The daily clinical scores were analyzed of animals and at 21 days after immunization, the mice were perfused with 4% PFA and the brain and spinal cord were collected. All experiments were approved and conducted in accordance with the ethical principles in animal research adopted by the Institute of Biomedical Sciences local Animal Care Committee, University of São Paulo (n. 03/book 03/page 03 CEUA ICB-USP). **Results** We found that DEX treatment delayed the score evolution compared to EAE group; however the neuronal degeneration, assayed by FlouroJadeB staining, was similar between EAE and EAE+DEX animals. Moreover, immunofluorescence assay showed astrogliosis and high nuclear translocation of p65 (RELA) NFkB subunit in the hippocampus and cortex of EAE+DEX group. Our results showed that glucocorticoids treatment, indeed, diminished EAE symptoms, however did not decrease either inflammation or neurodegeneration, suggesting a dual function of glucocorticoids. **Financial Agencies:** CNPQ

04.026 Potential protective effect of silymarin on irinotecan induced steatohepatitis in mice.

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Background: The Colorectal Cancer (CRC) is the third most prevalent neoplastic disease in the world and is one leading cause of death. Irinotecan is a drug used as first line treatment for CRC and its liver metastases and has markedly improved the overall survival of patients. However, irinotecan-related side-effects, which include intestinal mucositis and non-alcoholic steatohepatitis (NASH), significantly increase the risk of dose reduction. It is reported that the use of irinotecan-based regimens as preoperative chemotherapy is associated with a 3.45-fold increased risk of steatohepatitis when compared to individuals who are chemotherapy naïve. The pathogenesis of steatohepatitis is still unknown. However is characterized for lipid acumutation in the hepatocytes, culminating in inflammatory injury, necrosis and fibrosis. Recently, we developed an animal model that mimics all relevant clinical findings of irinotecan-related steatohepatitis. Currently, there is no specific therapeutic protocol for irinotecan-related steatohepatitis. Silymarin, a flavonoid isolated from "Silybum marianum", has demonstrated hepatoprotective potential in animal models of thioacetamide and carbon tetrachloride-induced hepatic lesions and is clinically used to treat liver diseases. Then, we investigated the potential protective effect of silymarin on irinotecano-induced steatohepatitis. **Design / Methods:** Swiss male mice (n=6) were divided into groups and injected with saline (5ml/kg, i.p.), irinotecan (50 mg/kg, i.p.), Silymarin (150 mg/kg p.o.) or irinotecan (50 mg/kg i.p) + silymarin (silymarin 1.5, 15 or 150 mg/kg p.o.) thrice a week for 7 weeks. At the end of this period, blood was collected to determine serum concentration of hepatic enzymes ALT and AST. The animals were killed and the livers were removed to perform lipid dosage and histopathological analysis. Survival curves were also obtained. Data were analyzed through ANOVA/Bonferroni's test or Kruskal Wallis/Dunn, as appropriate. $P<0.05$ was accepted. CEPA:21/12. **Results:** Irinotecan-injected mice presented a significantly ($P<0.05$) increase in serum ALT and AST, hepatic lipids accumulation, liver histopathological damage and neutrophil infiltrate/field (94.83 ± 21.73, 103.7 ± 6.126, 27.30 ± 6.568, 6.250 ± 0.4787, 3.90 ± 0,55) when compared with the saline group (48.25 ± 3.250, 41.50 ± 2.754, 7.728 ± 0.9532, 1.400 ± 0.6782, 0,32 ± 0,10), which was prevented by silymarin (1.5 mg/kg) (34.25 ± 12.27, 33.88 ± 6.443, 11.45 ± 1.795, 3.500 ± 0.9574) and neutrophil infiltrate/field, which was prevented by silymarin (15 mg/kg) (2,35 ± 0,43). However, all the studied groups showed similar survival curves ($p>0.05$). **Conclusions:** Our data showed that silymarin was able to reduce the hepatic damage. However, more studies are needed in order to demonstrate the mechanism involved. **Financial support**: INCT/CNPq, CAPES and FUNCAP.

04.027 Granulocytopoietic activity of satphylococcal enterotoxin type A (SEA) and B (SEB) in mice: **A possible mechanism to explain the pulmonary allergic exacerbation induced by these toxins in mice**. Torres ASP[1], Duarte APF[1], Squebola-Cola DM[2], Mello GC[2], Antunes E[2], De Souza IA[1] [1]FMJ – Fisiologia, [2]Unicamp – Farmacologia

Staphylococcal enterotoxins (SEs) are proteins produced and secreted by the gram-positive bacterium *Staphylococcus aureus*, and are responsible for the most pathological conditions associated with *S. aureus* infections, including pulmonary infections in hospitalar environment. Clinical evidences have shown a strong association between *Staphylococcus aureus* infections and exacerbation of bronchial asthma. However, few studies have attempted to evaluate the mechanisms involved on the exacerbation of allergic cell pulmonary infiltration induced by prior exposure to SEA and SEB. The aim of this study was investigated if the mechanisms whereby SEA and SEB cause exacerbation of allergic pulmonary inflammation are correlated with changes on granulocytopoese and citokines/chemokines production on mice bone marrow (BM). Male BALB/C mice were intransally instilled with SEA or SEB (0.3-1 µg). Broncoalveolar lavage (BAL) and BM were examined (4 to 48 h post-SEs instillation). Mice intranasally instilation with SEA significantly increases the eosinophil (at 4 h) and neutrophil (at 16 h) recruitment to BAL accompanied by the increase number of immature and mature forms of granulocytes on BM. A marked eosinophilia and neutrophilia was observed at 16 h and 24 h post-SEB to BAL fluid which was also correlated with BM increase on immature and mature forms of granulocytes. We also evaluated the levels of cytokines (GM-CSF, IL-5 e IL-4 and chemokines (eotaxin and KC/CXCL1) on the BM supernatant from mice submitted to airways exposition of SEs. Our results showed that both SEs are able to induced increase levels of eotaxin at 16 h when compared with control group (Control: 59.7 ± 7.8; SEA: 146.1 ± 14.6 and SEB: 322.8 ± 77.6 pg of eotaxin/mg of protein). A similar results was observed for the levels of the granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 after SEA or SEB instillation (Control: 4.78 ± 1.0; SEA: 10.3 ± 1.4; SEB: 47.5 ± 13.3 pg of GM-CSF/mg of protein; Control:16.4 ± 3.5; SEA: 29.6 ± 3.79; SEB: 49.5 ± 13.8 pg of IL-4/mg of protein). However, while SEB induced increased levels of IL-5 at 16 h (Control: 52.6 ± 8.8; SEB: 266.6 ± 103.0 pg of IL-5/mg of protein) in mice BM, SEA induced a marked reduction on the levels of this cytokine (17.7 ± 2.8 pg of IL-5/mg of protein). In addition the levels of the chemokine KC/CXCL1 was increased at 4 h after SEA (Control: 23.4 ± 5.16; .SEA: 54.0 ± 8.0 pg of KC/CXCL1/mg of protein) and 16 h after SEB (67.2 ± 12.5 pg of KC/CXCL1/mg of protein). In conclusion our study shows that SEA and SEB have granulocytopoietic activity by cytokine/chemokine release which displays a major role on production and trafficking of granulocytes (neutrophil/eosinophil) from mice BM to pulmonary inflammatory site. Taken in consideration the high number of clinical infections induced by *Staphylococcus aureus*, and their connection with allergic respiratory diseases (through SEs release) our findings can be useful to provide advancement to explain the allergic respiratory disease exacerbation after gram-positive bacterial exposition in humans. Approval of Brazilian ethics committee in animal experimentation: 492/2012; Financial Support: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP: 2009/16522-0).

04.028 Down-modulation of activated human neutrophil by LMW-fucoidan: **Role of microparticles**. Moraes JA[1], Frony AC[1], Barcellos-de-Souza P[1], Boisson-Vidal C[2], Barja-Fidalgo C[1]
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Introduction: During migration, neutrophils (PMN) interact with several mediators, which can lead to their activation, interfering with cell survival and inflammation resolution. Fucoidans are sulfated polysaccharides which are able to inhibit selectin-mediated events. A low-molecular-weight-Fucoidan (LMW-Fuc) fraction extracted from the brown algae *Ascophyllum nodosum* exhibits potent antithrombotic and proangiogenic properties, although its effects on inflammatory cells are still unknown. So we aimed to evaluate the effect of LMW-Fuc on activated PMN. **Methods:** PMN and monocyte (further macrophage) were isolated from healthy volunteers (Percoll gradient). Chemotaxis (1h) was performed in Boyden chamber. Apoptosis (20h) was assessed by Annexin V, JC1 and morphological analysis. Actin cytoskeleton rearrangement was analyzed by Falloind-rhodamin staining. ROS production was quantified by lucigenin, luminol and CM-DCFDA, according the ROS localization to be analyzed. Actin, AKT, Bad and p47 expression was evaluated by immunoblotting. L-selectin and AnnexinV+ microparticles (MP) were quantified in FACS. **Results:** LMW-Fuc inhibited PMN migration induced by LPS, fMLP or migration of PMN primed with LPS and further challenged to fMLP. Corroborating this data, in LPS/fMLP-activated PMN the LMW-Fuc attenuated the induced alterations on actin cytoskeleton dynamics and inhibited AKT phosphorylation. We also observed that LMW-Fuc was able to accelerate apoptosis of PMN treated with LPS, fMLP or primed with LPS and further treated with fMLP and, corroborating this result, LMW-Fuc prevented Bad degradation (an AKT target) induced by LPS/fMLP treatment. Then we showed that LMW-Fuc was able to inhibit extracellular, but not the intracellular ROS production induced by LPS/fMLP treatment. Furthermore, in stimulated PMN LMW-Fuc inhibited the MP release, which are the key actors of extracellular ROS production in these cells, mainly by its property to carry p47, a NOX2 subunit. Finally, we demonstrated that PMN MP was also able to induce extracellular ROS in macrophage, also in a p47-dependent mechanism. **Discussion:** In this work we pointed LMW-Fuc as a promising anti-inflamtory molecule, once it was able to inhibit LPS/fMLP effect on PMN migration, apoptosis and MP release. We showed for the first time that the potent proinflammatory stimulus LPS/fMLP induces strongly PMN MP release, which have an autocrine role. LPS/fMLP effect occurs partially via MP, once these MP can induce extracellular ROS production via p47. Furthermore we showed that PMN MP also can have a paracrine role, inducing extracellular ROS in macrophages. On the other hand, LMW-Fuc has no direct effect on MP, once it was not able to inhibit MP effect on PMN or macrophages. So, we hypothesized that LMW-Fuc acts inhibiting MP vesiculation, abrogating its auto/paracrine effects. Thus LMW-Fuc presents a potent ability to attenuate neutrophil activation that might be potentiated by its ability in inhibits MP release. **Funding Support:** FAPERJ, CAPES, CNPq.

04.029 N-acylhydrazone derivative LASSBio-294 suppresses inhibits lung inflammation caused by intransal siica in mice. Sá YAPJS[1], Ferreira TPT[1], Arantes ACS[1], Ciambarella BT[1], Barreiro EJ[2], Fraga CAM[2], Martins MA[1], Silva PMR[1] [1]Fiocruz – Inflamação, [2]UFRJ – Avaliação de Substâncias Bioativas (LASSBio)

Introduction: Silicosis is an occupational disease caused by prolonged inhalation of dust containing free crystalline silica particles, which is characterized by an intense pulmonary inflammation with formation of fibrotic nodules. Estimates indicate that silicosis kills million of workers every year worldwide and, in Brazil more that 6 million of workers are at a risk of silicosis. As there is no effective treatment for fibrotic diseases, in this study we investigated the effect of the 1,3-N-acylidrazonic benzodioxolic derivative LASSBio-294 on the experimental silicosis in mice. The effect of the compound was also evaluated in some target cells in vitro. **Methods:** Swiss-Webster mice were anesthetized and intranasally instilled with silica (10 mg/50 uL) and the analyses performed after 28 days. The animals received daily administration of LASSBio-294 (1, 5 and 25 mg/kg, p.o.) for 7 days, starting 21 days after stimulation with silica. The evaluation of pulmonary mechanics was performed by invasive whole body plethysmography (Fine point - Buxco System), in the absence or the presence of methacholine (3-27 mg/mL). Classical histological techniques were used and included morphology and morphometry (H&E and picrus Sirius) and immunohistochemistry (α-smooth muscle actin). Quantification of collagen deposition and of cytokine/chemokine generation was made by Sircol and ELISA, respectively. Lung fibroblast proliferation was evaluated in vitro by means of 3H. thymidine incorporation. All procedures were approved by the Ethics Committee for Animal Use (CEUA) of FIOCRUZ (license 034-9). **Results:** Therapeutic treatment of silicotic mice with LASSBio-294 inhibited the increase of both lung resistance and elastance as well as airways hyperreactivity to methacholine. Granuloma formation, collagen deposition and the increased expression α-smooth muscle actin positive cells were suppressed by the LASSBio-294. The generation of proinflammatory and profibrotic cytokines (TNFα) as well as chemokines (MCP-1, KC and TARC) in the lungs of silicotic mice was sensitive to LASSBio-294. The in vitro analysis revealed that lung fibroblasts from silicotic animals were also sensitive to previous incubation with LASSBio-294. **Conclusions:** Our results show that compound LASSBio-294 inhibited the inflammatory response and fibrogenesis, including granuloma formation, caused by silica particles in the lungs of mice, by a mechanism which may be dependent on its suppressive activity on some important target cells such as fibroblasts. **Financial Support:** FIOCRUZ, INCT-INOFAR, PRONEX 2009, CNPq and FAPERJ.

04.030 Female sexual hormones modulate the febrile response induced by prostaglandin but not by morphine. Brito H[1], Leite MCG[1], Simões FC[1], Brito LM[2], Zampronio AR[1] [1]UFPR – Farmacologia, [2]UFMA – Medicina

Introduction: There is increasing evidence that immune responses are modulated by circulating sexual hormones. Interleukin(IL)-1β-induced fever has been described to be influenced by these hormones (Ashdown et al, Am. J. Physiol., R1667-74, 2007) but how these hormones affect central mediators of fever is unknown. We showed before that IL-1β-induced fever is dependent on prostaglandin (PG) but not on endogenous opioids synthesis and release (Fraga et al., Am. J. Physiol., 294:R411, 2007). In this study we evaluated if female sexual hormones could affect the febrile response induced byPG and morphine (MOR). **Material and Methods:** Studies were conducted in male and Sham-operated (Sham) and Ovariectomized (OVX) female Wistar rats (200 g). Three weeks after ovariectomy or sham surgery, animals were implanted with guide cannula in lateral ventricle (when necessary) and with data loggers for measurement of body temperature (T_b) in the peritoneal cavity under the same anesthesia. Experiments were conducted one after this surgery. Basal T_b was measured for at least 2 h before any injection and then the febrile response was induced by lipopolysaccharide (LPS, 50 µg/kg,i.p.) or prostaglandin E_2 ($PGE_{2,}$ 125 ng/2µl, i.c.v.) or morphine (10 µg/2µl,i.c.v.). Control animals received only saline. In some experiments, PG synthesis inhibitor indomethacin (IND, 2 mg/kg, i.p.) was injected in Sham-operated and OVX animals 30 min before LPS injection. T_b (in ◦C) was registered every 15 min. Room temperature was kept at 28˚C. Data are presented as changes in T_b (ΔT_b in °C ± S.E.M) in relation to the basal T_b. All procedures were previously approved by Institutional Ethics Committee under protocol # 626. **Results and Discussion:** Administration of saline in both male and female (Sham-operated and OVX) rats did not significantly change body temperature of the animals. Treatment with LPS induced a febrile response 40% lower in Sham-operated female animals (at 3rd hour, 0.85 ± 0.02) when compared to male rats (1.47 ± 0.11°C). In sharp contrast, OVX rats had a febrile response significantly higher (1.63 ± 0.09°C) than Sham-operated female rats but similar to males. PG synthesis inhibitor IND reduced the febrile response induced by LPS in OVX animal more intensely than in sham-operated animals (88% in OVX and 71% in Sham) suggesting that PGs might be involved in these differences. The administration of PGE_2 induced a febrile response in OVX female rats 30 min after injection that was significantly higher than that observed in sham-operated animals (OVX: 1.83 ± 0.19°C and Sham: 1.11 ± 0.12°C). On the other hand, both groups showed a similar febrile response after administration of morphine that peaked at 75 min (Sham: ∆T:1.65 ± 0.10°C and OVX: 2.17 ± 0.13°C). These results suggest that sexual hormones can modulate several aspects of neuroimmune system, such as LPS- and PGE_2-induced fever. But this modulation seems to be specific, considering that can involve prostaglandin, but not the opioidergic system. Further studies are necessary to understand how this specific modulation occurs and its relevance. **Financial support**: CNPq, CAPES and FAPEMA.

04.031 Anti-inflammatory effects of inosine in lung allergic inflammation: evidence for the involvement of A2 and A3 adenosine receptors. Costa FRL[1,2], Ligeiro de Oliveira AP[3], Accetturi GB[4], Martins OI[4], Domingos VH[4], Lima TW[4], Cabrini DA[5], Santos ARS[2] [1]UFSC – Pharmacology, [2]UFSC – Physiological Sciences, [3]Uninove, [4]USP – Pharmacology, [5]UFPR – Pharmacology

Introduction: Inosine has been suggested to exert anti-inflammatory effects in a wide range of inflammatory conditions, an effect related to adenosine receptors. In agreement, we have previously demonstrated that adenosine and inosine reduced neutrophils migration, pleural leakage and the levels of pro-inflammatory cytokines, in pleural exudates, with involvement of A_{2A} and A_{2B} adenosine receptors (Lapa et al., *Purinergic signal.*, 8(4):693-704 , 2012). **Objective:** This study intended to investigate the possible involvement of adenosine receptors in inosine anti-inflammatory effects in a murine model of ovalbumin-induced asthma. **Methods:** Female balb/c mice, sensitized (day 0) and boosted (day 7) subcutaneously with OVA (10 µg) were challenged at day 14 and 15 with aerosolized OVA (1%) and treated intraperitoneally 30 min prior with inosine (0.001 - 10 mg/kg) or with the selective adenosine receptors antagonists A1 (DPCPX, 2.5 mg/kg, i.p.), A2A (ZM241385, 1.5 mg/kg, i.p.), A2B (alloxazine, 5 mg/kg, i.p.), A3 (MRS 3777, 5 mg/kg, i.p) and caffeine (1.5 mg/kg, i.p.) 30 min before inosine injection (10 mg/kg). After 24 h, the bronchoalveolar lavage (BAL) was obtained to perform the cell counting; lungs fragments were collected for histological analysis and determination of cytokines in lung explants or bronchial responsiveness to methacholine (MCh, 100 mg/ml) were evaluated “in vivo”, using an animal ventilator (flexiVent 5.2). This study was approved by the ethics committee at the Institute of Biomedical Sciences of USP (approval protocol n. 58/2009) and UFPR (approval protocol n. 320/2008). **Results and discussion:** Inosine treatment caused a dose-dependent inhibition of cell counting in BAL, reducing the number of total leukocytes (98 ± 2%) with ID50 of 0.094 (0.023 - 0.37) mg/kg, macrophages (100 %), lymphocytes (92 ± 3%) and eosinophils (97 ± 2%) at dose of 10 mg/kg, when compared with the allergic control group. Pre-treatment with ZM241385 and caffeine, reverted 66 ± 8% and 86 ± 8% the inosine effect on total cell count and 100% and 86.5 ± 7.9 % the macrophages count, respectively. Also, MRS3777 treatment completely reverted total leukocyte, macrophage and lymphocyte cell count. DPCPX or alloxazine treatment did not cause any effect. These data suggest us the involvement of A2A and A3 receptors in inosine effect against macrophage and lymphocytes access to lung compartments. Moreover, inosine also reduced the levels of IL-4 (81 ± 8%) and IL-5 (100%) in explants, an effect reverted in 96.5 ± 3.4 % and 78.0 ± 8.4 % by pre-treatment with caffeine. The histological analysis stained with HE and toluidine blue confirmed that inosine prevented leukocyte accumulation, including mast cells in lungs, an effect that might involve adenosine A2A and A2B receptors. Furthermore, our results showed that inosine treatment prevented the augmentation of elastance induced by antigen challenge, an effect reverted by ZM241385, reinforcing the hypothesis that adenosine A2A receptors activation mediates inosine protective effects in allergic lungs and improves lung function. **Financial agencies**: CNPq; CAPES/ REUNI; PROCAD; FAPESC and FAPESP

04.032 Amyloid beta peptide induces neuroinflammatory response in the pineal gland and impairs melatonin synthesis. Cecon E[1], Fernandes PACM[1], Jockers R2, Markus RP[1] [1]IB-USP, [2] Institute Cochin

Introduction: Melatonin is the main hormone produced by the pineal gland and it is mainly related to the adjustment of circadian rhythms. Melatonin synthesis occurs only at night, in synchrony with the environmental light/dark cycle and under the control of the central pacemaker located in the suprachiasmatic nuclei. However, this central regulation of the pineal activity is overcome during the mounting of an inflammatory response (Markus et al., Neuroimmunomodulation 14:126, 2007). Pathogen-associated molecular patterns (PAMPs) act through toll-like receptors (TLRs) in the pineal gland, activating the nuclear factor kappa B (NF-kB) transcription factor pathway and leading to inhibition of melatonin synthesis. Alzheimer's disease (AD) is a pathological condition that involves a neuroinflammatory scenario triggered by the neurotoxic peptide amyloid beta (Ab) and AD patients are known to have impaired melatonin synthesis. Therefore, we aimed to investigate whether Ab acts in the pineal gland, modulating the biosynthetic activity of the gland. **Methods:** Pineal glands from 2 month-old male Wistar rats (Animal Committee Care IB-USP: 111/2010) were treated *in vitro* (37°C, 95% O2 - 5% CO2) with Ab peptide (fragment $_{1\text{-}40}$; 0.03 - 1 µM, 0.5h to 72h), before norepinephrine stimulation (100 nM, 5h) to induce melatonin synthesis. The cultured medium was collected to measure melatonin and cytokine release through HPLC and ELISA kits, respectively. The glands were processed to allow the analysis of gene expression (PCR array), to detect the nuclear contents of the transcription factor NF-kB (EMSA) and to analyze the expression of the inducible nitric oxide synthase enzyme (iNOS), by immunofluorescence. **Results:** Pineal glands cultivated in the presence of Ab (0.03 µM - 1 µM, 24h - 72h) show reduced melatonin synthesis induced by norepinephrine (50% - 75% inhibition). Temporal analysis of nuclear NF-kB showed a transient and oscillatory activation that peaks at 15 minutes (Ab 0.1 µM) or at 5 minutes (Ab 1 µM), leading to a 100% increase in the nuclear content of p50-p50 and p50-p65 dimmers. Tumor necrosis factor (TNF) release in the culture medium is increased in the presence of Ab (control: 20.65 ± 5.56 x Ab: 74.45 ± 19.27 pg/mL; n=4) and iNOS expression is also induced. From 84 analyzed genes, 45 were up-regulated in pineal glands incubated with Ab. Those genes are mainly related to inflammatory response and include interleukins (IL-1a, IL-6, IL-2, IL-10), receptors (all TLRs, TNFR1, IL-1R, IL-6R) and signaling molecules of the TLR-4 – NF-kB pathway. **Discussion:** Our data strongly suggest that the pineal gland is part of the neuroinflammatory response induced by Ab peptide, being able to detect it and to produce molecules that positively regulate the inflammatory response. Ab induces nuclear translocation of NF-kB, which was previously shown to block melatonin synthesis in the pineal gland. In addition, several inflammatory genes are under the control of NF-kB activity. Therefore, the pineal gland is also a sensor for endogenous molecules that present danger-associated patterns (DAMPs) and the direct effect of Ab on the pineal gland might explain the circadian dysfunctions commonly observed in AD patients.**Financial support:** FAPESP, CAPES and CNPq

04.033 Pharmacological activity of *Uncaria tomentosa* in an experimental model of cyclophosphamide-induced hemorrhagic cystitis. Benevides FT[1], Marques LM[2], Alencar NMN[2], Aragão KS[1] [1]Estácio – Pharmacology, [2]UFC – Physiology and Pharmacology

Hemorrhagic cystitis (HC) is an adverse effect resulting from the use of cyclophosphamide (CFS). Previous studies have demonstrated the anti-inflammatory activities of the plant *Uncaria tomentosa* (Rubiaceae). The aim of this work is investigate the effect of aqueous extract of *Uncaria tomentosa* (UT) in an experimental model of CH induced with CFS. Experimental protocols were registered on the Institutional Ethics Committee under number10724196-0/04. Swiss female mice (n = 10) were treated with aqueous extract of UT 2% *ad libitum* for 9 days. On the 10th day, the animals were treated again with UT (200 mg/kg by gavage) 1h before, 3, 6 and 9h after the administration of CFS (400mg/kg i.p.). The control group received only the aqueous vehicle, orally. Animals were sacrificed 12 h after the administration of CFS. The bladders of animals were removed to determine their wet weight (PUV) and scored macroscopically according to Gray's criteria. CFS was able to induce hemorrhagic cystitis. However, the treatment with UT significantly reduced the PUV in 30.5% compared to control group (130.1%) ($p<0.05$). Moreover, bladders from UT group showed less edema (0 [0 - 1]) compared to control group (1 [1 - 2]) ($p <0.05$). These findings demonstrated the anti-inflammatory activity of aqueous extract from *Uncaria tomentosa* in the model of cyclophosphamide-induced hemorrhagic cystitis. New approaches are being taken to elucidate the possible mechanisms involved.

04.034 Animal model of intestinal damage induced by the compound SN-38, the active metabolite of the anticancer agent irinotecan. Wong DVT[1], Costa ELF[1], Bem AXC[1], Leite CAVG[1], Freire RS[1], Brito GAC[2], Lima AAM[1], Lima-Júnior RCP[1], Ribeiro RA[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Morphology

Introduction: Severe diarrhea and the associated intestinal mucositis are common side effects (15-25%) of colorectal anticancer therapy with Irinotecan (IRI). SN-38, NPC and APC are important liver metabolites of IRI. It has been shown that SN-38 is 100-1000 times more potent than the pro-drug IRI in in vitro and in vivo cytotoxicity tests. It is also recognized that some anticancer agents, such as ifosfamide, when metabolized give origin to metabolites that differentially respond to the anticancer activity, isophosphoramide mustard, and the side-effect, acrolein that induces hemorrhagic cystitis. However, it is unknown whether the intestinal accumulation of SN-38 would also be responsible for the intestinal injury secondarily to IRI injection or if such injury is induced by other metabolite. Therefore, we aimed to investigate the potential harmful local effect of SN-38 injection in murine intestinal loops and to evaluate the in vitro effect of this metabolite on rat intestinal epithelial cells. **Methods:Protocol I**: C57BL/6 mice (20-25g, n=7) were anesthetized with tribromoethanol 2.5% (1mL/100g, ip), followed by laparotomy, intestinal loops identification and ligation (4 cm long). Then, the animals were injected either with saline (200 μL/intestinal loop), SN38 (30, 100 or 300 μg/loop), Irinotecan (473 μg/loop) or toxin cholera (Vibrio cholerae) (Tx, positive control, 10 μg/loop). Intestinal fluid volume/length (μL/mg) and weight/length (mg/cm) ratios (secretion parameters) were calculated 3 hours later. Intestinal tissues were collected for the measurement of mieloperoxidase (MPO, neutrophils/mg tissue), morphometry (Villus lenth and crypt/villus ratio) and histopathology. **Protocol II**: IEC-6 cells (rat undifferentiated crypt cell line) were exposed to SN38 at 4, 40 or 400 μM, Irinotecan (400 μM) or doxorubicin (0.5 uM, positive control). Then cellular changes were examined based on MTT assay, viability and cell morphology. Statistical analysis was performed with Kruskal Wallis/Dunn's test or ANOVA/ Bonferroni's test as appropriate. $P<0.05$ was accepted. (CEPA 99/10). **Results:** SN38 (100 and 300 μg) induced a significant ($P<0.05$) increase in MPO activity (4.24 ± 0.69 and 4.57 ± 0.88, respectively) compared with saline group (2.09 ± 0.48). SN38 did not alter any secretory parameter ($P>0.05$) versus the saline group. However, irinotecan (weight/length: 74.08 ± 4.54; volume/length: 10.34 ± 4.7) and the cholera toxin (weight/length: 90.29 ± 18.18; volume/length: 39.88 ± 13.45) significantly increased ($P<0.05$) these parameters when compared with the saline group (weight/length: 4.47 ± 50.75; volume/length: 0.00 ± 0.00). Villus length and villus/crypt ratio in SN38-injected mice was significantly decreased compared with saline ($p<0.05$). Additionally, SN38 showed to be more cytotoxic (IC50=1.43 uM) than irinotecan (IC50=120.8 uM) after 24 hours of cells incubation. SN38, IRI or Doxorubicin ($p<0.05$ vs vehicle treated cells) markedly reduced cell viability after a 12 and 24-hour period of incubation. **Conclusion:** Therefore, this study, though preliminary, suggested that irinotecan-related diarrhea seems to be dependent on the intestinal inflammatory damage caused by SN38 and not due to a direct effect of this metabolite on the intestinal epithelial cell lining. **Financial Support**: CNPq/FUNCAP/CAPES

04.035 Fibrinogen-induced experimental arthritis: **New method to sensitization**. Saraiva ALL, Talbot J, Veras FP, Peres RS, Lima KA, Cunha FQ, Alves-Filho JC FMRP-USP – Farmacologia

Introduction: Rheumatoid arthritis (RA), a chronic inflammatory disease of the synovial joints, afflicts up to 1,0% of the adult population worldwide. Synovial inflammation contributes to cartilage and joint destruction. Moreover, RA is also characterized by the production of autoantibodies, including rheumatoid factor and anticitrullinated proteins antibodies. Notably, citrullinated proteins can be generated replacing arginine by citrulline residues in a posttranslational process known citrullination. Previous reports have shown that citrullinated fibrinogen is a synovial-derived target and, other studies have determined that fibrinogen can participate on the pathogenesis of RA. However, there are few animal models that enable to study the role of fibrinogen and other citrullinated peptides on the pathophysiology of RA. Thus, the present work brings a new method to induce experimental arthritis using citrulinatted human fibrinogen (hFIB). **Methods:** To induce arthritis, mice C57/BL6 were sensitized with subcutaneous (s.c.) injection of hFIB (200 µg) added in an emulsion containing 100 µL of sterile saline and equal volume of complete Freund's adjuvant (CFA); 7 and 14 days later animals were boosted with the same emulsion, but CFA were replaced by incomplete Freund's adjuvant; sham mice received just saline and CFA or IFA. 7 days after the last sensitization, animals were challenged with of hFIB (10 µg) injected in femur-tibial joint and, articular nociception (electronic Von Frey apparatus) and cell migration to articular cavity were evaluated. Serum titers of total IgG anti-hFIB were determined by ELISA assay. Cells from inguinal lymph nodes were cultured and restimulated (*in vitro*) with hFIB during 96 hours; production of IL-23 and IL-17 were determined by ELISA assay in culture supernatant. Frequencies of cells producing IL-17and IFN-γ were determined by flow cytometry. All experiments were performed in accordance with protocols approved by the institutional Ethics Committee (protocol 146/2011). **Results:** Sensitization with hFIB resulted in significantly more severe arthritis. Mechanical nociception threshold was reduced in mice treated with hFIB (t_7=5,53; P<0.05) which also presented higher cell migration to joint cavity (t_8=5,60; P<0.05). hFIB sensitization induced the production of high titers of total IgG anti-hFIB. Cell culture revealed that cells from mice treated with hFIB, when restimulated with the same antigen, were able to produce higher levels of IL-17 (t_8=2,64;P<0.05) and IL-23 (t_7=2,32; P<0.05). Moreover, flow cytometry analyses showed that the immunization with hFIB increased frequencies of cells producing IL-17 (t_{10}=2,15; P<0.05), but no change were detected on profile of cells producing IFN-γ (t_{12}=1,05; P>0.05). **Discussion:** As knowledge of the etiology of human RA expands, it is important to adapt and modify animal models to better represent human disease. Recent work describes an animal model to induce arthritis using hFIB. However, spontaneous arthritis develops just in DBA/1J and SJL mice strain. Using C57/BL6, a more common mice strain, our results showed that the change in immunization process can reproduce the clinical features of arthritis such as articular pain, cellular infiltrate in the joint, production of antibodies and, the development of Th17 cells. **Financial support:** CAPES, CNPq, FAPESP

04.036 Is there a role for CXCR1/2 chemokine receptors in a model of OVA-induced allergic airway inflammation? Kraemer LR[1], Lima BHF[2], Lopes GAO[1], Garcia CC[2], Peixoto AC[1], Bertini R[3], Allegretti M[3], Teixeira MM[2], Russo RC[1] [1]UFMG – Fisiologia, [2]UFMG – Bioquímica e Imunologia, [3]Dompé

Introduction: Asthma is an inflammatory chronic disease characterized by recurrent attack of breathlessness and wheezing that affects more than 235 million people worldwide, mainly children. The airway inflammatory response is characterized mainly by an influx of eosinophils and neutrophils, which respond to cytokines such as IL-4, IL-5, IL-8, IL-13 released epithelial cells, smooth-muscle cells, lymphocytes, macrophages and mast cells that also migrate to the lung. Dexamethasone (DEXA) is a glucocorticoid, the gold standard treatment for asthma. We investigated the effects of a non-competitive allosteric inhibitor of CXCR1/2 in the airway inflammation induced by ovalbumin(OVA). **Methods:** Male Balb/c mice, were sensitized i.p. with 100 ug of OVA in 2% alum (aluminum hydroxide gel adjuvant) on day 0 and 15, and then challenged i.n. from day 21-24 with 10 ug of OVA or PBS (control) to induce airway inflammation. As treatment, mice received 5 mg/kg of DEXA subcutaneously or 10 mg/kg of CXCR1/2 antagonist in a suspension of Carboximethylcelulose 0.5% by gavage. 24 hours after the last OVA challenge, the Broncho-Alveolar Lavage (BAL) was performed to assess differential cell count, ELISA and Bradford assays. The lungs were collected for ELISA, EPO activity assays and histopathology by H&E. **Results&Discussion:** We show here that instillation of OVA is able to induce leukocyte influx into the airways in OVA-immunized mice but PBS is not. This influx is comprised by increased number of eosinophils, neutrophils and lymphocytes and that the treatment with a non-competitive allosteric inhibitor of CXCR1/2 can block this cell influx. Moreover, we show that the dose of 10 mg/kg of the CXCR1/2 inhibitor is as effective as DEXA. However, DEXA treatment was not able to reduce airway protein leakage like we the CXCR1/2 inhibitor was. In the lungs, OVA challenge in OVA-sensitized mice could induce eosinophil accumulation in the parenchyma, as assessed by EPO assay. Treatment with DEXA partially decreased pulmonary levels of EPO as well as the treatment with the CXCR1/2 inhibitor. Evaluation of pulmonary cytokine and chemokine levels showed increased levels of these inflammatory mediators both in the parenchyma and in the BAL after exposure to OVA in OVA-sensitized mice treated with vehicle. The treatment with the CXCR1/2 inhibitor could block the production of CXCL1, CCL2, CCL11, but not CXCL2-3 in the lung tissue and BAL, as well as DEXA. In addition, treatment with the CXCR1/2 inhibitor could prevent the production of IL-5 and IL-13 as well as DEXA, but not IL-4 production in lung tissue. The histopathological analysis confirmed the anti-inflammatory effects by non-competitive allosteric inhibitor as well DEXA administration. We conclude that this non-competitive allosteric inhibitor of CXCR1/2 had an anti-inflammatory effect in the airway inflammation induced by OVA reducing leukocyte influx and cytokine production, resembling those effects of DEXA. This suggests that this compound could be an important tool for asthma treatment and that it could be used in clinical trials too. Further studies will also dissect the role of this non-competitive allosteric inhibitor in pulmonary mechanics. Supported by FAPEMIG, CNPq and CAPES. License number: CETEA 218/11

04.037 Effect of phosphodiesterase Type 4 (PDE4) inhibitors, Rolipram and CILOMILAST ON the lung inflammatory response caused by silica particles in mice. Souza ET[1], Ferreira TPT[1], Azevedo GBZ[1], Nunes IKC[2], Lima LM[2], Martins MA[1], Silva PMR[1] [1]Fiocruz – Inflammation, [2]LASSBio-UFRJ

Introduction: Among the respiratory occupational diseases, silicosis is the most disabling one. This disease results from a chronic inflammatory process with granuloma formation generated in response to silica particle deposition in the lungs. There is no efficient treatment available for fibrotic diseases, which demands the search for effective therapies to control silicosis. PDE4 enzyme plays a crucial role in the intracellular signaling of inflammatory cells and is considered an important therapeutic target for the treatment of chronic inflammatory. This study investigated the potential anti-inflammatory and anti-fibrotic effects of the classics PDE4 inhibitor rolipram and cilomilast on the experimental model of silicosis in mice. **Materials and Methods:** Male Swiss-Webster mice (25 g) were intranasally instilled with crystalline silica particles (10mg, 50 µL) and treated orally with rolipram (5 mg/kg) and cilomilast (1 and 3 mg/kg), every day, starting on day 21 up to day 28 post-silica provocation. Control animals were instilled with the same volume of saline. After 24 h, lung function (resistance and elastance) and airways hyperreactivity to aerosolized methacholine were evaluated by invasive whole body plethysmography (Fine point, Buxco system). Animals were killed and the whole lung samples prepared for histology (H&E and Picrus Sirius stain) and cytokine/chemokine quantification (ELISA). All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license L034/09). **Results:** We showed that 28-day silicotic mice had an increase in the basal levels of lung resistance and elastance as well as hyperreactivity to methacholine aerosolization, when compared to control animals. In parallel, a marked fibrotic response with granuloma formation was noted in the lung tissue. Therapeutic treatment with PDE4 inhibitors rolipram and cilomilast suppressed the increase in lung resistance and elastance as well as airways hyperreactivity. Morphometric analysis revealed a significant reduction of granuloma formation in the lungs of silicotic mice treated with both compounds. The generation of the chemokines and cytokines was also sensitive to rolipram and cilomilast. **Conclusion:** Our results show that treatment with PDE4 inhibitors rolipram and cilomilast effectively suppressed the decreased of lung function and fibrogenic response associated with silicosis in mice, indicating that the blockade PDE4 seems to be a promising target for the treatment of lung chronic fibrotic diseases such as silicosis. **Financial support:** FIOCRUZ, FAPERJ, CNPq and INCT-INOFAR.

04.038 ***Aryl hydrocarbon receptor* gene polymorphism is associated with smoking-induced exacerbation of rheumatoid arthritis**. Talbot J[1], Peres RS[2], Oliveira RDR[3], Pinto LG[1], Almeida SCL[3], Silva JR[2], Franca RFO[1], Ryffel B[4], Cunha TM[1], Alves-Filho JC[1], Liew EY[5], Louzada-Junior P[3], Cunha FQ[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Imunologia, [3]HCFMRP-USP – Reumatologia, [4]CNRS Orleans, [5]University of Glasgow

Background: Rheumatoid Arthritis (RA) is a multifactorial autoimmune arthropaty with unknown etiology that affects ~1% of worldwide adult population. Genetic and environmental factors are associated with RA development, but the interaction of these factors is not well understood. Among the environmental factors, cigarette smoking is the most studied and has been associated with increased susceptibility to RA. However, the mechanisms by which smoking aggravates RA remain unknown. For instance, it was described that a pathogenic lymphocyte related to autoimmune diseases, the CD4+ lymphocyte subtype T helper 17 (Th17), express the aryl hydrocarbon receptor (AhR) which acts as an important factor in Th17 pathogenic functions. AhR is a ligand-dependent transcription factor that is activated by organic compounds as halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbons. These compounds are environmental pollutants present in smoke (eg. wood and tobacco) and herbi/insecticides. Even more, genetic polymorphisms in *AhR* can change human response to these compounds. **Aim:** The aim of this study was to evaluate the influence of AhR in RA development as well as the interaction between smoking and AhR in disease outcome. **Methods:** This study was approved by HCFMRP/USP Human Ethics Committee (2981/2009) and FMRP/USP Animal Ethics Committee (038/2009). We evaluated the distribution of two *AhR* single nucleotide polymorphisms (SNPs) in sex and smoking pared samples of healthy individuals (104) and RA patients (104) by using TaqMan Probes. Experimental arthritis was accessed by mBSA-induced arthritis (AIA) in C57BL/6 mice, *Ahr* or *Il-17ra* genetic-deficient mice (AhrKO or Il-17raKO), and by collagen-induced arthritis in DBA1/J mice (CIA). Mice were treated i.p. with PBS (vehicle), FICZ (AhR agonist) or CH223191 (AhR antagonist), or exposed to cigarette smoking on a controlled smoking machine. To access experimental arthritis activity we evaluated: articular hyperalgesia, articular histopathology and Th17 frequencies. **Results:** We found that an *AhR* haplotype (T-A) is overrepresented in RA patients. This haplotype contains SNP rs2066853 that enhanced AHR transactivation function and was related to higher autoantibodies production and Th17 frequencies in RA. Furthermore, it was observed an interaction between smoking and haplotype T-A in increasing susceptibility to RA development. Mainly, carriers of allele A that smokes are prone to develop a more severe form of RA than non smokers. In experimental arthritis, FICZ exacerbated AIA and CIA, whereas CH223191-treated or AhrKO mice develop a less severe form of AIA. Furthermore, smoking aggravated AIA in an AHR-dependent manner. Arthritis aggravation induced by AhR activation was dependent of AhR in CD4+T cells and IL-17ra signaling. **Conclusion:** Our data suggest that AhR could be associated with human rheumatoid arthritis by enhancement of Th17/IL-17 signalling. These data demonstrate that genetic polymorphisms at AHR are closely linked to smoking-induced RA aggravation, and that individuals with this genotype should be strongly warned against smoking. **Financial Support:** CNPq, FAPESP, CAPES, FAEPA and TIMER

04.039 Galectin-3 increases mortality of mice submitted to polymicrobial sepsis. Ferreira RG[1], Nascimento DC[1], Melo PH[2], Kanashiro A[1], Borges VF[1], Mota JMSC[3], Cunha FQ[1], Alves-Filho JC[1]
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AIM: The term sepsis defines a systemic inflammatory response initiated after infection, mostly caused by bacteria, that takes place after recognition of the infectious agent by the host, leading to activation of mechanisms of cellular and humoral defense. Galectin-3, a protein molecule capable of binding the polysaccharides expressed on the membrane of different cell types through carbohydrate recognition domains (CRD), has important immunoregulatory functions and participates in the development of inflammatory response. Thus, despite the importance of galectin-3 as a modulator of inflammatory response, there are no studies demonstrating its role in the development of sepsis. **Methods:** To assess the importance of galectin-3 in the development of acute systemic inflammatory response after sepsis, galectin-3 knockout (Gal-3 KO) and wild type (WT) BALB/c mice were subjected to cecal ligation and puncture (CLP). For this, mice were anesthetized, and an incision was made on the abdomen. The cecum was exposed and ligated below the ileocecal junction, and a single puncture was made through the cecum using a 18-gauge needle, then mice were stitched and received basic support (hydration). Six hours after CLP, mice were euthanized and peritoneal fluid, blood and lung were collected to assess markers of inflammatory response. In this way we evaluated neutrophils migration in peritoneal fluid using a Neubauer chamber and lung neutrophils sequestration using a myeloperoxidase assay. Lung tissue was also used to assess cytokine production by ELISA. Furthermore, blood serum was used to assess markers of tissue damage in liver (by aspartate aminotransferase activity), kidney (by blood urea nitrogen levels), and heart (by CK-MB activity). Finally, colony-forming units (CFU) were counted from peritoneal fluid and blood. **Ethical Commission in Animal Research**, Protocol No. 098/2012. **Results:** Gal-3 KO mice, compared with WT, showed greater resistance to polymicrobial sepsis characterized by high survival rate and migration of neutrophils to the focus of infection. Consistent with this data, when compared with WT, Gal-3 KO mice showed fewer CFU counted in the peritoneal fluid and blood. Moreover, Gal-3 KO mice showed lower markers of damage in kidney and heart and low neutrophils sequestration in the lung. Accordingly, Gal-3 KO mice also showed lower levels of IL-6 production in the lung that is compatible with lower systemic inflammation. **Discussion:** Taken together, our data suggest that galectin-3 is an important mediator of inflammatory response triggered during sepsis. The lack of galectin-3 leads to high neutrophil number in peritoneal fluid, associated with reduction of local and systemic bacterial load, less systemic inflammation and consequently, reduction in tissue damage that is directly related to improvement of survival rates. Pharmacological approach against galectin-3 pathway might be a possible target to the treatment of sepsis. **Financial support:** CNPq, FAPESP

04.040 The anti-inflammatory and antinociceptive effects of β-caryophyllene, a full agonist of cannabinoid receptor Type 2 (CB2), in experimental arthritis. Vieira R, Bento AF, Marcon R, Andrade EL, Calixto JB UFSC – Farmacologia

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects about 1% of world population, which leads to destruction of joints, especially the hands and knees, associated with painful inflammation symptom and motor disability. The β-caryophyllene (BCP) is a natural sesquiterpene known for its broad anti-inflammatory and analgesic activity and has been recently recognized as a full agonist of cannabinoid receptor type 2 (CB_2). Therefore, in this study we examined the anti-inflammatory and antinociceptive properties of BCP in mBSA-induced arthritis in rats and assessed whether these effects are mediated by CB_2. **Methods:** For the RA induction male Wistar rats (250 – 300 g) were initially anesthetized with a mixture of isoflurane and oxygen (2.5% - 2.25%), and then treated with an emulsion (1:1) containing Freund's Complete Adjuvant (CFA) and methylated bovine serum albumin (mBSA) (0.5 mg) diluted in PBS. Each animal received 1 mL of the emulsion into an intradermal injection at the base of the tail on day 0 and day 7. Fourteen days after the last injection of CFA/mBSA (day 21), animals were anesthetized as described above and were injected in the right knee joint cavity with mBSA (0.5 mg/animal). The protocol was previously approved by Ethic Committee for Animal Use (CEUA PP00843). The animals received BCP (10, 30, or 100 mg/kg) orally twice a day from day 21 to day 24, and were monitored for joint swelling and stiffness and mechanical nociceptive activity. Seventh two hours after mBSA challenge animals are killed and synovial fluid was extracted for measurement of IL-1β production, total leukocyte infiltration and T regulatory (Treg) cells population. Furthermore, to investigate the interaction of BCP and cannabinoid system we pre-treated animals with AM630, a selective CB_2 antagonist, plus BCP (30 mg/kg). **Results:** Oral preventive treatment with BCP (30 and 100 mg/kg) resulted in a significant reduction of joint swelling, joint stiffness and mechanic hyperalgesia. Furthermore, the preventive treatment with BCP (30 mg/kg, p.o.) significantly reduced total leukocyte count and IL-1β levels in synovial fluid when compared to the vehicle group. Interestingly, BCP seems to be effective in Treg cells up-regulation, since oral BCP (30 mg/kg) treatment enhanced $CD4^{+}CD25^{+}FoxP3^{+}$ cells population in synovial fluid compared to vehicle and control groups. In addition, the pre-treatment with the CB_2 antagonist AM630 (10 mg/kg, i.p., 30 min before), significantly reversed BCP (30 mg/kg, p.o.) beneficial effects in experimental arthritis. **Conclusion:** The present study demonstrated that the BCP oral treatment greatly improved mBSA-induced arthritis. Moreover, our results corroborated and extend previous data indicating that BCP exerts its anti-inflammatory effects through interaction with cannabinoid CB_2 receptor. **Financial support:** CAPES, CNPq and FAPESC.

04.041 A novel monocyte subset contributes to clearance of damage tissue during sterile inflammation in the liver. Dal-Secco D[1], Jenne C[1], Yipp B[1], Wong C[1], Petri B[1], Kolaczkowska E[1], Ransohoff R[2], Charo I[3], Kubes P[1] [1]University of Calgary – Immunology, [2] Cleveland Clinic – Neurosciences, [3]University of California – Medicine

Introduction: Monocytes are recruited from the blood to sites of inflammation, where they contribute to tissue injury clearance, wound healing and tissue repair. There are at least two subsets of monocytes: pro- ($CCR2^{hi}Cx3CR1^{low}$) and anti-inflammatory ($CCR2^{low}Cx3CR1^{hi}$). A recent study from our group using a murine model of focal hepatic necrosis induced by localized thermal injury has shown that within the initial 4 h post-injury, there is an increased directional recruitment of neutrophils to the focus of thermal-induced damage in the liver. However, it is presently unknown whether pro-inflammatory, as well, anti-inflammatory monocytes subsets also are recruited to the liver at later time-points after thermal-induced injury. Therefore, we have evaluated the dynamic of different sort of monocyte accumulation in the thermal-induced focal liver injury model from transgenic mice at later time points. **Methods and Results:** By using spinning disk confocal intravital microscopy, we have observed the generation of an intravascular chemokine (MCP-1) gradient directed pro-inflammatory monocyte migration through healthy tissue toward focus of sterile injury. Moreover, there are many pro-inflammatory (60-90 ± 0.3%/area of injury), as well as anti-inflammatory (30-90% ± 0.4/area of injury) monocytes inside the hepatic thermal lesion, as identified by transgenic animals (CCR2-RFP and Cx3CR1-GFP, respectively), after 12 to 72 h of sterile thermal injury. Interestingly, we have also identified after 24 to 72 h of thermal injury in transgenic CCR2-RFP/Cx3CR1-GFP mice, a novel double-positive population of monocytes (60-90% ± 0.7/area of injury) in the liver. These monocytes are expressing both chemotaxis receptors ($CCR2^{hi}/Cx3CR1^{hi}$). Furthermore, it was also demonstrated a significant reduction (1.5% ± 0.8 cells/area) of double positive monocyte migration, as well as insufficient necrotic cells elimination and collagen redeposition evaluated by multi-photon excitation microscopy in the hepatic thermal injury in CCR2 ($CCR2^{-/-}$), but not in Cx3CR1 ($Cx3CR1^{-/-}$) deficient mice. **Discussion:** Therefore, our results, by dynamic in vivo imaging, revealed a third population of monocytes in the liver. Moreover, it was also demonstrated that chemotaxis receptor CCR2 is crucial for the double positive monocyte recruitment, which contributes to clearance of damaged tissue and collagen redeposition during hepatic sterile inflammation. **Financial support:** CAPES (Brazil), CIHR/IRSC (Canada).

04.042 Evaluation of anti-inflammatory action of the hydroethanolic extract of *Macrosiphonia longiflora* (Desf.) Müll. Arg on acute models of inflammation. Silva AO[1], Almeida DAT[1], Martins DTO[1] [1]UFMT – Ciências Básicas em Saúde

Macrosiphonia longiflora (Desf.) Müll. Arg (Apocynaceae) popularly known as velame or velame-branco, is a native subshrub that grows in the Brazilian Cerrado. The plant is widely used in traditional medicine in the form of decoction and infusion, particularly as anti-inflammatory, depurative, anti-rheumatic, anti-syphilitic and antiulcerogenic. Although its use is very widespread, especially in the treatment of inflammatory processes, there are no studies that have evaluated the pharmacological activity of *Macrosiphonia longiflora*. Thus, the present study aimed evaluates the anti-inflammatory pharmacological profile of the hydroethanolic extract (70%) of *Macrosiphonia longiflora* (Desf.) (HEMl) in experimental models of acute *in vivo* inflammation. HE*M* was followed by anti-inflammatory studies, in the models of paw edema by 1% carrageenin and 1.5% dextran, then carrageenan induced pleurisy via intrapleural injection of 2% in rats and peritonitis induced by lipopolysaccharide (LPS) in mice. All procedures were approved by the Committee for Ethics in Animal Research of UFMT, with number 23108.028369/12-4. The results obtained in the study demonstrate that HE*Ml* produced significant (p <0.05) inhibition, with the highest dose (200 mg/Kg), at the peak of paw edema by carrageenin (3rd and 4th h) with inhibition of 20,9 % (0,53 ± 0,05 mL, p < 0,05) and 26,1 % (0,48 ± 0,01 mL, p < 0,01), respectively. On dextran induced edema, 2 hours after induction with the phlogistic agent, the HE*Ml* effectively reduced the edema, in all doses (20, 50 e 200 mg/Kg), in 23,6 % (0,53 ± 0,03, p < 0,01), 31,9 % (0,47 ± 0,02, p < 0,001) e 31,8 % (0,47 ± 0,02, p < 0,001), respectively. Furthermore, HE*Ml* reduced both exudate and the number of cells at all doses, promoting its greatest effect at a dose of 200 mg / kg, at 32,2 % (0,98 ± 0,03 mL, p < 0,001) and 42,2 % (65,33 ± 5,13 x 106 cél., p < 0,01) in the model of carrageenan-induced pleurisy. The HE*Ml* also caused a significant reduction (p <0.001), at all doses not-dependent, on the leukocyte migration (75,6 % - 6,49 ± 0,73; 52,9 % - 9,678 ± 0,83 and 70,1 % - 7,95 ± 0,54 x 106 cells) and the number of neutrophils (74,8 % - 5,99 ± 0,72; 62,9 % - 8,81 ± 0,75 and 81,3 % - 4,45 ± 0,35 x 106 cells) present in the peritoneal fluid of mice with LPS-induced peritonitis. This study validates from the pre-clinical point of view, the popular use HE*Ml* in the acute inflammatory conditions, necessitating further studies to elucidate its anti-inflammatory mechanism of action by evaluating of important mediators involved.
Acknowledgment: CNPq, FAPEMAT.

04.043 Anti-inflammatory effects of aqueous extract of flowers from *Kalanchoe pinnata* and its flavonoid. Ferreira RT[1], Malvar DC[1], Coutinho MAS[2], Costa SS[2], Carvalho RRN[1], Vanderlinde FA[1]
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Introduction: *Kalanchoe pinnata* (KP), or saião-roxo, is a folk medicinal plant used to treat inflammatory processes and skin healing. This study aim to investigate the anti-inflammatory effects of aqueous extract of flowers from KP (AEFL) and its flavonoid (KP5), through *in vivo* and *in vitro* assays. **Methods:** In pharmacological methodology appreciated by institutional ethics commission (n°3403-COMEP/UFRRJ), were used adult male Swiss mice (25-35g), results expressed as mean ± S.E.M. and groups compared by one-way ANOVA and post-test Tukey, considering $p<0.05$. **Ear edema test**: In groups (n=8) pretreated (*s.c.*) with vehicle, AEFL (3, 10 and 30mg.kg^{-1}) or dexamethasone (2mg.kg^{-1}), the weight difference (Δ) between the right ears (croton oil, 2.5%) and left ears (acetone) was measured (Tubaro *et al.*, *Agents Actions,*v.17,p.347, 1985). **Pleurisy test**: 1h post treatment (*s.c.*) of groups (n=8) with vehicle, AEFL (300mg.kg^{-1}), KP5 (1.25; 2.5 or 5.0mg.kg^{-1}), dexamethasone (2mg.kg^{-1}) or indomethacin (10mg.kg^{-1}), pleurisy was induced by carrageenan (1%) intrapleural injection. After 4h, the number of leukocytes was counted in Newbauer chamber (Costa *et al.*, *Peptides*,v.27,p.2967, 2006). ***Ex vivo* dosage of TNF-α and MPO:** using aliquots from groups treated with AEFL in pleurisy test, and specific enzymatic kits, TNF-α and MPO concentrations in pleural fluid were measured. ***In vitro* activity of COX-1 and COX-2:** using an enzymatic kit (ELISA), COX-1 and 2 activities were evaluated by using KP5 in concentrations ranging 3.125 to 100μg.mL^{-1}. **Results:** In ear edema test, pretreatment with AEFL (3, 10 and 30mg.kg^{-1}), produced a dose related effect by 49.6% (Δ=2.9 ± 0.5mg), 52.6% (Δ=2.7 ± 0.7mg), 63.7% (Δ=2.1 ± 0.5mg) and dexamethasone 81.2% (Δ=1.1 ± 0.3mg) , comparing with vehicle (Δ=5.9 ± 1.0mg), with DI_{50}=4.3mg.kg$^{-1.}$ In pleurisy test, pretreatment with AEFL (300mg.kg-1) and indomethacin, inhibited the leukocyte (LK) migration by 56.3% (2.1 ± 0.2x10^{6} LK.mL^{-1}) and 61.6% (1.8 ± 0.3x10^{6} LK.mL^{-1}) comparing with vehicle (4.8 ± 0.2x 10^{6} LK.mL^{-1}). The treatment with KP5 (1.25; 2.5 or 5.0mg.kg^{-1}) and dexamethasone, inhibited 29.2% (7.2 ± 0.8x10^{6} LK.mL^{-1}), 70.3% (3.0 ± 0.4x10^{6} LK.mL^{-1}), 70.4% (2.9 ± 0.4x10^{6} LK.mL^{-1}) and 79.3% (2.1 ± 0.3x10^{6} LK.mL^{-1}) respectively, comparing with vehicle (10.1 ± 0.9x 10^{6} LK.mL^{-1}). The *ex vivo* dosages of TNF-α and MPO showed reduction of 46.4% (56.0±6.2mU.mL^{-1}) and 55.3% (4.7 ± 0.03pg.mL^{-1}), respectively when compared with its negative controls. COX's activities were reduced by KP5 in a concentration dependent profile, inhibiting both COX-1 (IC_{50}=4.2x10^{-5}M) and COX-2 (IC_{50}=31.4x10^{-5}M) enzymes. **Discussion:** The anti-inflammatory activity of AEFL and KP5, obtained by *in vivo* and *in vitro* assays, may explain some of the popular indications of this species. **Financial support:** CAPES/CNPq

04.044 Selective effects of corticosterone pre-treatment in LPS-induced NFKB nuclear translocation in mixed primary cortical cultures. Duque EA, Lopes DCF, Novaes LS, Santos NB, Wiezel G, Silva NG, Scavone C, Munhoz CD [1]ICB-USP – Farmacologia

Introduction: Although the anti-inflammatory and immunosuppressive actions of glucocorticoids (GCs) secreted by the adrenal glands during stress are well established, evidence suggests that GCs can potentiate some aspects of inflammation in the brain, and this could be due to the modulation of the transcription factor NFKB. Recent work suggests that the pro-inflammatory effect of GCs in the brain is cell type specific and corticosterone (CORT) pre-treatment increases lipopolysaccharide (LPS)-induced NFKB activation in astrocytes, yet in neurons, CORT still acts as an anti-inflammatory hormone (Munhoz, C.D., SFN. 356.18/BB33, 2008). In this study, we verified whether CORT pre-treatment selective augmented and/or changed LPS-induced NFKB nuclear translocation in microglia, astrocytes and neurons of mixed primary cortical cultures. **Methods:** Primary mixed cortical cultures were obtained from newborn rats (P1-P4) as described previously (Ahlemeyer, B., J Neur. Meth., 149:110, 2005). Cultures were maintained in DMEM High Glucose media supplemented with 10% Fetal Bovine Serum (FBS), 10% Horse serum (HS), and 0,5% penicillin/streptomycin (P/S) or in DMEM High Glucose media supplemented with 2% FBS, 2% HS, 0,5% P/S during the experiments performed on day 7 after culturing. Cultures were treated with ethanol (1%, 24 h) or CORT (1 μM, 24 h) and/ or RU486 (10 μM, 24 h) followed by saline or LPS (10 μg/mL, 1 h) and submitted to double immunofluorescence assay as described previously (Piccioli, P., J Neur. Res, 66(6):1064, 2001) with the following antibodies: anti-GFAP (astrocyte marker, 1:1000), anti-MAP2 (neuron marker, 1:1000), Lectin-FITC (microglia marker, 1:2500), anti-RELA (NFKB subunit marker, 1:1000) and DAPI (nuclear marker, 1:100.000). **Results:** RELA nuclear localization in neurons was independent of all treatments, with high basal nuclear localization in all groups, approximately 75 ± 10% (EtOH, CORT, LPS, CORT+LPS), that were attenuated by GR antagonist addition (15,9 ± 5,6%, p<0.001; 32,1 ± 6,8%, 35 ± 7,2%, 35,7 ± 7%, p<0.01). Microglia RELA nuclear localization was higher in all LPS-treated groups (LPS-87 ± 5,8%, RU+LPS-66,8 ± 7,3%, CORT+LPS-89,8 ± 6,2%, RU+CORT+LPS-61 ± 7,2%) compared to ethanol control (12,8 ± 8,8%, p<0.001), which were attenuated by GR antagonist addition in all LPS-treated groups (p<0.05, p<0.01). Astrocytes RELA nuclear localization was higher in all LPS-treated groups (LPS-70 ± 4,5%, RU+LPS-74,4 ± 3,2%, CORT+LPS-79,1 ± 1,9%, RU+CORT+LPS-55 ± 6%) compared to ethanol control (22,4 ± 4,3%, p<0.001), which were only attenuated by GR antagonist addition in the presence of CORT pre-treatment (p<0.001 vs CORT+LPS alone). Microglia and astrocytes controls do not change compared to respective ethanol control. **Discussion:** CORT did not modulate LPS-induced effects at neurons or microglia. At these two cell types, GR signaling seemed important for the physiological maintenance of nuclear RELA, irrespective to CORT or LPS stimuli. Astrocytes were responsive to LPS-induced NFKB activation, on the other hand, CORT did not prevent RELA nuclear translocation which was decreased by the GR antagonist, revealing a CORT role for the astrocyte maintenance of LPS-induced NFKB nuclear translocation. **Financial support**: FAPESP, CAPES e CNPq. Animal Ethics Committee number: 132/2010.

04.045 Effect of high-carbohydrate diet intake in metabolic and inflammatory response of mice IL18-/-. Yamada LTP[1], Oliveira MC[1], Lana JP[2], Batista NV[2], Fonseca RC[2], Pereira RV[2], Cara DC[2], Ferreira AVM[3] [1]FF-UFMG – Ciência de Alimentos, [2]ICB-UFMG – Morfologia, [3]UFMG – Nutrição

Introduction: Interleukin-18 (IL18) is a pro-inflammatory cytokine involved in immune responses and is associated with atherosclerotic progression, obesity, metabolic syndrome and type 2 Diabetes. Recently IL18 has been suggested to be an adipogenic cytokine, associated with body mass index, excess adiposity, insulin resistance, hypertrigliceridemia and metabolic syndrome. In a recent study, male $IL18^{-/-}$ mice ate more low-fat chow than C57BL/6J wild-type (WT) and administration of IL18 suppressed appetite, feed efficiency and weight regain, suggest that IL18 signaling modulates food intake, metabolism and adiposity (NETEA et al., *Nat. Med.* 12: 650, 2006; SMART et al., Nutr Metab Cardiovasc Dis, 21: 476, 2009). Here, we show that the high refined carbohydrate-containing diet also affects appetite, weight gain, adiposity and inflammation of adipose tissue in male $IL18^{-/-}$. **Methods:** Mice deficient in IL18 and C57BL/6J wild-type (WT) were kept in an environmentally controlled room under a light-dark cycle, with free access to water and food, in according to ethical guidelines of the Animal Ethics Committee of the University (protocol 060/2010). The animals were fed standard laboratory chow (LABINA) or a high-refined carbohydrate diet (HC) for 8 weeks. The HC diet was composed of 74,2% carbohydrate (30% of sucrose), 3,1% fat and 31,1% protein (4,4 kcal/g). Food intake, weight gain and adiposity index was measured. At the end of the experiment, the oral glucose tolerance was performed and the animals were euthanized to collect blood, tissue and organs. Levels of TNF-α and IL-6 are measured. **Results and Discussion**: $IL18^{-/-}$ mice had more weight than WT mice with chow diet and equal to animals with HC diet. The diet intake was higher in $IL18^{-/-}$ mice with chow diet and equal for WTs groups and $IL18^{-/-}$ HC group. The adiposity index was increased in mice fed with HC diet (WT and $IL18^{-/-}$). Mice deficient in IL18 had more glucose intolerance than WT, as much chow diet as HC diet. The animals fed with HC diet also showed high levels of TNF-α and IL-6 on adipose tissue than WT with chow diet group, but equal to $IL18^{-/-}$ with chow diet group. The data suggest that the diet composition have more effects of adiposity index than the IL18 but, this cytokine player significant role in the glucose tolerance. Like demonstrate in literature, the $IL18^{-/-}$ mice ate more chow diet than WT mice, but were not observed in mice with HC diet, unlike the high-fat diet (ZORRILA et al., PNAS, v.104, p.11097, 2007). Has been described by our group as the HC diet promote a increase of adiposity and metabolic changes in adipose tissue, like the increase of TNF-α and IL-6 cytokines (OLIVEIRA et al., Obesity, 2013. In press). Here the increase of these pro-inflammatory factors in WT mice with HC diet was equal in IL18 deficient mice, suggesting that the composition of diet have influence in the expansion and inflammation of the adipose tissue as well as the IL18 cytokine. **Acknowledgements**: FAPEMIG and CNPq for financial support.

04.046 Characterization of anti-inflammatory properties of passion fruit seed oil. Lima CKF[1], Moreira CC[1], Silva CS[1], Lima JA[2], Miranda ALP[1] [1]LEFEx-ICB-UFRJ, [2]Assessa

Introduction: Passion fruit seed oil (PFSO) contains Omega-3 and Omega-6 fatty acids which play important roles in the development of inflammatory response. Omega-3 fatty acids are precursors of many anti-inflammatory mediators such as resolvins of series D and E (Sommer, C. F1000 Med. Reports. 2011, 13:19). Furthermore, we decided to investigate the anti-inflammatory activity o passion fruit seed oil in a model of TPA-induced ear edema. **Methods:** TPA-induced ear edema was performed in Swiss mice weighing between 18-25 g. Mice received PFSO topically (20 µl/ear) and TPA solution (20 µl/ear; 100 µg/ml) was applied 15 min later. Edema formation was verified at 2, 4, 6 and 24h after TPA stimulation. At 6h and 24h after TPA ears were removed and homogenized for quantification of TNF-α production by ELISA (BD Bioscience), MPO activity and for western blot analysis in order to verify p38 MAPK activation and COX-2 expression. Mice peritoneal macrophages stimulated with LPS (100 ng/ml) were also used to evaluate if PFSO could interfere in TNF-α production *in vitro*. Animal protocols were approved by UFRJ ethical animal care committee (FARMACIA03/CEUA/UFRJ). **Results and discussion:** Previous treatment with topical application of 20 µl of PSFO reduced edema formation induced by TPA at 4h and 24h (4h= 62% and 24h= 52% of inhibition; n=6-8). This inhibition was followed by reduction in MPO activity (47%, n=5) 24h after TPA, which is indicative of diminished neutrophil infiltration. It was also observed decrease in TFN-α production in the ear, although 6h after TPA application, which had been shown by Murakawa and colls. (Biochem. Pharmacol. 2006, 71:1331) as the peak time for the production of this cytokine. PFSO treatment also reduced the expression of COX-2 and p38 MAPK activation in the ear after TPA stimulation. In order to investigate if PFSO can interfere directly in TNF-α production in macrophage, we stimulated peritoneal macrophage with LPS and verified that only at lower concentration (1 µg/ml) PFSO can inhibit TNF-α production, since at 100 µg/ml or 10 µg/ml we did not observe any interference. **Conclusion**: PFSO topically is effective in dermatitis model induced by TPA, by a mechanism dependent on inhibition of TNF-α production, p38 MAPK activation, COX-2 expression and reduction of neutrophill infiltration. Thus, PFSO arises as a new pharmacological tool to treat inflammatory disorders. **Acknowledgement**: FAPERJ, CAPES, PIBIC/CNPq, CNPq, EXTRAIR.

04.047 Role of B1 and B2 bradykinin receptors in sepsis induced by cecal ligation and puncture. Oharomari Jr LK, Trevisan SC, Cunha FQ FMRP-USP – Farmacologia

Introduction: Sepsis is defined as inflammatory response during a current infection. This disease is characterized by vascular dysfunction that can culminate with severe hypotension and multi-organ failure (ALVES-FILHO et al., 2008). Bradykinin is an inflammatory mediator released in sepsis and recognized by two G-protein coupled receptors, B1 and B2. The first is inducible in leukocytes and vessels cells mainly by IL-1beta, while the second is constitutive and involved in control of tonus and vascular permeability (MGHEBREHIWET e KAPLAN, 2010). Despite its importance, the function of bradykinin receptors in sepsis is poorly understood. Our aim was to evaluate the role of bradykinin B2 and B1 receptors in pathogenesis of sepsis. **Methods:** C57BL/6 (wild type- WT), B1 deficient mice ($B1^{-/-}$) and B2 deficient mice ($B2^{-/-}$) were given cecal ligation and puncture (CLP) and survival rates were evaluated during seven days. Six hours after CLP were measured: number of neutrophils in peritoneal cavity, number of colony forming units in blood and peritoneal cavity, neutrophil sequestration in lungs, CXCL2 , CXCL1 and TNF-alpha in serum. All procedures are in according with ethical principles in animal research and were approved by Ribeirao Preto Medical School, University of Sao Paulo (protocol 119/2012). **Results:** Mice deficient of B2 or B1 bradykinin receptor showed lower survival rates compared to WT (80% of survival for WT versus 30 and 20% of survival for $B1^{-/-}$ and $B2^{-/-}$ mice). Interestingly, the absence of bradykinin B2 receptor increased neutrophil migration to focus of infection/inflammation (9.28 ± 1.84 vs 5.36 ± 1.6, for mean ± SD). In spite of the higher neutrophil recruitment to peritoneal cavity, the mice deficient of B2 showed similar number of colony forming units in peritoneal exudates (4.8 ± 0.6 vs 4.77 ± 0.6, median ± SD) and number of neutrophils trapped in lungs (3986 ± 696 vs 4573 ± 271, mean ± SD). In addition, mice deficient of B2 have higher levels of CXCL2 (947.5 ± 44 vs 478.842 ± 197, mean ± SD pg/ml) and TNF-alpha (3.50 ± 0.74 vs 1.12 ± 0.68 in serum than WT. Different of $B2^{-/-}$, $B1^{-/-}$ mice showed neutrophil recruitment (5.75 ± 1.08) to peritoneal cavity similar to WT as well as neutrophil lung infiltration (5109 ± 554), however these mice showed higher number of bacteria in peritoneal lavage (5.35 ± 0.3). **Discussion:** Deficiency of bradykinin B2 receptor leads an enhanced neutrophil migration to infectious focus, however, does not control the systemic inflammatory response after CLP induced sepsis. Deficiency of bradykinin B1 receptor impaired sepsis mortality as well as B2 deficiency, but through uncontrolled infection. **References:** Mghebrehiwet, B.; Kaplan, A.P. The plasma bradykinin-forming pathways and its interrelationships with complement**.** Mol Immunol., v.47, n.3, p.2161 - 2169, 2010. Alves-Filho, J.C.; De Freitas, A.; Spiller, F.; Souto, F.O.; Cunha, F. Q. The role of neutrophils in severe sepsis. Shock, Suppl 1, p.3 - 9, 2008.

04.048 IL-10 exerts a dual effect on rat pineal melatonin production. Santos GC, Markus RP, Fernandes PA IB-USP – Fisiologia

Introduction: Our group and others have shown that, besides its phototransducer function, pineal gland is also an important immunomodulator. In this context, named immune-pineal axes (Markus et al., Neuroimmunomodulation, 14, 126, 2007), immunological modulators related with the mounting of inflammatory processes (LPS and TNF) reduce pineal melatonin production, while the Th1 cytokine, interferon-gamma (IFN-γ), potentiates the hormonal production of pineal gland. Interleukin-10 (IL-10) is a cytokine with pleiotropic effects in inflammation, reducing, for example, the production of TNF and IFN-γ by macrophages and Th1 cells (Ng et al., Front Immunol. 4: 129, 2013), but the direct effect of IL-10 on pineal melatonin production is unknown. The aim of this work was to evaluate the effects of IL-10 on the noradrenaline induced production of melatonin and the precursors serotonin (5-HT), 5-HIAA and N-acetilserotonin (NAS) in cultured rat pineal glands. **Methods:** Pineal glands of male Wistar rats (250-350 g), cultivated for 48 h in BGJb medium, (95% O^2, 5% CO^2, 37°C) were incubated with IL-10 (0, 3, 10, 30 e 100 ng/mL, 30 min) and then stimulated with noradrenalin (NA, 10 nM, 5 h, still in the presence of IL-10). The content of 5-HT, 5-HIAA, NAS and melatonin were determined in the medium by HPLC. All the procedures were approved by the animal ethical committee of the Biosciences Institute (protocol: 174/2013). **Results:** IL-10 (3 ng/mL) increased the NA-induced production of 5-HIAA (782.2 ± 62.42), NAS (51.67 ± 14.32) and melatonin (76.09 ± 9.15) when compared to the control group (5-HIAA: 461.8 ± 59.08; NAS: 20.25 ± 2.37; melatonin: 54.19 ± 4.09). When higher concentrations of IL-10 were used, no effect was observed on 5-HIAA production, the production of NAS was still elevated in comparison to the control group but, there was a dose-dependent inhibitory effect on melatonin production. 5-HT levels were not altered by IL-10 treatment. **Discussion:** IL-10 exerts differential effects on the biosynthetic pathway of melatonin by a mechanism still under investigation, but that might be related to the modulation of the enzymes (transcription and/or activity) participating on pineal hormonal production. The dual effect on melatonin suggests that IL-10 may change melatonin production according to the phase of the immune response. The present work increased our understanding of how pineal gland integrates immunological signals present in different contexts of a defense response. Financial Support: FAPESP (2010/52687-1; 2012/23122-1).

04.049 Improvement of anti-edematogenic activity of friedelin with cyclodextrin complexes.

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Introduction: From the hexane extract of the stem bark of *Clusia nemorosa* Mey (Clusiaceae), the friedelane triterpenoid named friedelin has been isolated. However, the poor solubility of friedelin in water has limited advances in pharmacological studies. Thus, the aim of this study was to prepare the inclusion complex of friedelin (FD) and hydroxyl-propyl-β-cyclodextrin (HP-βCD) with improved solubility, stability and to study the anti-edematogenic effect of HP-βCD inclusion complex in mice. **Methods:** The inclusion complex of FD with HP-βCD was prepared by direct mixing in dissolution vessel. Fixed volumes of FD solution (2.3×10^{-4} mol/L) were added to HP-βCD solution (in a 1:2 molar ratio) and kept stirring for 24 h. The association constant of FD:HP-βCD complex was estimated by the Benesi–Hildebrand method, based on the spectrophotometric quantification of free HP-βCD at 228 nm and 278 nm. Male mice (25-30 g) were treated by intraperitoneal route (i.p.) with FD, FD/HP-βCD or vehicle (NaCl, 0.9%), and 1 h later, paw edema was induced by carrageenan (1%) being the edematogenic response evaluated at 4 after stimulus. The results were compared with that the FD and FD/HP-βCD inclusion complex in equal amounts (1 or 10 mg/kg). Results were expressed as mean ± standard error of the mean and statistically analyzed using the ANOVA followed by post-test Newman-Keuls, P values < 0.05 were considered significant. All experiments were approved by the Institutional Ethics Committee (License no. 9244/2009-45). **Results:** The results demonstrated that FD/HP-βCD solid powders showed improved stability and solubility in aqueous solution, when comparing with free FD. The formation of inclusion complex were proved by UV-Vis analysis that indicated the constants of dissociation (KD) and formation (KF) at 228 nm as being 4.1×10^{-6} and 2.4×10^{5} mol/L, respectively, while to 278 nm the KD and KF were 3.8×10^{-6} and 2.6×10^{5} mol/L, respectively. Intraplantar injection of carrageenan induced a significant edematogenic response (52.0 ± 2.9 μl) into paw from mice. Administration of single intraperitoneal doses (1mg/kg or 10 mg/kg) of FD significantly reduced the carrageenan induced paw edema in mice by 38% and 18%, respectively. Pretreatment with the complex (1mg/kg or 10 mg/kg) improved the anti-edematogenic activity of the FD for 54% and 59%, respectively. **Discussion**: Results for anti-edematogenic activity showed that the stronger effects were found in the treatment group of friedelin/hydroxyl-propyl-β-cyclodextrin inclusion complexes in comparison with those of free friedelin. These results suggest that HP-βCD inclusion system might be a promising formulation for the delivery of friedelin. **Financial support:** CNPq, CAPES.