04. Inflammation

04.001

Microbiota is important to 5-fluorouracil-induced intestinal mucositis in mice.

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Introduction: Mucositis is a major side effect caused primarily by anti-cancer chemotherapy in the mucosal surfaces of gastrointestinal tract. It is characterized by nausea, vomiting, diarrhea, constipation, and abdominal pain, which may lead to decrease of the chemotherapeutics dosages or to postpone chemotherapy treatment. Gastrointestinal tracts colonized by millions of microorganisms, collectively called microbiota. The microbiota is involved in various inflammatory bowel diseases, such as colitis and Crohn's disease, but it has not been demonstrated its role in development of mucositis. Thus, the aim of this work was to study the role of microbiota in the development and severity of chemotherapy 5-fluorouracil (5-FU)-induced mucositis. **Methods**: The use of animals in this study was approved by the "Ethics Committee in Animal Experimentation at UFMG" (protocol N°: 264/08). The 5-fluoruoracil (5-FU) chemotherapy was injected i.p. in conventional (CV) and germ-free (GF) mice at a dose of 450 mg/kg once a day for 3 consecutive days to induce mucositis (5-FU) or vehicle (C). On day 4 and 5 (24h and 48h after the last injection of 5-FU), animals were euthanized and blood and intestine were removed for analysis. To deplete microbiota, conventional adults or newborn mice were treated with antibiotics cocktail for 30 days or 3 months, respectively. The antibiotics cocktail consisted of ampicillin 1g/L, neomycin 1g/L, metronidazole 1g/L, vancomycin 0,5g/L and ciprofloxacin 1g/L. Subsequently, the animals received 5-FU to induce mucositis as described above. To determine if mucositis was associated with dysbiosis, mice stools were plated on specific medium to determinate the enterobacteria and *Bacteroide* concentration. To evaluate the role of enterobacteria and *Bacteroide* in mucositis, germ-free mice were monocolonized with members of these groups. The statistical significance across all groups was assessed by one-way analysis of variance (ANOVA). Data were represented by means ± SEM of 5 animals per group. **Results:** We have observed intestine shortening CV-5-FU mice compared to CV-C, however in GF groups did not show changes. In addition, CV-5-FU animals showed increased of MPO and EPO activity enzymes in intestine, indicating neutrophils and eosinophils influx, respectively, but not in GF mice. Furthermore, only CV-5-FU group presented increased in IL-1β, TNF-α, CXCL-1, CCL-11 and CCL-24 levels when compared to CV-C mice. This protection observed in GF animals resulted in 100% survival and clinical recovery after 5-FU injection, whereas all animals CV succumbed within 7 days after treatment start. In addition, adults and newborn mice, previously treated with cocktail of antibiotics to deplete microbiota, present with results similar to germ-free mice. In CV mice, after treatment with 5-FU, there were increased in enterobacteria group but no change was observed in *Bacteroide* number. The monocolonization of GF mice with *Escherichia coli* (a represented of enterobacteria group), reversed the protection of this group after 5-FU injection, but not in *Bacteroides fragilis*. **Discussion**: Briefly, the data obtained so far allow us to conclude that the microbiota presence is involved in the development and severity of chemotherapy-induced mucositis. The microbiota contributes to the exacerbating of inflammatory response which results in greater intestinal injury. Furthermore, enterobacteria group is important to severity of chemotherapy-induced mucositis. **Financial Support**: CNPq, FAPEMIG

04.002

Effect of high dose intravenous immunoglobulin (IVIG) therapy in the treatment of severe dengue. Rocha RPF[1], Costa VV[1], Fagundes CT[2], Valadão DF[1], Avila TV[1], Cisalpino D[1], Souza PR[1], Ribeiro LS[1], Queiroz CMJ[3], Silva TA[3], Dias ACF[1], Verri WA[4], Teixeira MM[5], Souza DG[1] [1]ICB-UFMG – Microbiologia, [2]UFMG – Microbiologia / Trinity Biomedical Sciences, [3]FO-UFMG – Patologia Oral, [4]UEL – Patologia, [5]UFMG – Bioquímica e Imunologia

Introduction: Dengue is caused by one of the four serotypes of *Dengue virus* (Denv-1-4). The hallmarks of severe dengue may include thrombocytopenia, increased vascular permeability, cytokine storm associated with an exacerbated inflammatory response and shock. High dose intravenous immunoglobulin (IVIG) therapy has been used in the treatment of a large range of autoimmune and inflammatory diseases. Here we evaluated the potential therapeutic effects of the administration of high dose IVIG therapy in a model of DENV infection in mice. **Methods:** BALB/c mice (8 to10 weeks) were inoculated with an adapted strain of DENV-3 via the i.p route. Mice were given vehicle or IVIG daily, via i.v route, at dose of 1g/kg from day 0 to 6 of infection. Seven days after infection, mice were euthanized and blood and tissues (spleen and liver) collected for several analysis described below. Mice were accompanied for lethality until day 14th of infection. All experimental procedures were approved by CETEA/UFMG access number 113/2009. **Results:** Seven days after DENV-3 infection vehicle-treated mice presented severe disease manifestation characterized by markedly thrombocytopenia, increased hemoconcentration, elevated levels of hepatic transaminases in serum and massive liver damage. Higher levels of TNF-a and IFN-g and elevated viral loads were found in serum and tissues of infected mice. Conversely, high dose IVIG administration to mice clearly inhibited the major manifestations of the disease, including lethality. Mechanistically, IVIG treatment resulted in massive release of the immunomodulatory cytokines IL-33 and IL-10 in comparison to the vehicle-treated mice. Of note, the protection afforded by the IVIG administration during DENV infection occurred without loss of control of viral replication and IFN-γ production and was completely dependent on IL-33 production. IVIG administration to mice deficient for IL-33 receptor ($ST2^{-/-}$ mice) failed to mediate protection after DENV-3 inoculation. **Conclusion:** IL-33 production induced by IVIG treatment is pivotal for disease amelioration during DENV infection. Interfering in the exacerbated inflammatory response induced by DENV through high dose IVIG administration can be used as an alternative therapeutic to the severe manifestations of dengue disease. **Financial support:** CNPq, FAPEMIG, Capes, INCT em dengue and PRONEX (Ministério da Saúde).

04.003

Expression of inducible nitric oxide synthase (iNOS) in heart and kidney of mice with collagen-induced arthritis (CIA). Zochio GP[1], Carlos CP[2], Girol AP[3], Taipeiro EF[4], Chies AB[1] [1]FAMEMA – Farmacologia, [2]FACERES – Medicina, [3]FIPA – Imunohistoquímica, [4]FAMEMA – Bioquímica Básica

Introduction: Studies have demonstrated changes of endothelial function in rheumatoid arthritis (RA) as well as in the adjuvant-induced arthritis (AIA). However, there were not observed modifications of endothelial function in aorta taken from mice with CIA, a model that is more similar to RA. In a previous study we also have not observed modifications of vascular responsiveness to norepinephrine or acetylcholine in these preparations. Nevertheless, such data do not exclude modifications of mechanisms that control the tonus in the microcirculation. Thus, the aim of this study was to investigate the expression of iNOS in heart and kidney of CIA mice. **Methods:** DBA/1J mice (n=5-6) were immunized intradermally at the base of the tail with 100 µg of emulsified CII plus Complete Freund's adjuvant. On the day 21°, the animals received a boost with100 µg of emulsified CII plus Incomplete Freund's adjuvant. The first signs of joint inflammation appeared on the 26° – 33° day. Age-matched DBA/1J mice without CIA were employed as controls. The iNOS expression was investigated by immunohistochemistry 15 days after appearance of the first signs of joint inflammation. In the heart, the expression of iNOS was analyzed in the myocardium while that in the kidney this analysis was performed in the cortical (glomerular and interstitial) and medullar regions. **Results:** The optical densitometric analysis revealed that the CIA induced increased expression of iNOS in myocardium (from 133.7 ± 4.8 to 172.0 ± 3.4; $p<0.0001$) as well as in renal cortex (glomerular: from 132.1 ± 2.3 to 179.9 ± 7.0; $p<0.0001$; interstitial: from 128.0 ± 3.0 to 184.9 ± 9.1, $p<0.0001$) and medulla (from 131.4 ± 3.3 to 180.1 ± 12.8; $p<0.01$). **Conclusion:** CIA increases the expression of iNOS in the mice cardiac and renal tissues which may influence the vascular resistance in these organs.

04.004

In vitro and _in vivo_ immunomodulatory mechanisms involved in the anti-inflammatory activity of subfraction 4 (SF4) of _Echinodorus macrophyllus_ (Kunth.) Mich (Alismataceae)

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Introduction: Echinodorus macrophyllus (Kunth.) Mich (Alismataceae), known as "chapéu de couro" in Brazil is used popularly to treat rheumatic and inflammatory diseases. Immunoregulatory effects (Pinto, *J Ethnopharmacol*, 111: 435, 2007) were previously described by our group for the aqueous extract of the aerial parts of E. macrophyllus (AEEm) and its ethanolic fraction (Fr20). Thus, the aim of this work was to evaluate the effects of subfraction 4 (SF4), obtained from Fr20, as well as the mechanisms involved in this response. **Methods:** Chromatographic profile of SF4 was obtained by HPLC-DAD using a C18 reverse phase column, by monitoring at 264 nm, 318 nm and 369 nm. *in vitro* NO and IL-1β production from supernatant of RAW 264.7 macrophages pre-incubated or not with SF4 was performed by Griess reagent and ELISA assay, respectively, and the cell viability by reducing mitochondrial activity (MTT). Additionally, the effects of SF4 on the expression of iNOS and TNF-α were evaluated in cell line RAW264.7 stimulated with LPS, by RT-PCR. DCFH-DA probe was used to measure intracellular ROS level in RAW264.7 stimulated or not with LPS and treated or not with SF4. Thioglycollate (4%) was used to induce peritonitis model (SW mice, 25-35 g, n=4) (Frimodt-Moller, 1993) and the effects of SF4 on macrophage peritoneal migration and Mac1/Gr1 integrin modulation was evaluated by flow cytometry (Approved by CEA-IBRAG committee/protocols 05/2009 and 05/2013). Results were expressed as mean ± SD and compared using ANOVA followed Dunnet's test. **Results and discussion:** SF4 showed a majority peak with RT= 13.44 minutes when observed at 318 nm. The treatment with SF4 inhibited the LPS-induced NO production, showing highest inhibition of 42.6% (25.83 ± 5.69 μM) at 100 μg/ml compared to control culture (60.6 ± 11.5 μM). Moreover, SF4 also induced decreased on IL-1β level, achieving inhibition of 66% (172 ± 98.77 pg/ml), compared to control culture (503 ± 178 pg/ml). However, SF4 did not show cytotoxic effects on RAW264.7 cell line. In addition, LPS stimulation induced a significant increase on expression of iNOS and TNF-α. Therefore, cells treated with SF4 showed an inhibition in a concentration-dependent manner, showing the maximal inhibition of 64.3% and 78% at 150 μg/ml, respectively. LPS stimulation also induced an increase in intracellular ROS when compared to unstimulated cells. However, cells treated with quercetin showed an inhibition on ROS production of 80%, while SF4 showed inhibition of 40.28% and 48.87% at 50 μg/ml and 150 μg/ml, respectively. Thioglycollate induced an increase ($5.96 \times 10^6 \pm 0.63$ to $23.9 \times 10^6 \pm 5.59$) of cell number in the peritoneum. Nevertheless, daily treatment with SF4 at 2.5 mg/kg inhibited up to 66% of cell migration ($8.11 \times 10^6 \pm 0.98$), while the integrins expression has not changed among the groups tested. In addition, the treatment with dexamethasone or SF4 was able to significantly reduce the number of $Gr1^-Mac1^+$ cells in the peritoneum. These results confirm the anti-inflammatory potential suggested for this plant and provide a basis for understanding their molecular mechanisms of action.

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04.005

Role of advanced glycation end-products on glucocorticoid-induced mastocytopenia and mast cells hyporeactivity. Santoro TT, Torres RC, Silva PMR, Martins MA, Carvalho VF IOC-Fiocruz – Inflamação

Introduction: Glucocorticoid (GC) therapy is the most effective treatment for chronic inflammatory and autoimmune diseases. One metabolic effect of GC is the increase of glycaemia and it may enhance the formation of Advanced Glycation And-products (AGEs). Examples of AGEs are fructosamine and glycated hemoglobin. AGEs induce mast cell apoptosis through activation of its receptors (RAGE and Galectin-3) leading an increase of reactive oxygen species generation. The aim of this work was evaluate the role of AGEs in reduction of mast cell numbers and reactivity induced by prolonged treatment with GC. **Methods:** The animals were obtained from the Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (CEUA-Fiocruz, license LW 23/11). Male Wistar rats were treated with GCs (dexamethasone or prednisolone 0.1 mg/kg, sc) for 21 days. On the third day, some animals were co-treated daily with aminoguanidine (AG) (50 or 250 mg/kg, vo) or L-NAME (30 mg/kg,vo). Pleural mast cell numbers were evaluated by toluidine blue stain. To assess mast cell reactivity, these cells were obtained from peritoneal cavity and after isolated by a continuous Percoll gradient. Then, 10^5 cells were plated and triggered by 48/80 compound or antigen, and the supernatant was used to measured histamine released by fluorescence. **Results:** Treatment with dexamethasone (DEXA), a high power GC, induced a reduction in the pleural mast cell numbers compared to controls (from 451.5 ± 28.89 to 308.17 ± 25.3 mast cells ($x10^3$)/cavity, respectively; mean ± SEM, n=6; $p<0.05$). Treatment with AG, which prevents AGEs formation, reverses the reduction of mast cells numbers in rats that received DEXA (434.39 ± 19.17 mast cells ($x10^3$)/cavity, mean ± SEM, n=6; $p<0.05$). *In vivo* treatment with DEXA induced reduction of mast cell reactivity triggered by antigen or 48/80 compound *in vitro* compared to mast cells obtained from control rats, and AG reverses this hyporeactivity-induced by DEXA. Similar results were noted using prednisolone (PRED) as GC therapy. Furthermore, AG in the exclusive dose of NO synthase inhibition or L-NAME was unable to reverse the reduction in the number and reactivity of mast cells induced by DEXA. In addition, neither DEXA nor PRED were able to alter glycaemia and fructosamine levels. **Discussion:** The results showed an influence of AGEs on reduction of the number and reactivity of mast cells induced by prolonged treatment with GCs. Once GC treatment did not change glycaemia and fructosamine levels, we hypothesized that GC could be increase the expression of AGEs receptors in mast cells, which leads to increased intracellular signaling cascade of activation pathways of apoptosis in mast cells. **Sources of research support:** CNPq, Faperj, Capes, PAPES-VI/ Fiocruz.

04.006

Epigenetics alterations is involved in reduced inflammatory response in intrauterine undernourishment. Ballico MM[1], Carvalho MHC[1], Câmara NOS[2], Landgraf RG[3], Landgraf MA[1] [1]ICB-USP – Farmacologia, [2]ICB-USP – Imunologia, [3]Unifesp – Inflamação e Farmacologia Vascular

Introduction: It has been demonstrated that adverse environmental factors in the prenatal period cause changes in the normal pattern of growth and development of the fetus. Indeed, failure of the maternal-placental supply to match fetal nutritional demand results in a range of adaptive changes in fetal development. In this study, we have investigated the impact of intrauterine undernutrition on the levels of inflammatory markers, and their correlation with the lung dysfunction. The global methylation pattern in the lung was also investigated. **Methods:** All procedures used in this study were approved and performed in accordance with guidelines established by the ethics committee of the ICB/USP (CEUA–67/2013). Female Wistar rats were randomly divided into 2 groups: nourished (NR; ad libitum diet) and undernourished (UR; 50% food restriction). At 8 weeks of age, lung global methylation pattern was determined and serum levels of corticosterone, leptin, IL-1ß, TNF-α, IL-5 and IL-13 were evaluated by Bioplex in NR and UR offspring. The influence of intratracheal instillation of LPS (750 μg/400 μL) on leukocyte accumulation in the lung was also evaluated in both groups. **Results:** We did not observe differences in corticosterone, leptin, IL-1β and TNF-α levels between UR and NR offspring. Global lung methylation (150%) as well as IL-5 (375%) and IL-13 (83%) levels were higher in UR than NR offspring. Inflammatory cell infiltration into airways after LPS instillation was increased (3.5 times) in NR offspring as compared to UR offspring. **Conclusion:** These preliminary results indicate that epigenetic alterations might be involved in the altered pattern of inflammatory response displayed by UR offspring. **Financial support:** Fapesp (2012/51104-8, 2010/01404-0) and CNPq

04.007

Arginase contributes to lung fibrotic response in silicotic mice. Dias DF, Ciambarella BT, Carvalho VF, Martins MA, Silva PMR IOC-Fiocruz

Introduction: Inhalation of silica particle induces a lung inflammatory disease called silicosis, which is mainly characterized by intense fibrosis and granuloma formation. Arginase is an enzyme considered as important contributor for fibrogenic responses and shown to act secondary to IL13 generation. Recently we described IL13 to play a critical role in experimental silicosis (Ferreira TP *et al*., *J Immunol*., 191(10), 5220, 2013). Thus, in this study we investigated the potential contribution of arginase to fibrosis and granuloma formation in the experimental model of silicosis in mice. **Methods:** Male Swiss-Webster mice were anesthetized and received an intranasal instillation of silica particles (10 mg/50 µL) or saline (control). Analyzes were made 3, 7 and 28 days after silica provocation and included: i) lung function and hyper-reactivity to methacholine (invasive plethysmography, Buxco system), ii) leukocyte population in the blood and bronchoalveolar lavage (BAL) (Neubauer chamber and cytospin preparations), iii) morphometry and immunohistochemistry of lung tissue. The animals received daily administration of arginase inhibitor Nor-NOHA (1 mg/kg, po), for 5 days, starting 23 days after stimulation with silica. The analyses were made at day 28. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (L-034/09). **Results:** We showed that silica challenge induced progressive inflammatory and fibrotic responses in the lungs of mice, as attested 7 and 28 days post-stimulation. In parallel, by means of immunohistochemistry technique, an increase in the labeling for F4/80 and arginase was detected in the lungs of silicotic mice, suggesting a potential correlation with macrophages and possibly those with M2 phenotype. Further, silica-challenged mice were treated therapeutically with the arginase inhibitor Nor-NOHA. We noted that treatment with Nor-NOHA decreased granuloma area (5.5% ± 0.7%) as compared to non-treated animals (17.3% ± 5.1%) (mean ± SEM; $p<0.05$, $n=7$). Also, Nor-NOHA restored lung function and suppressed airways hyper-reactivity to methacholine in silicotic mice. **Discussion:** Our findings show that mice challenged with silica particles exhibit marked tissue fibrosis and granuloma formation, which parallel with increased expression of arginase in the lungs. Treatment with arginase inhibitor Nor-NOHA suppressed fibrosis and lung function decrease in silicotic mice, suggesting that inhibition of arginase might be a promising strategy in innovative anti-fibrotic approaches for treatment of silicosis. **Financial support**: Fiocruz, Faperj and CNPq.

04.008

Intrauterine undernourishment reduced leptin receptor and toll-like receptor-4 expression and modulate acute lung inflammation. Balbino AM[1], Torres TC[1], Fernandes L[1], Landgraf MA[2], Landgraf RG[1] [1]Unifesp-Diadema – Inflamação e Farmacologia Vascular, [2]ICB-USP – Farmacologia

Introduction: Lung diseases characterized by acute inflammation arise from various causes including Gram-negative bacterial infections. The outer cell wall of Gram-negative bacteria contains lipopolysaccharide (LPS) that activates cells such as macrophages and endothelial cells to produce pro-inflammatory mediators, cytokines and chemokines leading to expression of adhesion molecules and recruitment of inflammatory cells. Inflammatory response to endotoxins is largely mediated through Toll-like receptor 4 (TLR4). Lung microvascular endothelial TLR4 is involved in the recruitment of neutrophils in inflamed lungs and TLR4 on neutrophils is critical for maximal neutrophil recruitment into the inflamed lungs. Intrauterine undernourishment can induce a range of fetal adaptations, which can lead to permanent alterations in adult life, such as reduced inflammatory response. **Objectives**: In this study we standardized a model of intrauterine undernourishment in mice and we have investigated the impact of intrauterine undernourishment on the TLR-4 expression, and correlated with the development of lung inflammatory response. **Methods**: All procedures used in this study were approved and performed in accordance with guidelines established by the ethics committee of the Unifesp (CEP–1666/09). Female C57Bl/6 mice were randomly divided into 2 groups: nourished (NR, ad libitum diet) and undernourished (UR, 25% food restriction). After birth, each litter was left with the mother for 28 days. 5-6 male C57BL/6 mice at 8-9 wk of age were used for each group. Control group was given saline intranasally (i.n.,*30µL*). *Experimental groups were given LPS* (*i.n.,1.5µg/g/30µL*). 6h after instillation the bronchoalveolar lavage fluid (BALF) was collected to evaluate cellular infiltration in lung. Lungs were removed for measurement of the cellular infiltration and TLR-4 expression and long-form leptin receptor by western blot. **Results:** Intrauterine undernourishment mice stimulated by LPS were presented significantly reduced in total cell (45.9%) and neutrophils (76.2%) in bronchoalveolar lavage fluid when compared to nutrition group. Besides, cellular infiltration in the peribronchial area was also significantly reduced (37.8%). Western blot assay showed that expression of long-form leptin receptor is decreased (39.8%) and the TLR-4 is reduced (45%) when compared to nutrition group. **Conclusion:** Our preliminary results suggest that intrauterine undernourishment mice dowregulates leptin receptor expression and TLR-4 and modulate acute lung inflammatory response stimulated by LPS. **Financial support**: Fapesp (2010/01404-0, 2012/51104-8) and CNPq.

04.009

Involvement of adenosine receptors in inflammation induced by copper in zebrafish larvae. Cruz FF[2,1], Leite CE[1], Maboni LO[3], Pereira TCB[2], Bogo MR[3,2], Bonan CD[3], Campos MM[4,1], Battastini AMO[5], Morrone FB[2,6] [1]PUCRS – Toxicologia e Farmacologia, [2]PUCRS – Medicina e Ciências da Saúde, [3]PUCRS – Biociências, [4]PUCRS – Odontologia, [5]UFRGS – Bioquímica, [6]PUCRS – Farmácia

Introduction: Zebrafish (Danio rerio) is an aquatic vertebrate that is becoming a popular model organism in biomedical research (Kalueff, *Trends Pharmacol Sci* V. 35(2), P. 63, 2014). Adenosine receptors are a family of G-coupled-protein receptors divided into four subtypes: A_1, A_{2A}, A_{2B}, and A_3 (Burnstock, *Curr Top Med Chem*, V. 4(8), P. 793, 2004). For the A_{2A}, there were described two genes in zebrafish, identified as $A_{2A.1}$ and $A_{2A.2}$ (Boehmler, *Gene Expr Patterns*, V. 9(3), P. 144, 2009). Adenosine signaling is involved in the resolution of pathological conditions, promoting protective effects from excessive inflammatory responses (Antonioli, *Nat Rev Cancer*, V. 13(12), P. 842, 2013). Copper is a heavy metal that can induce the production of reactive oxygen species and oxidative stress, and previous studies demonstrated its effect in purinergic signaling (Rosemberg, Toxicology, V. 236, P. 132, 2007). **Objectives**: To verify the effect of copper exposure in apoptosis and in adenosine receptors expression in zebrafish larvae. **Methods:** *Determination of apoptotic cells*: 7 dpf larvae were exposed to 10 µM $CuSO_4$ for 1 h. The animals were treated for 30 minutes with 2 mg/mL acridine orange. To carry out the pictures, the animals were anesthetized with tricaine and tissue labeling was evaluated by optical microscopy. *Molecular Analysis*: The expression of A_1, $A_{2A.1}$, $A2_{A.2}$ and A_{2B} receptors was determined 4 and 24 hours after 10 µM $CuSO_4$ exposure using qRT-PCR. Statistical comparison was performed by one-way ANOVA followed by Tukey's test, and $p<0.05$ was considered as significant. All protocols were approved by the Institutional Animal Care Committee (09/00135, CEUA–PUCRS). Results: Cell death of neuromasts was visually confirmed in the trunk region and in the skull of the larvae. Animals exposed to copper showed an increase in A_1, $A_{2A.1}$, $A_{2A.2}$ e A_{2B} receptors gene expression during 4 h (14.2 ± 5.2; 3.7 ± 1.16; 1.5 ± 0.31; 1.54 ± 0.23, respectively), and 24 h copper exposure (17.42 ± 6.62; 3.69 ± 0.88; 1.69 ± 0.16; 1.7 ± 0.11, respectively). Discussion: Our data corroborate the study of Olivari *et al.* (2008) demonstrating that cell death is a consequence of oxidative stress induced by copper (Olivari, *Brain Res*, V. 1244, P. 1, 2008). This death can occur either through necrosis and apoptosis, depending on the concentration and time of exposure. The results agree with previous data from Leite *et al.* (2013) who showed an altered expression of different subtypes of ADA and a decrease in the activity of ecto-5'-NT and ADA enzymes with consequent increase of AMP and adenosine in zebrafish larvae treated with copper (Leite, Toxicol Appl Pharmacol., V. 272(3), P. 681, 2013). Therefore, our results suggest that adenosine signaling is involved in inflammation induced by copper in zebrafish larvae. **Financial Support**: PUCRSINFRA, CNPq, FAPERGS/PRONEX. FAPERGS/CNPq n. 008/2009 (PRONEX).

04.010

Protease inhibitors promote resolution of the acute inflammation associated with increased intact form of annexin-A1. Vago JP[1], Lima GLN[1], Caux TR[2], Tavares LP[1], Lima KM[1], Ribeiro ALC[1], Pinho V[1], Perretti M[4], Teixeira MM[5], Sousa LP[1] [1]FF-UFMG – Análises Clínicas e Toxicológicas, [2]ICB-UFMG – Morfologia, [4]QMUL, [5]ICB-UFMG – Bioquímica e Imunologia, ICB

Introduction: Annexin-A1 (AnxA1) is a glucocorticoid (GC)-induced protein of 37KDa that is known as a mediator of several GC functions. Its intact form (37KDa) is considered a mediator of the anti-inflammatory and pro-resolution actions of AnxA1. However, this protein may be cleaved *in vivo* at the N-terminal region by neutrophil proteases including elastase and proteinase-3 (PR3), generating the 33KDa isoform of unknown proprieties. In this study, we investigated the dynamics of AnxA1 expression and the role of synthetic (Sivelestat, Eglin) and natural (Elafin) protease inhibitors on resolution of LPS-induced neutrophil inflammation. **Methods:** All procedures described here had prior approval from the Animal Ethics Committee of UFMG, Brazil (CETEA/UFMG, Protocol number 15/2011). We used the murine model of pleurisy induced by LPS. BALB/C mice were challenged by administration of LPS (250 ng/mouse, i.pl.) or PBS. The cells present in the pleural cavity were collected, by repeated washings with PBS, at different times of LPS challenge or 4h after treatment with specific neutrophil elastase inhibitors (Sivelestat, 5 mg/kg, i.p; Elafin, 10 µg/kg, i.p), the inhibitor of elastase and cathepsin g (Eglin, 100µg/kg, i.p), a pan-inhibitor of serine proteases (PMSF, 30 mg/kg, i.p) and processed for viable and apoptotic leukocyte count and western blot analysis. **Results and discussion:** The injection of LPS induced a time-dependent influx of neutrophils into the pleural cavity of mice which was maximal at 8 h and resolved at 48 h. Intact AnxA1 was detected in the pleural cells of PBS-challenged mice, and AnxA1 cleavage was maximal at 8 h after LPS, when neutrophil recruitment was maximal. Expression of intact AnxA1 was regained during the resolution phase of this inflammatory response. Similarly, activity of endogenous elastase peaked at 8 h and decreased at 48 h. Treatment of 4 h LPS-challenge mice with sivelestat inhibited elastase activity and decreased neutrophils influx into the pleural cavity without modifying mononuclear cell numbers. This effect of elastase inhibition was associated with increased numbers of neutrophils displaying apoptotic morphology, caspase-3 cleavage and accumulation of intact (37KDa) AnxA1. Resolution of neutrophilic inflammation (i.e. decrease in number of total neutrophils, but enhanced number of apoptotic neutrophils) was also observed when mice were administered with Elafin and Eglin. PMSF caused a similar effect on neutrophils, but also decreased the number of mononuclear cells. **Conclusion:** Our results show that protease inhibition resolves inflammation associated with increased levels of intact AnxA1 and neutrophil apoptosis, suggesting that therapeutical strategies that increase AnxA1 may be an alternative treatment to acute inflammation. **Financial Support:** CNPq, PRPq-UFMG, FAPEMIG and Capes.

04.011

Purinergic signaling involving NTPDase 2 and P2Y1 receptors contributes to endothelial leukocyte-adhesion in schistosomal inflammation. Oliveira SDS[1], Oliveira NF[1], Savio LEB[2], Meyer-Fernandes JR[3], Ornelas FGI[4], Ferreira ZS[5], Coutinho-Silva R[2], Silva CLM[1] [1]ICB-UFRJ, [2]IBCCF-UFRJ, [3]IBqM-UFRJ, [4]IB-USP, [6]UFRJ

Introduction: Schistosomiasis is caused by the intravascular parasite *Schistosoma mansoni*. Vascular homeostasis depends on the signaling of purinergic receptors and nucleotide metabolizing extracellular enzymes (NTPDases). As the disease alters vascular physiology (Oliveira *et al.*, PLoS One 6: e23547, 2011; Oliveira *et al.*, Purinergic Signal. 9: 81, 2013), our aim was to investigate the influence of chronic schistosomiasis on endothelial purinergic signaling involving $P2Y_1$ receptor ($P2Y_1R$) and NTPDases. **Methods:** *S. mansoni*-infected and control mice (60-80-days old; CEUA/UFRJ: DFBC-ICB-011) were used to obtain primary culture of mesenteric endothelial cells (MECs) and mononuclear cells (MC). We evaluated $P2Y_1R$-mediated MC adhesion. MECs were treated with 500 µM ATP (5 min) and the NTPDase activity was assayed. The extracellular ADP concentration was determined by HPLC analysis. MECs lysates were used to quantify the $P2Y_1R$ and NTPDases expression by Western blot or RT-PCR analysis. Data are expressed as mean ± SEM. **Results and discussion:** In the control group $P2Y_1R$ activation (2-MeSATP, 60 µM, 4h) increased MC adhesion to endothelial cells from 9.4 ± 1.3 (basal) to 29 ± 5.1 cells/field (n=21-33 replicates, $P<0.001$). The $P2Y_1R$ antagonist (MRS2179, 0.3 µM) blocked the agonist effect (9.2 ± 1.2 cells/field, n=36, $p<0.05$). However, MECs from infected mice showed an increased basal MC adhesion (22 ± 2.0 cells/field, n=33, $p<0.05$), and 2-MeSATP (60 µM) only caused a marginal increment. The antagonist MRS2179 also blocked the agonist effect, but, more important it also reduced basal adhesion values (to 9.6 ± 1.0 cells/field, n=33, $p<0.05$). We did not observe alteration of $P2Y_1R$ expression, but we observed that the extracellular ATP hydrolyis was higher in MECs from infected mice than controls (17 ± 3 and 7 ± 1 pmol Pi/µg ptn, n=16-14, $p<0.05$, respectively). As an important endogenous $P2Y_1R$ agonist (ADP) may be produced during ATP hydrolysis mainly by NTPDases 2 (and 3), firstly we characterized which NTPDases isoforms are expressed in MECs. We found that NTPDases 1, 2 and 3 isoforms are expressed in control MECs. Besides, qRT-PCR assays revealed an increased expression of the NTPDase 2 (and 3) in the infected group and, HPLC preliminary data showed a corresponding increased ADP production by MECs primed during infection. **Conclusion:** Altogether, these data suggest that leukocyte adhesion to MECs is enhanced in schistosomiasis. NTPDase 2 and $P2Y_1R$-mediated leukocyte adhesion contribute to this vascular dysfunction. These alterations could contribute to the mesenteric inflammation observed in schistosomiasis. **Support:** CNPq, Faperj-PRONEX, Faperj, INCT-INPeTAm/CNPq/MCT.

04.012

High doses of inhaled corticosteroid and periodontal disease alter NTPDase activity in blood serum of rats. Medeiros LF[1], Scarabelot VL[2], Cavagni J[2], Detanico BC[2], Rozisky JR[1], Daudt LD[2], Gaio EJ[2], Battastini AMO[3], Ferreira MBC[1], Rosing CK[2], Torres ILS[1]
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Background: certain drugs such as glucocorticoids may interfere with the modulation of periodontal disease. In contrast, corticosteroid treatment has been associated with a protective effect with regard to periodontal breakdown, depending on the dose, pathway, and exposure time. Considering that previous study demonstrated a role for purinergic signaling in periodontal disease and the potential relevance of nucleotidases in coordinating the cardiovascular system and inflammation processes, the aim of this study was to investigate the nucleotidase activities in the blood serum of rats with periodontal disease exposed chronically to inhaled corticosteroids. **Methods:** Adult male Wistar rats (n=26) were randomly assigned to one of the following 4 study groups: a **control group** (**C**) that received no intervention; a periodontal disease (PD) group that received saline solution; a 'low dose' (LD) group that received 30 µg of budesonide daily; and a corresponding 'high dose' (HD) group that received 100 µg daily over a 15-day time course. The hydrolysis of ATP, ADP, and AMP were analyzed in blood serum. **Results:** periodontal disease diminished the hydrolysis of ATP (CT = 1.98 ± 0.21, PD= 1.26 ± 0.22, HD= 0.84 ± 0.21, LD= 1.12 ± 0.25; two-way ANOVA, $p<0.05$; $F_{(1,22)}= 12.89$) and enhanced the hydrolysis of ADP (CT = 1.86 ± 0.25, PD = 4.52 ± 0.44, HD= 3.29 ± 0.37, LD= 3.39 ± 0.56, two-way ANOVA, $p<0.05$; $F_{(1,21)}= 16.62$). The association of periodontal disease model and repeated administration of low or high dose of inhaled corticosteroids reversed the observed increase in ADP hydrolysis, and only repeated administration of low doses of inhaled corticosteroid was able to reverse the decrease in the hydrolysis of ATP induced by periodontal disease. **Conclusion:** The variables investigated in this study may be involved in the pathophysiology of periodontal disease and may participate in the mechanisms that mediate the development of some of the side effects of inhaled corticosteroids. In conclusion, it can be suggested that soluble nucleotidases may have an important role in the maintenance of periodontal disease and in the relationship between periodontal disease and cardiovascular diseases. **Financial Support:** CNPq, Capes

04.013

Inflammatory effects in isolated intestine during ischemia and reperfusion in rats.

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Introduction: Intestinal ischemia and reperfusion (i-I/R) accounts for local inflammation that is characterized by leukocyte migration, increased microvascular permeability and edema. In addition to local injury, reperfusion of the intestine leads to remote organ dysfunction. The isolation of the intestine during ischemia and reperfusion was found to reduce the degree of subsequent lung injury avoiding the local cytokines influx to systemic circulation (Narita, *Surg Today*, 34: 937, 2004). Here we aimed to investigate the ascites obtained after the isolation of the intestine during ischemia and reperfusion and the local inflammatory status. **Methods:** Animals were housed and used in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the Institute of Biomedical Sciences, University of Sao Paulo. Anesthetized male rats (Wistar, 60 days old) were submitted to an occlusion of the superior mesenteric artery for 45 min, followed by 2 h of reperfusion (i-I/R), during this period the intestine was placed in a plastic bag and maintained in the abdominal cavity. As control were used Sham-operated animals (Sham i-I/R), also with the intestinal bag. The ascites were collected from the intestinal bag in order to quantify volume, total and differential leucocyte counts (optical microscopy) and IL-10 levels (ELISA kit). The neutrophil presence into the gut after i-I/R was evaluated by myeloperoxidase (MPO) activity assay; the microvascular permeability was assessed using the Evans blue (EB) dye extravasation method (20 mg/kg, i.v., 20 min before euthanasia). Comparisons between groups were made by one-way ANOVA followed by Bonferroni posttest. **Results:** The ascites volume decreased in rats submitted to i-I/R compared to sham (Sham i-I/R = 1.26 ± 0.16 vs i-I/R = 0.73 ± 0.07/mL; n = 6), but the leucocyte number (Sham i-I/R = 30.31 ± 7.9 vs i-I/R= 143.1 ± 24.34 x 10^4 cells/mL; n = 6) and the IL-10 concentration increased (Sham i-I/R = 535.2 ± 106.8 vs i-I/R = 1,303 ± 288 pg/mL; n = 6). In Sham animals the cell content of ascites was predominantly neutrophilic, but after i-IR the number of neutrophils decreased and the mononuclear cells increased (Mononuclear cells: Sham i-I/R = 13.5 ± 1.55 vs i-I/R = 72.6 ± 7.27%; n = 5; Neutrophils: Sham i-I/R = 87.4 ± 2.82 vs i-I/R= 24.4 ± 6.4%; n = 5). Intestinal MPO activity did not change after i-IR, but the EB dye extravasation was increased (Sham i-I/R = 91.92 ± 12.53 vs i-I/R = 172.7 ± 22.35 mg/g of dry weight; n = 5). **Discussion:** Our data support that ischemia and reperfusion changes the profile of local homeostasis characterized by increased IL-10 levels, mononuclear cells and reduced ascites extravasation. In conclusion, we suggest that the remote organ dysfunction caused by ischemia and reperfusion is not only modulated by humoral factor but also by locally activated mononuclear cells. Ethics Committee number: 111/10/03 – CEUA – ICB/USP. Supported by Fapesp 2013/15291-0.

04.014

In vivo **PCB126 exposure impairs leukocyte activation by gpcr-induced pathway.** Shimada ALB, Cruz W, Rodrigues S, Azevedo R, Dorr F, Fock R, Pinto E, Farsky S FCF-USP – Análises Clínicas e Toxicológicas

Introduction and Objectives: Polychlorinated biphenyls (PCBs) are persistent organic pollutants widely used in industrial processes. PCB126 is the most toxic PCB in this group of pollutants. Once the respiratory tract is an important pathway of PCB126 absorption, this work aimed at investigating the effects of instilled PCB126 on the immune system, especially on mechanisms of leukocyte host defense. **Material and Methods:** Male Wistar rats were exposed to PCB126 (0.1; 1 or 10 µg/kg of body weight; during 15 days; once a day) or vehicle (0.5% DMSO in saline solution) through nasal instillation, and experiments were performed five hours later. The samples were evaluated as following: a) PCB126 quantification in lung and liver tissues by gas chromatography/mass spectrometry; b) aryl hydrocarbon receptor (AhR) expression in lung, liver, kidney and adipose tissue by Western Blot; c) bone marrow cells count and total number of cells in blood (per mm^3), using a hemocytometer; d) differential bone marrow cells count and circulatory leukocytes were quantified in blood stain smear; e) expression of adhesion molecules on circulating leukocyte membranes, blood cell viability and expression of FPR-1 were evaluated in basal condition or after formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation, *in vitro* (10^{-7}M; 1 h), by flow cytometer; f) ROS production after fMLP or phorbol-12-myristate-13-acetate (PMA; 100ng/ml; 15 minutes) stimulation, quantified by flow cytometer; g) measurement of soluble adhesion molecules levels; h) number of adherent leucocytes in mesenteric postcapillary venules, visualized by intravital microscopy; and i) neutrophils chemotaxis in Boyden chamber. All the experiments were conducted and approved by the Ethics Committee in Animal Experiments (CEUA/FCF/315). **Results and Conclusions:** PCB126 exposure was characterized by enhanced AhR expression in liver, lung, kidney and adipose tissues. Additionally, high levels of PCB126 were found in lung and liver tissues. The largest concentration of PCB126 reduced the leukocyte production in bone marrow and number of lymphocytes in the circulation, independently on necrosis or apoptosis. Moreover, PCB126 exposure modified expression of membrane receptors on blood leukocytes, especially CD62L, CD18 and CD31 adhesion molecules, after *in vitro* stimulation with fMLP, a G-protein coupled receptor activator (GPCR). The altered membrane expressions were also visualized *in vivo* at the mesenteric tissue, by impaired blood leukocyte adhesion to the postcapillary venules induced by fMLP stimulation. The demonstration that *in vivo* PCB126 exposure affects the pathways of GPCR activation was shown by the reduced fMLP receptor expression on blood neutrophils, diminished chemotactic response and blunted oxidative burst activation after fMLP stimulation. Therefore, our data highlight PCB126 *in vivo* exposure impairs the leukocyte activation induced by a GPCR pathway, which is pivotal for the immune system host defense. **Financing:** Fapesp

04.015

Involvement of TLR2/MYD88/NF-KB pathway regulating the expression of IL-18 in the pathogenesis of irinotecan-induced intestinal mucositis. Wong DVT[1], Batista GLP[1], Borges VF[2], Wanderley CWS[1], Bem AXC[1], Gonzales RH[1], Andrade CL[1], Teixeira MA[1], Brito GAC[3], Lima-Júnior RCP[1], Cunha FQ[2], Ribeiro RA[1] [1]UFC – Physiology and Pharmacology, [2]FMRP-USP, [3]UFC – Morphology

Introduction: Severe diarrhea and the associated intestinal mucositis (IM) are common side effects (15-25%) of colorectal anticancer therapy with Irinotecan (IRI). Gut injury induced by chemotherapeutic agents may result in bacterial/endotoxin translocation from the intestine to the systemic circulation. Pathogens recognition is partially due to the activation of toll-like receptors (TLR) by the pathogens-associated molecular patterns (PAMPs). Furthermore, necrotic cells and extracellular matrix, which release damage-associated molecular patterns (DAMPs), activate the synthesis of pro-inflammatory mediators through TLRs, causing intestinal injury. We have demonstrated the important role of TNF-α, IL-1β, IL-8, IL-18 and nitric oxide in the pathogenesis of intestinal mucositis induced by IRI and that this drug induces a significant damage on the gastrointestinal tract followed by bacterial translocation to peripheral organs. However, the role of toll-like receptors in the IRI-induced IM is not completely understood. Then, we aimed to evaluate the involvement of TLR2/MyD88/NF-kB pathway and its role in IL18 activation in the pathogenesis of irinotecan-associated intestinal mucositis. **Methods:** C57BL/6 (WT) mice (20-24g, n=6-7) and TLR2 ($TLR2^{-/-}$) or MyD88 ($MyD88^{-/-}$) knockout mice were given either saline or IRI (75 mg/kg i.p/4 days). On day 7, weight loss, diarrhea, blood leukocyte and bacterial count were assessed. Following euthanasia, ileum samples were obtained for myeloperoxydase (MPO) assay, morphometric analysis, NFκB immunohistochemistry, IL-1β dosage and NFκB and IL18 gene expression by (q)RT-PCR. Kruskal Wallis/Dunn's test or ANOVA/Bonferroni's test were used for statistical analysis. $P<0.05$ was accepted. (CEPA 99/10). **Results**: Irinotecan induced a marked weight loss, diarrhea, leukopenia, increased bacteremia, MPO activity, morphometric alterations, NFκB immunoexpression, as well as increase IL-1β level and IL18 and NFκB RNAm expression in intestinal samples of WT animals versus saline-injected group ($P<0.05$). On the other hand, IRI-injected $TLR2^{-/-}$ and $MyD88^{-/-}$ mice showed a milder ($P<0.05$) weight loss, diarrhea (0[0-1]; 0[0-1] respectively), lower neutrophil infiltration (1888 ± 545; 1310 ± 443, respectively), bacterial clearance, increase villus length (149 ± 6.8; 178.6 ± 6.2), reduced NFkB (1.75 ± 0.1; 1.06 ± 0.07) and IL18 expression (2.67 ± 0.4; 2.52 ± 0.6), as well as IL-1β level (0.85 ± 0.1; 1.11 ± 0.1) when compared with IRI-administered WT mice (diarrhea: 2[1-3]; MPO:4725 ± 1231; villus length: 122.1 ± 5.7; NFkB: 2.0 ± 0.23; IL18 expression: 3.25 ± 0.38 and IL-1β level: 3.9 ± 1.2) ($P<0.05$). **Conclusions:** This study suggests the involvement of TLR2/MyD88/NF-kB pathway regulating the expression of IL-18 in the pathogenesis of IRI-induced intestinal mucositis. **Financial support**: CNPq/Capes/FUNCAP.

04.016

Kinin B1 receptor lack influences bone healing in a mouse model of femur critical-size defect in streptozotocin-induced type-1 diabetes. Cignachi NP[1], Pesquero JB[2], Oliveira RB[1], Etges A[3], Campos MM[4] [1]PUCRS – Odontologia, [2]Unifesp – Biofísica, [3]UFPel – Odontologia, [4]INTOX-PUCRS

Introduction: A previous study (Pesquero JB *et al. J Clin Periodontol* 2013; 40:653) revealed a protective role for kinin receptors, by demonstrating that B_1R knockout (B_1RKO) mice presented increased bone resorption following ligature-induced periodontal disease. Herein, the effects on kinin B_1 receptor (B_1R) deletion were examined on bone regeneration in streptozotocin (STZ)-type-1 diabetic mice, subjected to a model of femoral critical-size defect. **Methods:** Male C57/BL6 wild-type (WT) or B1RKO mice (25 to 32 g; N=7-11 group) were used. The protocols were approved by the Local Animal Ethics Committee (13/00037). Type-1 diabetes was induced by 5 daily injections of streptozotocin (STZ; 50 mg/kg i.p.), dissolved in citrate buffer (50 mM; pH 4.5). Non-diabetic groups received citrate buffer vehicle alone i.p., at the same schedule of administration. The critical-size defects were created 7 days after the last STZ injection. After anesthesia with ketamine and xylazine (100 + 10 mg/kg, i.p.), the left mouse femur was assessed, and a monocortical bone defect (4-mm in length and 1-mm in diameter) was created. Following 21 days, the animals were euthanized, and the femurs were collected for histological procedures. One-way ANOVA followed by Bonferroni's test was used. P values <0.05 were considered significant. **Results:** Diabetes induction in WT C57/BL6 mice was allied to significant decrease of body weight and hyperglycemia, in relation to the non-diabetic group of the same strain ($p<0.05$). The lack of B_1R did not affect STZ-elicited body weight loss, although it partially prevented hyperglycemia. Type-1 diabetic mice presented significant delayed bone regeneration (17 ± 1%) with large areas of loose connective tissue within the defects, when compared to wide areas of newly-formed woven bone in non-diabetic WT C57/BL6 mice (29 ± 4%) ($p<0.01$). Either non-diabetic (24 ± 2%) or diabetic B_1RKO mice (22 ± 2%) displayed bone regeneration levels comparable to that seen in control WT C57/BL6 mice. WT C57/BL6 STZ-diabetic mice presented a marked reduction of collagen contents (57 ± 10; $p<0.01$) within the bone defect gap, whereas diabetic B_1RKO displayed collagen levels (100 ± 8) comparable to those observed in non-diabetic WT C57/BL6 (90 ± 16) or B_1RKO mice (94 ± 17). The enhanced bone regeneration in diabetic mice lacking B_1R does not seem to be associated to lessened osteoclast activity, as no prominent difference was detected in the levels of tartrate-resistant acid phosphatase (TRAP) positivity, or even in the immunolabeling for the proteins of the RANK/RANKL/OPG system. **Discussion:** Altogether, data presented in this study extends previous evidence on the relevance of kinin B1R under type-1 diabetes, and provide novel evidence on the role of this receptor in bone regeneration. We hope this data might help, in a near future, to develop new therapeutic strategies to provide superior outcomes for type-1 diabetic patients subjected to bone surgeries. **Financial support:** PUCRSINFRA #01.11.0014-00, Capes, FINEP and CNPq.

04.017

Lipoxin A4 protects mice during severe malaria by modulation of HO-1 and ICAM-1 expression. Pádua TA[1], Souza MC[1], Torres ND[1], Costa MF[2,1], Candea AP[1], Costa T[2,1], Seito LN[1], Penido C[2,1], Estato V[3], Antunes B[3], Silva L[4], Pinheiro AA[4], Caruso-Neves C[4], Tibiriçá E[3], Carvalho L[5], Henriques MG[1,2] [1]Farmanguinhos-Fiocruz – Applied Pharmacology, [2]INCT-IDN-CDTS-Fiocruz, [3]IOC-Fiocruz – Cardiovascular Investigation, [4]IBCCF-UFRJ, [5]IOC-Fiocruz – Malaria Research

Introduction: Severe malaria (SM) is characterized by inflammatory cell accumulation in the brain vasculature as well as breakdown of the brain-blood barrier (BBB) induced by both the killing of endothelial cells by cytolytic CD8 + T cells and increased endothelial cell activation. Lipoxins (LX) present anti-inflammatory activities such as inhibition of cytokine production, impairment of endothelial adhesion molecules expression by a mechanism dependent on HO-1. Previous results obtained by our group demonstrated that endogenous LXA_4 contributes to tolerance to experimental (E)SM. Herein we study the mechanism by which exogenous LXA_4 induces tolerance in ESM susceptible mice. **Methods:** The animals procedures were approved by the Committee on Ethical Use of Laboratory Animals of Fiocruz (L052/12). Male C57BL/6 mice (n=6/group) were *i.p.* injected with $5x10^6$ *P. berghei*(*Pb*) ANKA-infected red blood cells (RBC) or saline solution. Treatment was performed daily with LXA_4 (0.5µg/kg/day). The following parameters were evaluated 6d post-infection: measure of Evan's blue (EB) extravasation to brain tissue (BBB breakdown), peripheral parasitemia; microcirculation of *pia mater* by intravital microscopy, activation of T cells from brain and spleen by flow cytometry and ICAM-1 and HO-1 expression in brain tissue by western blot. For *in vitro* assays, endothelial murine cell line (tEnd.1) and human cell line (ECV-304) were incubated with LXA_4 (10 nM), BOC-2 (ALX antagonist, 40 nM) or ZnPPIX (HO-1 inhibitor,50 µM). CFSE-stained RBCs, *Pb*RBC or *P. falciparum* (*Pf*) RBC were then added to the cultures (50 erythrocytes/cell, 5% parasitemia) for cytoadherence assay and ICAM-1 immunocytochemistry. **Results:** The treatment of *Pb*-infected C57BL/6 mice during 5d with LXA_4 prevented BBB breakdown (LXA_4 21.7 ± 3.2 *vs Pb* 40.7 ± 7.6 EB mg/tissue g) and increased *Pb*-infected mice survival. Microcirculation analysis showed that LXA_4 treatment did not modulate leukocyte adhesion, neither CD8 + T cells accumulation in brains of *Pb*-infected mice. As well, LXA_4 treatment did not inhibit α-L integrin expression, neither TNF-α and IFN-γ production by CD3 + splenocytes from *Pb*-infected mice. On the other hand, it was observed increased functional capillary density (*Pb* 288 ± 34.9 *vs* LXA_4 420 ± 39.7 capillaries/mm2). In accordance, treatment with LXA_4 restored HO-1 expression (LXA_4 0.4 ± 0.1 *vs Pb* 0.01 ± 0.01 arbitrary units, AU) while suppressed ICAM-1 expression (LXA_4 47.4 ± 6.3 *vs Pb* 68.7 ± 6.9 AU) in brain tissue of *Pb*-infected mice. As well, LXA_4 treatment inhibited ICAM-1 expression *in vitro* in endothelial cell stimulated by *Pb*RBC or *Pf*RBC. In addition, *Pb*RBC and *Pf*RBC failed to adhere to LXA_4-treated endothelial cells, however adhere to LXA_4-treated endothelial cells upon further treatment with an HO-1 inhibitor. **Discussion:** The results suggest that LXA_4 exerts a protective role during experimental severe malaria inducing HO-1-dependent modulation of endothelial cell ICAM-1 expression. **Supported by:** CNPq/Faperj/Fiocruz.

04.018

Endogenous LXA_4 contributes to tolerance to experimental severe malaria. Torres ND[1], Souza MC[1], Padua TA[1], Costa MS[1], Candea AP[1], Costa TEMM[1], Seito LN[1], Penido C[2], Estato V[3], Antunes B[3], Antunes B[3], Tibiriçá E[3], Tibiriçá E[3], Carvalho L[4], Henriques MG[1]
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Introduction: Malaria remains a major global health problem, especially in tropical areas. Severe malaria (SM) is mainly characterized by increased intracranial pressure, acute lung injury and coma. The literature on inflammatory response-triggered by SM reveals an uncontrolled leukocyte/lymphocyte activation as well as endothelial activation leading to multi organ dysfunction such as brain-blood barrier breakdown, pulmonary function impairment and kidney damage. The lipoxins (LX) are described as eicosanoids with anti-inflammatory activity. Interestingly, LXA_4 is able to diminish the expression of adhesion molecule in TNF-α stimulated endothelial cells. In this study, we investigated the role of LXA_4's during malaria infection in susceptible or tolerant mice. **Methods:** The animals procedures were approved by the Committee on Ethical Use of Laboratory Animals of Fiocruz (L052/12). BALB/c and C57Bl/6 (n=6/group) mice were infected or not with 5 x 10^6 parasitized red blood cell (*P. berghei* (*Pb*) ANKA) intraperitoneally. C57BL/6 mice were treated daily with LXA_4(0.5µg/kg/day) and Balb/c mice were treated daily with BOC-2(100 mg/kg/day). Blood-brain barrier breakdown (BBB) was measured by Evan's blue (EB) extravasation to brain tissue. Behavioral analysis was performed as described in SHIRPA protocol. TNF-α were analyzed in brain tissue by ELISA. Microcirculation of pia máter was assessed by intravital microscopy. Statistical analysis was done by ANOVA followed by the Bonferroni test (mean ± SEM, $p > 0.05$). **Results:** Experimental (E)SM-tolerant BALB/c mice show higher baseline LXA_4 plasma levels than ESM-susceptible C57BL/6 mice. The lower baseline LXA_4 levels in C57BL/6 mice were associated with disease severity: treatment of *Pb*-infected C57BL/6 mice during 5 days with LXA_4 prevented BBB breakdown (*Pb* 40.7 ± 7.6 *vs* LXA_4 21.7 ± 3.2 EB mg/g tissue). Increased survival and improves behavioral and functional analysis. Accordingly, *Pb*-infected BALB/c mice treated with the LXA_4 receptor antagonist BOC-2 exhibited BBB breakdown (*Pb* 11.5 ± 2.2 *vs* BOC-2 23.0 ± 3.0 EB mg/g tissue). Both C57BL/6 and BALB/c-*Pb*-infected mice showed increased levels of TNF-α in brain tissue, which was not modulated by LXA_4 or BOC-2 treatment, respectively. Histological examination of the brain tissue showed that *Pb*-infected C57Bl/6 mice treated with LXA_4 present fewer occluded vessels than the untreated mice; In addition, it was observed increased numbers of occluded vessels in *Pb*-infected BALB/c treated with BOC-2 when compared with non-treated *Pb*-infected mice (*Pb* 68.7 ± 2.4 *vs* LXA_4 44.7 ± 6.9 % occluded vessels). Microcirculation analysis showed that neither LXA_4 nor BOC-2 treatment modulate leukocyte adhesion to brain microvasculature, however LXA_4 treatment improve functional capillary density in *Pb*-infected C57BL/6, while BOC-2 treatment impaired functional capillary density in *Pb*-infected BALB/c mice. **Discussion:** In summary, our results suggest that efficient endogenous LXA_4 production contributes to tolerance to ESM. Supported by CNPq/ Faperj/ Fiocruz.

04.019

Effect of acute melatonin administration in a model of chronic orofacial pain.

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Introduction/OBJECTIVE: the temporomandibular joint dysfunction (TMD) is a common type of orofacial pain disorders, and it is characterized by persistent pain in the temporomandibular joint (TMJ), masticatory muscles, causing difficulty for chewing and speaking. It is known that inflammatory processes can contribute for the induction and/or maintenance of pain, and, nowadays the melatonin has been used for treating inflammatory and neuropathic pain. Based on that, our aim was to evaluate the effect of acute administration of melatonin in a model of chronic orofacial pain induced by Freund's Adjuvant (CFA). **Methods:** we used 33 *Sprague-Dawley* male rats at 60 days old. The animals were maintained under a standard L/D 12:12 cycle (lights-on at 07:00h and lights-off at 19:00h), in a controlled environment (22 ± 2°C), with rat water and chow available *ad libitum.* The animals were randomized by weight and divided into six groups: **Control** (naive); **Pain** (50 µL CFA into TMJ and no treatment); **Pain + Melatonin** (CFA into TMJ plus melatonin 1 mL/kg i.p.); **Pain + Vehicle** (CFA into TMJ plus vehicle 1mL/kg i.p.); **Sham Pain + Melatonin** (saline into TMJ plus melatonin 1mL/kg i.p.); **Sham Pain + Vehicle** (saline into TMJ plus vehicle 1mL/kg i.p.). The nociceptive behaviors (mechanical alodinia and thermal hyperalgesia) were assessed by the Von Frey electronic and hot plate tests, respectively. The tests were applied 30, 60, 90 and 120 min and seven days after the melatonin treatment. Serum BDNF levels were measured by ELISA Method: Behavioral data were evaluated by Generalized Estimating Equation (GEE) and Bonferroni test, whereas BDNF levels were analyzed using One-way ANOVA/Student Newman Keuls tests. This study was approved by Ethical Committee of Animal Use of Clinics Hospital of Porto Alegre (GPPG: 12-0104). **Results:** we found an interaction between treatment *vs* time in the mechanical alodinia (Wald $\chi 2$ =2.03;29; P<0.001). The acute dose of melatonin was able to reverse the nociceptive behavior and this effect lasted up to seven days after melatonin use. We found an interaction between treatment *vs* time in the thermal hyperalgesia (Wald χ^2 =6.04;27; *P*<0.001), without long-lasting effect as found in the mechanical alodinia. In addition, we did not observe any differences in the BDNF serum levels between the groups (P>0.05). **Conclusion**: our findings demonstrate that melatonin promotes long-lasting antiallodynic effect, maybe related to an anti-inflammatory action, and corroborates previous studies that highlight the melatonin as a therapeutic option to manage the chronic pain. Chronic pain patients often can display symptoms like mood and sleep disorders, and melatonin may help in both aspects, thus improving their life quality. **Financial support**: CNPq, Capes, FIPE/HCPA.

04.020

Targeting the sphingosine pathway to resolution of inflammatory response induced by LPS. Perez D, Athayde RM, Reis AC, Menezes PVA, Teixeira MM, Sousa LP, Pinho V
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Introduction: Sphingosine, an important sphingolipid derived from plasma membrane, plays a fundamental role in many cellular processes including cell proliferation, angiogenesis, senescence and apoptosis but its role to inflammation resolution remains unclear. Here, we propose to evaluate the role of sphingosine pathway on neutrophil accumulation at pleural cavity of LPS-challenged mice. Methods and Results: Mice received i.pl. administration of LPS (250 ng/cavity) or PBS. LPS induced neutrophil recruitment that was increased at 4 h, peaked at 8–24 h, and declined thereafter (number of neutrophil: PBS: 0,02 ± 0,03; 4h: 9,4 ± 3,8; 8h: 10,1 ± 5,2; 24h: 7,12 ± 4,8; 48h: 1,5 ± 0,7). Intraperitoneal treatment with sphingosine pathway modulators such as the L-cycloserine, DL-threo-Dihydrosphingosine (DTD), Cay 10444, FTY720, at 4 h after LPS administration, decreased the number of neutrophil (number of neutrophil: PBS: 0,1 ± 0,06; LPS: 7,3 ± 2,2; L-cyclo: 2,1 ± 1,2; DTD: 7,6 ± 0,8; Cay10444: 2,3 ± 1,4; FTY720: 3.6 ± 1,8) and increased the percentage of apoptotic cells (% of apoptotic neutrophil: PBS: 0,2 ± 0,04; LPS: 0,9 ± 0,5; L-cyclo: 7,2 ± 2,7; DTD: 1,8 ± 0,9; Cay10444: 4,1 ± 1,3; FTY720: 5,0 ± 1,6) and phagocytosis of apoptotic cells (efferocytosis) (% of efferocytosis: PBS: 0,1 ± 0,1; LPS: 1 ± 0,7; L-cyclo: 3,4 ± 1,4; DTD: 4,9 ± 1,9; Cay10444: 4,9 ± 1,0; FTY720: 4,7 ± 2,1; n=6; $p<0,05$). Conclusion: We suggest that regulation of sphingosine pathway *in vivo* may represent a pro-resolving strategy for the treatment of neutrophilic inflammation. **Financial support**: CNPq and FAPEMIG.

04.021

Effect of pipecolyl xylidide (PPX), a non-anesthetic bupivacaine metabolite in a short-term A/J murine model of asthma marked by resistance to steroid therapy. Cotias AC[1], Serra MF[1], Rodrigues VC[1], Olsen PC[1], Pão CRR[1], Costa JCS[2], Cordeiro RSB[1], Silva PMR, Silva PMR[1], Martins MA[1] [1]IOC-Fiocruz – Inflamação, [2]Farmanguinhos-Fiocruz

Introduction: Glucocorticoids (GCs) are highly effective anti-inflammatory agents, although a small subgroup of patients shows persistent tissue inflammation and hyper-reactivity of the airways (AHR) despite treatment with high doses of GC. Unfortunately, a proportion of patients develop severe disease, which is relatively or totally refractory to glucocorticoid therapy. Several approaches have shown the efficacy of nebulized local anesthetic in reducing the use of oral GCs in patients with moderate and severe asthma. We studied the effect of nebulized bupivacaine and its non-anesthetic Metabolite pipecolyl xylidide (PPX) on allergen-induced asthma changes in mice. **Methods:** Mice of strain A/J were subcutaneously sensitized on days 1 and 14 by a mixture of Al(OH)3 + ovalbumin (OVA) and daily challenged from days 19 to 22 by 25 µg OVA (25 µl, intranasal instillation), to establish a murine model of acute asthma characterized by airways inflammation and AHR. Bupivacaine and PPX (1-2%, nebulized) were administered immediately after the ovalbumin challenge, and dexamethasone (DEX) (3 mg/kg, orally) was administered 1 hour before ovalbumin challenge in sensitized A/J mice. AHR, peribronchial eosinophil density, interleukin (IL)-4, IL-13, eotaxin-1 and KC, epithelial mucus and extracellular-matrix deposition were evaluated. The impact of treatments on DO11.10 T cell survival and proliferative responses was assessed *in vitro*. Anesthetic and safety properties were assessed *in vivo*. License number for this study is LW-23/10. **Results and discussion:** We found that allergen provocation increased AHR (9,7 ± 1 to 17,7 ± 1, AUC) (Mean ± SEM, n=7) it was significantly inhibited by PPX (12,1 ± 1,2, AUC) but not DEX (17,4 ± 2,8, AUC) and Bupivacaine (18,7 ± 2, AUC) in this model. In addition, we observed that mice A/J challenged of antigenic provocations developed marked leukocyte infiltration, mucus exacerbation, peribronchial fibrosis and increased levels of IL-4, IL-13, KC and eotaxin-1. All these changes being clearly sensitive to PPX. In contrast, these changes remained unaltered after bupivacaine or dexamethasone treatment. Both bupivacaine and PPX (10-1000 µM) inhibited in a concentration-dependent manner T cell survival and proliferative response survival following exposure to allergen *in vitro*. Furthermore, while the local anesthetic activity of PPX was drastically reduced (33,2 ± 1,9) in comparison to bupivacaine (158,7 ± 3,1), both lethality (in mice) and hemodynamic changes (in rats) were clearly more pronounced in those animals treated with bupivacaine as compared to those treated with PPX. Our findings show that nebulized PPX, but not bupivacaine, inhibits pivotal features of asthma in a murine model expressing a certain degree of refractoriness to glucocorticoids. These effects were likely due to down-regulation of pro-inflammatory cytokines and chemokines and subsequent blockade of eosinophilic infiltration in the lung tissue. **Financial Support:** Faperj, CNPq, PDTIS and Capes

04.022

cAMP elevating agents induce resolution of acute inflammation dependent on annexin A1. Lima KM[1], Caux TR[2], Vago JP[1], Tavares LP[3], Aribada RG[2], Carmo AAF[1], Galvão I[3], Costa BRC[2], Soriani FM[4], Perretti M[5], Silva PMR[6], Pinho V[1], Teixeira MM[3], Sousa LP[7]
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Introduction: Annexin A1 (AnxA1) is a glucocorticoid-induced 37kDa protein known for its anti-inflammatory and proresolving effects. We previously showed that Rolipram (ROL), a phosphodiesterase-4 inhibitor, induces resolution of acute inflammation and AnxA1 expression. In this study, we investigated the ability of ROL and other cAMP elevating agents such as db-cAMP (cAMP mimetic) and Forskolin (an adenylate cyclase activator) to modulate AnxA1 expression and evaluated whether AnxA1 is involved in the pro-resolving ability of these compounds. **Methods:** All procedures described here had prior approval from the Animal Ethics Committee of UFMG, Brazil (CETEA/UFMG, Protocol number 15/2011). BALB/c mice were challenged with an intrapleural (i.pl) injection of LPS or PBS and 4h later received an injection of ROL (6 mg/kg/i.p.), db-cAMP (100µg/mouse/i.pl.) or Dexamethasone (DEXA, 2 mg/kg/i.p.) as a control. A nonselective AnxA1 receptor antagonist (BOC-1 -5 mg/kg/i.pl.) or a PKA inhibitor H89 (100µg/kg/i.pl.) were given before the drugs. The cells in the pleural cavity were harvested 4h after treatments and processed for total and differential leukocyte counts and western blot analysis for AnxA1. *in vitro* studies were carried out in PMA-differentiated THP-1 cells (a human monocytoid cell line), which were treated with ROL, db-cAMP or Forskolin at different times and concentrations. To investigate whether the effect of Rolipram was dependent of PKA pathway, we used two PKA inhibitors: H89 and Rp. **Results and discussion:** ROL and db-cAMP promoted resolution of neutrophilic inflammation which was associated with increased AnxA1 expression. *in vitro* studies showed that ROL, db-cAMP or Forskolin induced AnxA1 expression in a dose- and time-dependent manner and such effect was prevented by PKA inhibitors, suggesting the involvement of PKA on ROL-induced AnxA1 expression. Akin to these *in vitro* findings, PKA inhibition prevented ROL and db-cAMP-induced resolution and it was associated with AnxA1 inhibition. Interestingly, BOC1 prevented ROL and db-cAMP-induced resolution, suggesting AnxA1 involvement in resolution induced by these compounds. **Conclusion:** Our results showed that cAMP elevating agents increase the levels of AnxA1 and this protein is involved in the pro-resolving abilities of cAMP elevating agents and mimetic drugs. **Financial support:** CNPq, FAPEMIG and Capes.

04.023

Healing activity of a protein fraction of latex *Himatanthus drasticus* (HdLP) for topical use in an experimental model of cutaneous ulcers. Paiva YTCN[1], Souza TFG[1], Vasconcelos MS[2], Carmo LD[1], Moreno LMC[1], Figueiredo IST[1], Fonseca SGC[3], Ramos MV[2], Teixeira MDA[1], Alencar NMN[1] [1]UFC – Farmacologia e Bioquímica, [2]UFC – Bioquímica e Biologia Molecular, [3]UFC – Farmácia

Introduction: Several substances of natural origin have proven effective as healing, including the latex released by plants, which feature popular usage indicated for the treatment of ulcers. On this basis, this study aims to investigate the action of latex proteins *Himatanthus drasticus* (HdLP) in an experimental model of cutaneous excisional wounds. **Methods:** Swiss male mice (20-25 g, n = 10/group) were used for induction of back injury (1.0 cm2) and distributed according to established topical treatment: Negative control (vehicle), positive control (Regederme®) and treated with ointment containing HdLP (0.5%, 1.0% and 2.0%). Treatments were performed daily and measured the area of the wound for 14 days. Moreover, the percentage of epithelized wounds was observed from the 10th to the 14th day. Were performed ulcers skin biopsies for histological analysis using the dose that showed best result previously (HdLP 2.0%) described on days 2, 9 and 14 after surgery. The results were expressed as the mean ± standard error of the mean (SEM) and frequency of re-epithelialization, the results of ANOVA, followed by Bonferroni post-test and Kruskal-Wallis test followed by Dunn being applied where the statistical difference found considering $p<0.05$. The project was accepted by the ethics committee on animal research (CEPA) under number 39/2014. **Results:** The results showed that the three groups treated HdLP 0.5%, 1.0% and 2.0% showed greater reduction in wound area than the control group. HdLP 2.0% showed a significant difference on day 4 (0.68 ± 0.06), on the 9th day (0.12 ± 0.03) and at day 12 (0.01 ± 0.004), respectively, controls at day 4 (0.94 ± 0.10), day 9 (0.56 ± 0.10) and day 12 (0.07 ± 0.05). Regarding the frequency of reepithelialization, the 12th day the HdLP 1.0% (60%) and HdLP 2.0% (70%) groups showed a significant difference compared to control (20%), which is repeated on the 14th day that 100% of the wounds of groups HdLP 1.0% and 2.0% compared to the control (60%). Histological analysis of skin wounds showed no difference on the intensity of infiltrating leukocytes between experimental groups on day 2. On the 9th day, the group HdLP 2.0% showed intense proliferation of fibroblasts, re-epithelialization partial, evolving to complete epithelialization with characteristics of stratified squamous epithelium on day 14. While the sham control and vehicle groups showed moderate proliferation of fibroblasts on the 9th day, prosing to partial reepithelialization of the ulcer on the 14th day. The results were expressed as the mean ± standard error of the mean (SEM), and the median variations, gresand the percentage of reepithelialization for each experimental group the results being applied to ANOVA followed by Bonferroni post-test (multiple comparisons) and chi-squared (percent), where the statistical difference was found for $p<0.05$. Histological analysis was qualitative. **Discussion:** In the wound healing process reepithelialization is important for restoration of injured tissue. Treatment with the protein fraction (HdLP 2.0%) favored a reduction in the wound area and promoted a complete re-epithelialization of ulcers in less time than controls, showing greater activity in the proliferative phase and tissue remodeling. Nonetheless, further studies to investigate this process of healing of skin ulcers are needed. **Financial support**: CNPq, Capes AND FUNCAP.

04.024

Lymphatic system influence on acute lung injury after intestinal ischemia/reperfusion in female rats. Fantozzi ET[1], Breithaupt-Faloppa AC[2], Ricardo-da-Silva FY[1], Bernardez-Amorim MB[1], Oliveira-Filho RM[1], Tavares-de-Lima W[1] [1]ICB-USP – Pharmacology, [2]FMUSP-HC-InCor

In this study we investigated the involvement of lymphatic system on the lung inflammation caused by intestinal ischemia/reperfusion (I/R) in female rats. Intestinal I/R cause local and remote injuries that are multifactorial and essentially inflammatory in nature. Studies indicate that female rats are relatively resistant to organ injury caused by hemorrhagic shock and that gut of female is more resistant than the male to deleterious effects of ischemic injury. We previously reported that obstruction of thoracic lymphatic flow during intestinal I/R in male rats blunts pulmonary neutrophil recruitment and microvascular injury. Here, we focus on the effects of the obstruction of thoracic lymphatic flow in female rats. **Methods and Results:** Upon anesthesia Intestinal I/R was induced by 45 min occlusion of the superior mesenteric artery, followed by 2 h reperfusion in female Wistar (60 days old). Ovaries removal (OVx) was carried 7 day before IR induction. In parallel, a group of rats had the thoracic lymphatic duct obstructed before the ischemic procedure. OVx rats not submitted to I/R and OVx Sham-I/R rats were used as controls. Lung and gut myeloperoxidase activity (MPO) and microvascular leakage were determined. Intestinal I/R induced neutrophils migration and increased lung vascular permeability in female OVx rats compared to non-OVx. Thoracic lymphatic duct ligation before intestinal I/R induced elevation in lung and gut MPO activity and microvascular leakage in the non-OVx group. The number of cells on the bone marrow compartment was also increased after the duct obstruction before I/R in OVx and non-OVx rats. Regarding the O_2 saturation the duct obstruction before I/R reduced its values in the non-OVx group. In addition the respiratory rate was increased in rats with the duct obstructed. **Conclusion:** Our data indicates that inflammatory responses in the lung and in the gut after intestinal I/R in female rats is influenced by lymphatic circulation and that neutrophil recruitment, microvascular permeability and lung function are altered when the thoracic lymphatic flow is obstructed. Ethics Committee: 111/10/03 – 2013. **Financial support**: CNPq and Fapesp (2013/15291-0)

04.025

Lung inflammatory process after brain death in rats: sexual dimorfism. Breithaupt-Faloppa AC[1], Ferreira SG[1], Kudo GK[1], Armstrong Jr R[1], Tavares-de-Lima W[2], Sannomiya P[1], Moreira LP[1] [1]FMUSP-HC-InCor – Cardiovascular Surgery, [2]ICB-USP – Pharmacology

Introduction: Solid organ transplantation has become a routine procedure for treatment of patients with end-stage organ diseases. Lung transplantation is an established option for patients with a wide variety of lung diseases. Brain death (BD) affects organ function by multifactorial mechanisms, which include alterations in hemodynamics, hormonal changes and a systemic inflammatory status. Lung neutrophil sequestration is a consequence of the systemic inflammation and results in increased lung microvascular permeability. Because sex hormones possess inflammatory and immune mediating properties, the immune response may differ between men and women. These differences could influence the donor state and the results of the transplant. In this study, we investigated the sex differences on the evolution of the lung inflammatory process in a model of brain death in rats. **Methods:** BD was induced by a sudden increase in intracranial pressure by rapid inflation of a balloon catheter in the intracranial space. Groups of male, female (from high estradiol secretion to heat period) and ovariectomized rats were used throughout the experiments. Ovaries removal (OVx) was carried 10 days before BD induction. Vascular permeability and myeloperoxidase activity (MPO) were assessed at 6 h as markers of lung inflammation. White blood cell counts and female sex hormones levels were analyzed. Serum concentrations of CINC-1 and VEGF were quantified by ELISA. In addition, lung sections were analyzed by histology and ICAM-1 expression quantified by immunohistochemistry. **Results:** After 6 h of BD, the estradiol (E) and progesterone (P) concentrations in serum of female rats were significantly reduced (E (pg/ml) initial: 37.32 ± 8.5; 6h:2.2 ± 1.5* and P (ng/ml) initial: 1097 ± 207.2; 6h: 200.9 ± 52.7*, *$P<0.05$). Female rats maintained the circulating leukocyte number, whilst male rats showed pronounced leukopenia after 6 h (Female initial: 10509 ± 572; 6h: 9213 ± 694 and Male initial: 12550 ± 1034; 6h: 7880 ± 689*, *$P<0.05$). Lung microvascular permeability was increased in female rats compared to male rats (Female: 154.4 ± 12.1* and Male: 104.9 ± 8.3, *$P<0.05$). CINC-1 and VEGF concentrations were increased in female rats in comparison with male (CINC-1 Female: 675 ± 75.2*; Male: 269.7 ± 66.69 pg/ml and VEGF Female: 89.86 ± 11.85* and Male: 12.88 ± 5.7 pg/ml, *$P<0.05$). Regarding lung MPO activity and ICAM-1 expression, there were no significant differences between groups, but female rats presented more important leukocyte infiltrate in the lung parenchyma (Female: 4.06 ± 0.12* and Male: 3.4 ± 0.19 cells/mm^2, *$P<0.05$). **Discussion:** The lung transplant can prolong life substantially; however, the survival statistics for lung transplants are still pale compared with other solid organ transplants. Our results evidenced important differences between genders after BD with more pronounced lung inflammatory compromise in female animals under higher estradiol influence. The data allowed us to suggest that sex hormones may exert a role on the inflammatory events triggered by brain death and deserve, therefore, attention as a potential factor influencing the organ status. *Ethics protocol: nº 344/12.* Supported by Fapesp (2013/20282-0).

04.026

Pravastatin and rosuvastatin *in vivo* or *in vitro* inhibit platelet adhesion to fibrinogen.

Goulart G, Naime ACA, Lopes Pires ME, Monteiro FP, Marcondes S Unicamp – Farmacologia

Introduction: Statins are lipid lowering molecules which reduce serum cholesterol, but a number of other benefits have been reported by these agents. Statins may affect multiple types of cells, including platelets. Works have demonstrated that statins inhibits platelets aggregation especially through reduction of intraplatelet calcium mobilization and by inhibition of TXA2 synthesis. However, the studies about the effect of statins on platelet adhesion are scarce. Therefore, in the present work we decided to study the effects of pravastatin and rosuvastatin *in vitro* and *in vivo* on platelet adhesion to fibrinogen. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – Unicamp, protocol number 3046-1). For *in vivo* experiments, male Wistar rats (250-320 g) were separated in two main groups. In the first group, rats were treated with saline (once a day, by gavage, for 7 days) and in the second group, rats were treated with pravastain (20 mg/kg) or rosuvastatin (10 mg/kg) once a day, by gavage, for 7 days. In the eighth day, the animals were euthanized and blood was collected. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min and the platelets were washed using citrated buffer (pH 6.0). Washed platelet adhesion was evaluated using fibrinogen-coated 96-well microtiter plates. Platelets were maintained in the plate for 30 min. After that, the plate was washed and adherent platelets were incubated with the acid phosphatase substrate for 1h. The plate was read by a microplate reader set at 405nm. For *in vitro* experiments, platelets were incubated with pravastain or rosuvastatin (5 or 50 for 15 min before the adhesion experiments. Cyclic AMP levels were measured in platelets of statin-treated rats using enzyme immunoassay kit. **Results:** Treatment of rats with rosuvastatin inhibited adhesion of non-stimulated and stimulated platelets (42%, 39% and 52% reduction for non-stimulated, thrombin (50 mU/ml) or ADP (10 μM)-stimulated platelet adhesion, respectively). Similarly, pravastain inhibited ADP-stimulated platelet adhesion by 43%, however, non-stimulated or thrombin-stimulated platelet adhesion was not affected by this statin. Incubation of platelets with pravastatin (15 min, 5μM or 50 μM) inhibited by 20% the spontaneous adhesion and by 25% the ADP (10 μM) or thrombin (50 mU/ml)-induced platelet adhesion. Adhesion to fibrinogen was also inhibited by the incubation of platelets with rosuvastatin (32 ± 4%, 19 ± 1% and 26 ± 5% of reduction of non-stimulated, thrombin (50 mU/ml) or ADP (10 μM)-stimulated platelet adhesion, respectively, n=4). Incubation with rosuvastatin (50 μM, 15 min) with platelets did not change the intraplatelet cAMP levels compared to the platelets incubated with saline. **Conclusion:** Rosuvastatin and pravastatin, in both *in vivo* and *in vitro*, inhibit platelet adhesion of healthy rats to fibrinogen. The treatment of rats with rosuvastatin is more effective in inhibiting platelet adhesion than *in vitro* condition. The present results show that statins may be used to inhibit platelet activation even in non hypercholesterolemic condition and that inhibitory effect is mediated by cAMP-independent mechanisms. **Supported by:** CNPq

04.027

Treatment with *Saccharomyces boulardii* ameliorated the function gastrintestinal and reverted the inflammatory events in experimental intestinal mucositis induced by 5-fluorouracil. Xavier AF[1], Justino PFC[1], Morais CM[1], Nogueira AF[1], Malveira CB[1], Girão DKFB[1], Souza EP[2], Lima MAS[3], Mendes TS[2], Lima RF[1], Souza MHLP[1], Ribeiro RA[1], Soares PMG[2] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Morfologia, [3]UECE – Ciências Fisiológicas

Introduction: Anticancer drugs may induce dyspeptic syndromes associated with cancer. This syndrome can is associated with inflammatory cytokines and with a dysfunction of gastrointestinal smooth muscle. *Saccharomyces boulardii* (SB) is used widely in the treatment of gastrointestinal disorders associated with diarrhea. However, the interactions of this probiotic in intestinal mucositis induced by 5-FU in mice is not totally defined. Objective was to evaluate effect of *Saccharomyces boulardii* (SB) in inflammatory aspects and functional of intestinal mucositis induced by 5-FU in mice. Our hypothesis is that treatment with SB is able to reduce inflammatory cytokines and normalize the contractile response of gastrointestinal smooth muscle during intestinal mucositis induced by 5-FU. **Methods**: Swiss male mice (25-30g) were treated with 5-FU (450 mg/kg, ip) or SB + 5-FU (16.10^9 UFC/kg, daily for 3 days), other group received saline. After animals were sacrificed and sample of ileum (I) were collected for assessment cytokines concentrations (pg/ml) were also evaluated by ELISA. The mechanical response obtained after electrical stimulation was recorded using an isometric force transducer coupled to a data acquisition system. To determine the intestinal permeability test was used lactulose / mannitol (L / M). Ethics committee: Protocol 34/10. *Statistical analysis*: Tests ANOVA followed by Bonferroni and $p<0.05$. **Results**: SB treatment was cable reverted the increase of the TNF-alfa (C= 667.8 ± 89.2; 5-FU= 2496.0 ± 320.1; SB + 5-FU= 888.3 ± 157.5), IL-1beta (C=1059.0 ± 122.0; 5-FU= 2197.0 ± 149.3; SB + 5-FU = 726.0 ± 98.4), CXCL-1 (C= 512.3 ± 113.7; 5-FU= 1991.0 ± 837.5; SB + 5-FU = 66.3 ± 50.5) and recovered the concentrations of IL-10 (C= 177.0 ± 5.7; 5-FU= 84.5 ± 8.3; SB + 5-FU = 126.5 ± 4.7) during intestinal mucositis induced by 5-FU. Furthermore, 5-FU induced higher sensitivity of the contractile response to electrical stimulation (20Volts: C= 0.21 ± 0.04, 5-FU= 0.45 ± 0.05; 4Hz: C= 0.15 ± 0.04; 5-FU= 0.38 ± 0.03g), this was blocked by SB (20Volts: SB + 5-FU= 0.20 ± 0.09; 4Hz: SB + 5-FU= 0.20 ± 0.05). 5-FU also alters the ratio L/M (C=0.54 ± 0.03, 5FU=2.12 ± 0.77) that were reverted by SB treatment (L/M= 0.62 ± 0.02). **Conclusions**: Our results suggest that the treatment with SB reverted the inflammatory and dysfunction gastrointestinal presents in the experimental intestinal mucositis induced by 5-FU. **Financial Support:** CNPq, Capes and FUNCAP.

04.028

Anti-inflammatory activity of the aqueous extract of the bark of *Chrysobalanus icaco Linnaeus*. Oliveira TB, Guerra ASHS, Mota FVB, Carvalho JA, Silva BIM, Silva TG UFPE – Antibióticos

Introduction: *Chrysobalanus icaco* Linnaeus (Chrysobalanaceae) is a plant used in traditional medicine in Brazil to control various diseases, such as diabetes, diarrhea and inflammation. The present study aimed evaluates the anti-inflammatory effect of the aqueous extract of the bark of *Chrysobalanus icaco* (AECI). **Methods**: The decoction was prepared using 200 g of the bark in 1000 mL of water and was heated to 100 °C for 30 minutes. The decoction was filtered and lyophilized. For the paw oedema, male Wistar rats (180-200 g) were treated orally with vehicle (0.9% saline, 10 mL/kg), AECI (100, 200, 400 mg/kg) or indomethacin (10 mg/kg) 60 min before injection of carrageenan (1%, 0.1 mL) in the subplantar region of the right hindpaw. After 1, 2, 3, and 4h from the carrageenan administration, the paw diameter was determined using a plethysmometer (Ugo Basile). The edema was expressed in mL as the difference between the final and initial volume of the paw. The plantar tissues were removed, homogenates and the supernatant were used for measured the activity of MPO (Bradley, Priebat, 1982). The anti-inflammatory activities of AECI were tested by the formation of air pouches on the dorsal cervical region of mice via a subcutaneous injection of 2.5 mL of sterile air on days 0 and 3. On day 6, the mice orally (p.o) received AECI (400 mg/kg), indomethacin (10 mg/kg) or vehicle. After 1 h, inflammation was induced by injecting 1 mL of carrageenan suspension 1% into the air pouch. After 6 h, the animals were euthanized in a CO_2 chamber, and the pouches were washed with 3 mL of saline solution containing 3 μM EDTA. Polymorphonuclear leukocytes were counted using an ABX Micros 60 hematology analyzer (Guerra *et al.*, 2011). The results are presented as the means ± S.D. Differences between the groups were determined by analysis of variance (ANOVA – one way), followed by Bonferroni's post hoc test. The results were considered statistically significant when $p<0.05$. All procedures were approved by the Committee for Ethics in Animal Research of UFPE, with number – CEEA/PE Nº 23076.050728/2010-84. **Results and discussion:** AECI (100, 200 and 400 mg/kg) and indomethacin (10 mg/kg) significantly inhibited paw oedema formation in the 3rd hour (0.86 ± 0.02, 0.80 ± 0.05, 0.78 ± 0.05 and 0.51 ± 0.04, respectively) when compared the vehicle (1.65 ± 0.08). Moreover, AECI (200 and 400 mg/kg) and indomethacin inhibited MPO activity (0.321 ± 0.002, 0.273 ± 0.004 and 0.158 ± 0.03, respectively) compared to the vehicle (0.620 ± 0.02). AECI (400 mg/kg) and indomethacin decreased cell migration in air pouch (4.72 ± 0.94 and 3.88 ± 0.69, respectively) compared to the vehicle (11.30 ± 1.62). These results indicate that AECI possesses anti-inflammatory activity in acute inflammation induced by carrageenan. Studies are in progress in the attempt to clarify the action mechanism in the inflammatory process. **References:** Bradley PP, *et al.* Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol*, v.78, p.206-9, 1982. Guerra ASHS, *et al.* Anti-inflammatory and antinociceptive activities of indole-imidazolidine derivatives. *Int Immunopharmacol.* 11 (11): 1816-1822, 2011. Winter CA. *Proc Soc Exp Biol Med.* 111, 544, 1962. **Financial support:** UFPE/FACEPE.

04.029

The polyphenol resveratrol prevents the allergic pulmonary eosinophilic inflammation in obese mice via AMPK activation and insulin resistance amelioration. André DM, Calixto MC, Alexandre EC, Sollon CS, Anhê GF, Antunes E Unicamp – Farmacologia

Introduction: Asthma and obesity are major public health problems worldwide. Obesity increases the prevalence and incidence of asthma in humans (Huang *et al.*, 1999). High-fat diet (HFD)-induced obesity enhances the pulmonary eosinophilic inflammation in a murine model of allergic disease (Calixto *et al.*, 2010). Recently, insulin resistance associated with obesity has been shown to play an important role in the asthma exacerbation in obese mice (Calixto *et al.*, 2013). Resveratrol (RESV) is a polyphenol stilbene contained in red grapes, cranberries and peanuts, and exhibits anti-inflammatory and antioxidant effects in some pathological conditions. Resveratrol activates AMP-activated protein kinase (AMPK), an important metabolism regulatory protein (Um *et al.*, 2010). In mice, AMPK activation improves HFD-induced insulin resistance and ovalbumin (OVA)-induced airway inflammation (Calixto *et al.*, 2013). We have hypothesized that insulin resistance in obese mice aggravates the pulmonary eosinophilic inflammation in allergic animals, and that resveratrol may present beneficial effects in the eosinophilic inflammation and oxidative stress in these animals. **Methodology:** The experimental protocols have been approved by the Ethics Committee of Unicamp (Nº. 2709-1). Male C57Bl/6/JUnib mice received HFD or standard-chow diet for 12 weeks. Control and obese mice received daily administration of resveratrol (100 mg/kg, gavage, 2 weeks). Mice were sensitized with OVA (100 µg, s.c) and intranasally challenged with this antigen (10 µg) two weeks later. At 48 h after OVA challenge, cell infiltration, p-AMK expression and superoxide dismutase (SOD) activity were examined in the lung tissues. **Results:** Obese mice exhibited a 43% decrease ($P<0.05$) in glucose uptake in response to insulin stimulation compared with lean group, which was significantly restored by resveratrol treatment. In OVA-challenged obese mice, resveratrol significantly decreased the eosinophil counts in peribronchiolar region compared with respective untreated obese mice (42.2 ± 4.0 and 7.4 ± 1.5 cells/mm^2, respectively). Resveratrol treatment also increased by 58% ($P<0.05$) the p-AMPK expression in lung tissue of obese mice compared with obese untreated mice. Besides, OVA-challenged obese mice showed a decrease ($P<0.05$) in SOD activity in the lung tissue, which was greatly prevented by resveratrol treatment. **Discussion: and Conclusion**: Our present data show that resveratrol increases systemic glucose uptake and p-AMPK expression in lung tissue of OVA-challenged obese mice, which is accompanied by amelioration of the oxidative stress. Resveratrol may contribute to the resolution process in lungs of obese mice by accelerating the transit of eosinophils and its elimination to airway lumen. Our findings are consistent with a strong correlation between insulin resistance and asthma. **Financial support**: CNPq. **References:** Calixto MC *et al. Plos One*. 2013; 8(10): e76786. Calixto MC *et al. Br J Pharmacol*; 2010: 1; 159(3): 617-25. Huang SL. *et al. Clin Exp Allergy*. 1999; 29: 323-329. Um JH *et al. Diabetes*. 2010; 59: 554–563.

04.030

Down-modulation of activated human neutrophil by LMW-Fucoidan: Role of microparticle. Moraes JA[1], Frony AC[1], Barcellos-de-Souza P[1], Boisson-Vidal C[2], Barja-Fidalgo C[1] [1]UERJ – Biologia Celular, [2]INSERM U765

During migration, neutrophils (PMN) interact with several mediators, which can lead to their activation, interfering with cell survival and inflammation resolution. Fucoidans are sulfated polysacharides which are able to inhibit selectin-mediated events, inhibiting PMN rolling, a crucial step to inflammation resolution. 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. PMN was isolated from healthy volunteers (Percoll gradient). In a boyden chamber, after 1h of migration, 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, LMW-Fuc attenuated the induced alterations on actin cytoskeleton dynamics (immunocytochemistry) and inhibited AKT phosphorylation after 30min (western blotting). We also observed that LMW-Fuc was able to accelerate apoptosis of activated PMN (microscopy and annexin-V positive cells), 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 (lucigenin), but not the intracellular ROS production (DCF) induced by LPS/fMLP treatment. Furthermore, in stimulated PMN LMW-Fuc inhibited microparticle (MP) release, which are the key actors of extracellular ROS production in these cells, mainly, as we observed, by its property to carry p47. Interstingly we also oserved that PMN-MP was also able to induce extracellular ROS production in macrophages, which inspire us to suggest a paracrine role for these MP. Finally, we observed that LMW-Fuc inhibited MLC activation induced by LPS/fMLP, a pivotal pathway to vesiculation. Thus LMW-Fuc presents a potent ability to attenuate PMN activation that might be potentiated by its ability inhibiting MP release. **Funding Support:** Capes/CNPq/Faperj.

04.031

Effect of Helicobacter pylori urease (HPU) in endothelial cells. Souza MJ[1], Moraes JA[1], Nascimento-Silva V[1], Helal-Neto E[1], Morgado-Diaz J[2], De-Freitas-Junior J[2], Uberti A[3], Scopel-Guerra A[3], Morandi V[1], Carlini C[3], Barja-Fidalgo C[1] [1]UERJ – Dept de Biologia Celular, [2]INCA, [3]UFRGS

The urease of *Helicobacter* pylori (HP) has been extensively reported as an important bacterial product which enables HP colonizes and survives in the stomach, favoring the occurrence of gastric ulcer and adenocarcinoma. Recently, we demonstrated that HP urease (HPU) is a pro-inflammatory molecule that can contribute to the worsening of HP infection. Although the effects of HP on endothelial cells (EC) have been early described, the direct involvement of HPU in the vascular effects is not completely understood. We now investigate the effects of HPU on EC and the signaling pathways involved in those processes.

The treatment of EC with HPU (up to 10nM) did not affect cell viability. On the other hand, HPU enhanced EC permeability and reduced transendothelial resistance. Corroborating this data, HPU caused dissociation of cell–cell junctional cadherins, induced VE-cadherin phosphorylation, promoted alterations on actin cytoskeleton dynamics, and also increased FAK phosphorylation. Additionally, HPU induced ROS and NO production, that were impaired by apocynin, which inhibits NOX2 and by LY, a PI3K-AKT pathway inhibitor. HPU also increased adhesion of neutrophils to EC. The effects of HPU on EC seem to be modulated by integrins, as they were attenuated in the presence of RGD peptides. Finally, treatment with HPU induced tubulogenesis, evidencing its potential to induce EC differentiation.

The data indicate that HPU actives EC, probably through an integrin-associated signaling, supporting its role as a pro-inflammatory and also pro-angiogenic key molecule in *H pylori*-related diseases. Supported by: Faperj, Capes, CNPQ.

04.032

PKC and PKG activate NADPH oxidase and increase reactive oxygen species generation in platelets of rats treated with lipopolysaccharide. Lopes-Pires ME, Naime ACA, Antunes E, Marcondes S Unicamp – Farmacologia

Introduction: A previous work of our group showed that the treatment of rats with lipopolysaccharide (LPS) increased reactive oxygen species (ROS) production, which was mediated especially by NADPH oxidase. Phosphorylation of NADPH oxidase cytosolic subunitis by PKC and AKT corroborate to increase enzymatic activity. Furthermore, studies have shown that NADPH oxidase may still be activated by increasing cGMP synthesis and activation of PKG. Therefore, the aim of this study was to investigate the mechanisms that mediate NADPH oxidase activation in platelets of LPS-treated rats. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – Unicamp, protocol number 2097-1). Wistar rats were injected i.p. with saline or LPS (from *E. coli*, 1 mg/kg) and at 6 h or 48 h thereafter arterial blood was collected. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min and the platelets were washed using citrated buffer (pH 6.0). Production of ROS in washed platelets was measured by flow cytometry using DCFH-DA (5 μM) in absence or presence of the inhibitors of PKC (GF109203X, 10μM), sGC (ODQ, 10 μM), PKG (Rp-8-Br, 5μM) and Akt (API-1, 20μM). NADPH oxidase activation was evaluated by determination of p47phox subunit phosphorylation using western blotting assays. **Results:** ROS production in platelets from LPS-treated rats was 4.5-fold higher ($P<0.05$) than in saline-injected rats. Increase of ROS generation in platelets of LPS group was accompanied by increased phosphorylation of p47-phox subunit of NADPH oxidase at residue Ser345 (2.6 and 3-fold increase at 6h and 48h after LPS treatment, respectively compared to the saline group). Incubation of platelets from saline or LPS-treated rats with API-1 did not change ROS formation. GF109203X did not affect ROS generation in platelets of saline-treated rats but decreased about 42% and 26% the release of ROS in platelets of LPS-treated rats at 6h and 48h respectively. Furthermore, GF109203X reduced about 2 times the phosphorylation of Ser345 residue of p47 phox in rats treated with LPS. ROS generation in saline or LPS-treated rats at 6h was not change by ODQ or Rp-8-Br. However, ROS generation in platelets 48h after LPS treatment was significantly reduced by ODQ or Rp-8-Br (reduction of 35% and 39% after incubation with ODQ and Rp-8-Br, respectively). **Conclusion:** ROS generation in platelets of rats injected with saline or LPS is not mediated by AKT. PKC is involved in the activation of NADPH oxidase and consequently on the increase of ROS formation in platelets from rats injected with LPS 6h and 48h afterwards. The sGC and PKG modulate ROS release in platelets in the late phase of sepsis. These results show that the exposure time of rats to LPS determine the signaling pathways that take part on modulating ROS generation in platelets. **Supported by**: Fapesp

04.033

Evaluation of anti-edema activity of microspheres of poly-ε-caprolactone containing usnic acid. Barbosa JAP[1], Silva CVNS[2], Bezerra TO[1], Santana MAN[3], Silva TG[1], Magalhães NSS[2], Santos NPS[4], Maia MBS[5] [1]UFPE – Antibióticos, [2]LIKA-UFPE – Bioquímica, [3]UFPE – Histologia, [4]UFPE – Biotecnologia, [5]UFPE – Fisiologia

Introduction: Lichens are beings resulting from symbiosis between fungi and one or more algae. Natural sources of biologically active compounds that derive their primary and secondary metabolism (Kaffer *et al.* 2005) are important. Among secondary metabolites isolated from lichens, usnic acid, a hydrophobic liquenic metabolite, has been highlighted in the literature with diverse biological activities such as anti-inflammatory, analgesic, antimicrobial, antiviral, antiparasitic, gastroprotective, and antitumor (Nunes *et al.* 2010). Although the usnic acid (UA) has various biological activities, its use as a therapeutic agent is not possible due to their physicochemical properties drawbacks such as low solubility, high toxicity and difficult interaction with biological barriers (Muller *et al.* 2001). The incorporation of usnic acid in controlled release systems, such as microspheres, may allow the reduction of the toxicity of the compound and make it an alternative for therapeutic application. Therefore, the aim of this study was to assess the anti-edematous property of microspheres of poly-ε-caprolactone (PCL) containing usnic acid in rats. **Methods:** PCL microspheres were prepared by the method multiple emulsion W/O/A followed by solvent evaporation and characterized by the encapsulation efficiency of the microspheres usnic acid, particle size, polydispersity index (span) and the load surface. The anti-edema activity was analyzed using the paw edema induced by carrageenan and determined the level of myeloperoxidase in inflamed tissues. The control group received vehicle (NaCl 0.9% containing 5% cremophor), the treated groups received AU (25 mg/kg), Microspheres containing AU (Micro-AU/25 and 50 mg/kg) and indomethacin (standard drug, 10 mg/kg) oral administration. The experimental protocol was approved by the UFPE, Protocol Committee for Ethics in Animal Experimentation (EAEC). 23076.052269/2012-35. **Results:** The encapsulation efficiency of the microspheres usnic acid was 97.72%, the average diameter of the microspheres containing AU was 13.54 microns was negative zeta potential (ζ = -44.5 mV) and with homogeneous size distribution (Span 2:36). Treatment with microspheres containing AU and AU caused a significant reduction of the edema on the inhibition of the edema volume of 47, 60, 31 and 34% (AU/25 mg/kg), 49, 64, 46 and 48% (Micro-AU/25 mg/kg), 57, 68, 59 and 63% (Micro-AU/50 mg/kg) and 70, 76, 75 and 77% (Indomethacin / 10 mg/kg) for intervals of 1, 2,3 and 4 hours. Further, treatment with AU free and encapsulated in microspheres resulted in a reduction of cell migration to the site of injury in animals treated with UA (25 mg/kg), Micro-AU (25 and 50 mg/kg) and indomethacin (10 mg/kg) with inhibition of 73%, 65%, 68.9% and 75%, respectively. **Discussion:** Regarding the physicochemical characterization of PCL microspheres suggest acceptable for oral delivery system parameters (Ribeiro-Costa *et al*, 2009.). In accordance with the results obtained from the anti-edema activity, possibly AU interfere at different mediators of inflammation, in view of the significant reduction of the edema has occurred in different time intervals (Vijayakumar, F., V.71, p 564, 2000). **References:** (Kaffer, AB, 19 v, 815 p, 2005); (Muller, AMB, v.56, p.9, 2001); (Nunes, JTAC, V.99, p.1011, 2010; (Ribeiro-Costa, PT, V 111, p 190, 2009). (Vijayakumar, F., V.71, p 564, 2000). **Financial Support:** Propesq; CNPq; FACEPE; UFPE.

04.034

δPKC and AKT stimulate NO/CGMP/PKG pathway in platelets of rats treated with lipopolysaccharide. Frade JO, Lopes-Pires ME, Antunes E, Marcondes S FCM-Unicamp – Pharmacology

Introduction: Studies showing the involvement of platelets in inflammatory conditions such as sepsis have been growing over the past few years. Previous studies have shown that patients with sepsis present reduced platelet aggregation to ADP, collagen and arachidonic acid. Similarly, platelet aggregation is decreased in rats after intraperitoneal or intravenous LPS administration. Despite of works showing the inhibitory effects of lipopolysaccharide (LPS) on platelets, the intracellular mechanisms have not yet been elucidated. Therefore the objective of the present work was to investigate the signaling pathway involved on the inhibitory effect of LPS on ADP-stimulated platelet aggregation. **Methods**: The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – Unicamp, protocol number 3316-1). Wistar rats were injected i.p. with saline or LPS (from E. coli, 1 mg/kg) and after 6h arterial blood was collected. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min and the platelets were washed using citrated buffer (pH 6.0). ADP (10 μM)-induced platelet aggregation was evaluated using a two-channel aggregometer in absence or presence of enzymatic inhibitors. Nitrated proteins were determinate by western blotting assays. Cyclic GMP levels were measured in platelets using enzyme immunoassay kit **Results:** Platelet aggregation was significantly reduced by LPS (65% of reduction compared to control), which was accompanied by increased intraplatelet cGMP levels (4.7 fold higher compared to the control) and nitrated proteins (3.3 fold higher compared to the control). Incubation of platelets with the peroxynitrite scavenger epigallocatechin-3-gallate did not change the inhibition of platelet aggregation by LPS. On the other hand, ODQ and Rp-8-Br-PET-cGMPS (inhibitors of guanylyl cyclase and PKG, respectively) prevented LPS inhibitory effect. AKT inhibition by API-1 fully reversed the inhibitory effect of LPS treatment on aggregation, which was accompanied by reduction on cGMP levels. Incubation of platelets from LPS-treated rats with GF109203X (a non-specific PKC isoforms inhibitor) restored aggregation and intraplatelet cGMP levels. However, GF109203X inhibited 33% platelet aggregation from saline-treated rats and significantly augmented cGMP levels. As different PKC isoforms have opposite effects on nitric oxide synthase activity, we investigated the effect of δPKC isoform, which is express under oxidative stress condition, on platelet aggregation of LPS-treated rats. Incubation of rottlerin, a specific δPKC inhibitor, with platelets from LPS-treated rats increased aggregation by 45% and did not change platelet aggregation from saline-treated rats. **Conclusion:** our results show that platelet inhibition in sepsis is mediated by NO/cGMP/PKG-dependent mechanisms, and δPKC and AKT act upstream upregulating this pathway. **Supported by**: Capes and Fapesp.

04.035

Gamma delta T lymphocyte modulation by lipoxygenase-derived mediators. Negreiros C[1], Cascabulho C[2], Pons A[2], Henriques MG[1], Costa MF[3], Penido C[1] [1]Farmanguinhos-Fiocruz – Farmacologia Aplicada, [2]Fiocruz – Inovações em Terapias, Ensino e Bioprodutos

Introduction: γδ T lymphocytes represent an unconventional subset of T lymphocytes preferentially distributed in epithelial tissues, which presents a critical role in the modulation of inflammatory and infectious diseases, including tuberculosis (*Tsukaguchi K. et al.*, *J Immunol*, *v. 4*, *p. 1786*, *1995*). These cells directly recognize mycobacterial antigens, such as isopentenyl pyrophosphate (IPP) that induces proliferation and cytokine production. During the inflammatory response, lipoxygenase-derived lipid mediators, such as leukotrienes (LTs) and lipoxins (LX), exert pro-inflammatory, anti-inflammatory and pro-resolutive functions (*Borgeat and Naccache*, *Clin Biochem*, *v. 5*, *p. 459*, *1990*). Previous data from our group demonstrate that γδ T lymphocyte migration during *Mycobacterium bovis* BCG-induced response is mediated by LTB_4 and its receptor BLT1 (*Costa M.F.S. et al.*, *J. Leukoc. Biol. v. 87*, *p. 323*, *2009*). Moreover, it has been demonstrated that LXA_4 induces the chemotaxis of human monocytes and downmodulates the expression of BTL1 by T lymphocytes (Lin K.T. *et al.*, *J Pharmacol Exp Ther.*, *v. 277*, *p. 679*, *1996*; *Maddox J.F.& Serhan C.N.*, *J Exp Med.*, *v. 183*, *p. 137*, *1996*). In the present study, we have investigated the role of lipid mediators in γδ T lymphocyte activation, migration and cytotoxic function. **Methods:** Human γδ T lymphocytes were derived from PBMC after 14-day culture in the presence of IPP (5μg/ml) and IL-2 (12.5ng/ml). The surface expression of TCRγδ, BLT1, CD25 and CD107a by γδ T lymphocytes was analyzed by flow cytometry upon re-stimulation with IPP (10μg/ml) or stimulation with LTB_4 (100nM) and LXA_4 (100nM). Chemotaxis was evaluated by transwell assay (0.3 μm, Corning), cytotoxic activity was evaluated by Granzyme B Activity Fluorometric Assay Kit (Biovision) and intracellular calcium influx was determined by Calcium Assay Kit (Molecular Devices). All protocols were approved by the Research Ethics Committee of Fiocruz (CEP Fiocruz/IOC – 346.627). p values<0.05 were regarded as significant (ANOVA followed by Student-Newman-Keuls test, $n \geq 5$). **Results:** Expansion of γδ T lymphocytes from PBMC after 14-day stimulation with IPP plus IL-2 resulted in 92% of purity. IPP-stimulated γδ T lymphocytes upregulated BLT1 expression, in a manner dependent on time (day 1: 0.36%; day 7: 0.49%; day 14: 0.65%) and on concentration (medium: 0.36%; IPP 2μg/ml: 0.43%; IPP 5μg/ml: 0.65%; IPP 10μg/ml: 0.71%). Both LTB_4 and LXA_4 induced γδ T lymphocyte activation, as observed by intracellular calcium influx and chemotaxis (medium: 1.46 ± 0.2; LTB_4: 2.66 ± 0.5; LXA_4: 2.18 ± 0.4 $x10^3$ cells/well). Interestingly, the pre-incubation of γδ T lymphocytes with LXA_4 was able to inhibit IPP-induced CD25 expression and LTB_4-induced chemotaxis (LTB_4 + LXA_4: 1.09 ± 0.35 $x10^3$ cells/well). IPP-induced γδ T lymphocyte cytotoxicity was enhanced by LTB_4 stimulation, as observed by the increased surface expression of CD107a (medium: 0.65 ± 0.0; IPP: 29.88 ± 1.7; LTB_4 + IPP: 35.65 ± 1.8 % of γδ T lymphocytes $CD107a^{+)}$ and release of granzyme b (medium: 28.97 ± 6.8; IPP: 61.90 ± 5.4; LTB_4 + IPP: 92.30 ± 5.8 pg/well). **Discussion:** Our results indicate that the lipoxygenase-derived mediators different and directly modulate γδ T lymphocyte activation, migration and cytotoxic functions. Further studies are required to elucidate the involvement of these mediators on γδ T lymphocyte modulation during infections. **Financial Support:** Capes, Faperj, Fiocruz, CNPq.

04.036

PDTC inhibits UVB-induced skin inflammation and oxidative stress in hairless mice and exhibits antioxidant activity *in vitro*. Ivan ALM[1], Campanini MZ[1], Martinez RM[1], Ferreira VS[1], Steffen VS[1], Vicentini FTMC[2], Vilela FMP[2], Martins FS[2], Zarpelon AC[3], Cunha TM[4], Fonseca MJV[2], Baracat MM[1], Georgetti SR[1], Verri WA[3], Casagrande R[1] [1]UEL – Ciências Farmacêuticas, [2]FCFRP-USP – Ciências Farmacêuticas, [3]UEL – Ciências Patológicas, [4]FMRP-USP – Farmacologia

Introduction: UVB irradiation may cause oxidative stress- and inflammation-dependent skin cancer and premature aging. Pyrrolidine dithiocarbamate (PDTC) is an antioxidant and inhibitor of nuclear factor-κB (NF-κB) activation. Herein, the mechanisms of PDTC were investigated in cell free oxidant/antioxidant assays, *in vivo* UVB irradiation in mice and *in vitro* UVB-induced IκB degradation in keratinocytes culture. **Methods:** ***in vitro*** cell-free systems were: 2,2'-azinobis-(3-ethyl benzothiazoline-6-sulphonic acid) radical (ABTS), 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH), hydroxyl radical, iron chelation, iron-dependent and -independent lipid peroxidation assays. Hairless mice were divided in five groups (n=5): non-irradiated control, irradiated control (4.14 J/cm^2) and three group of irradiated and treated with PDTC (10-100 mg/kg, ip) 1 h before the irradiation and 8 h after the first dose. The UVB source used was a Philips TL/12 RS 40W with a peak emission at 313 nm. Mice were terminally anesthetized at 2h (cytokines) or 12h (others tests) after the end of the irradiation. The skin edema was measured as an increase in dorsal skin weight. The UVB-induced leukocyte migration was evaluated by myeloperoxidase (MPO) activity assay. IL-1β levels were determined by ELISA. Sodium dodecyl sulphate polyacrylamide gel electrophoresis substrate-embedded zymography was used to detect enzymes with gelatinase activity. The reduced glutathione (GSH) levels were determined by the 5,5'dithiobis (2 nitrobenzoic acid) (DTNB) assay and ferric reducing ability potential (FRAP) by colorimetric assay. UVB-induced IκB degradation in HaCAT keratinocytes was assessed by western blot. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's t test, $p<0.05$. This study was approved by the Ethics Committee on Animal Research of UEL (Process 33631.2010.82). **Results and discussion:** PDTC presented the ability to scavenge ABTS radical, DPPH radical and hydroxyl radical; inhibited iron-dependent and -independent lipid peroxidation and chelated iron with the following IC50 values: 0.74 µg/mL, 5.14 µg/mL, 66.53 µg/mL, 1.08 µg/mL, 3.77 µg/mL and 35.32 µg/mL, respectively. The *in vivo* treatment with PDTC at the doses of 10, 30 and 100 mg/kg significantly reduced UVB irradiation-induced increase of skin edema by 60%, 48% and 60%; MPO activity by 43%, 41% and 54%; IL-1β production by 43%, 48% and 47%; MMP-9 by 11%, 0%, 25% as well as prevented the reduction of GSH by 0%, 51% and 84%; FRAP by 45%, 21%; and 47%; ABTS by 6%, 0% and 55%, respectively. PDTC also reduced up to 84% of UVB-induced IκB degradation in keratinocytes. **Conclusion:** These results demonstrate that PDTC presents antioxidant and anti-inflammatory effects *in vitro*, which line up well with the PDTC inhibition of UVB irradiation-induced skin inflammation and oxidative stress in mice. These data suggest that treatment with PDTC may be a promising approach to reduce UVB irradiation-induced skin damages and merits further pre-clinical and clinical studies. **Financial Support:** Capes, CNPq, Fundação Araucária and UEL.

04.037

Carvacrol/beta-cyclodextrin inclusion complex reduces muscle inflammation in rats.

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Skeletal muscle inflammation causes pain and compromises the motor function. Carvacrol is a monoterpene with anti-inflammatory and antinociceptive, but its low solubility may limit its use. The inclusion in beta-cyclodextrin is well known to improve the solubility and pharmacokinetic profile of many compounds. The objective of this study was to evaluate the effect of carvacrol/beta-cyclodextrin (BCD-CARV) inclusion complex on the muscle inflammation induced by carrageenan. Male Wistar rats (n=6/group) were used in this study and the experimentation was approved by the Institution's Ethic Committee (number 02/12). Rats were pre-treated (v.o.) with BCD-CARV(2.5-10 mg/kg), free carvacrol suspension (CARV; 10 mg/kg) or vehicle one hour before the administration of carrageenan (3%, 100 uL) in the gastrocnemius muscle. Animals were submitted to the evaluation of nociceptive parameters using the electronic von Frey (to measure paw hyperalgesia) and the Grip strength meter (to measure motor performance) after 6 and 24 h of the induction. After 24 h, rats were euthanized and the muscle was collected for the analysis of myeloperoxidase activity, edema formation (muscle weight in mg/body weight in g), histological analysis and interleukin-1beta (IL-1beta) concentrations. Pre-treatment of rats with BCD-CARV (10 mg/kg) reduced the MPO activity (5.3 ± 1.7 UMPO/mg of tissue; $p<0.05$) and edema (7.00 ± 0.09 mg/g; $p<0.05$) when compared with vehicle-treated group (12.6 ± 1.6 UMPO/mg of tissue and 7.82 ± 0.15 mg/g), which was not observed by the pre-treatment with CARV (8.4 ± 1.3 UMPO/mg of tissue and 8.23 ± 0.06 mg/g). These results were confirmed by the histological analysis. Also, the pre-treatment with BCD-CARV reduced the concentrations of IL-1beta (342 ± 53 pg/mL; $p<0.05$) in muscle homogenate, when compared with vehicle-treated group (708 ± 130 pg/mL). Besides, the pre-treatment with BCD-CARV impaired the variation of the paw hyperalgesia (-0.7 ± 1.6 and -7.1 ± 1.5 g for 6 and 24 h respectively; $p<0.001$), when compared with vehicle-treated group (-21.9 ± 3.9 and -22.2 ± 3.2 g for 6 and 24 h respectively), which did not occur in the group pre-treated with CARV (-17.5 ± 5.4 and -18.3 ± 5.2 g for 6 and 24 h respectively). The motor performance was also reversed by the pre-treatment with BCD-CARV (-19 ± 61 and -7 ± 63 g for 6 and 24 h respectively; $p<0.001$), when compared with vehicle-treated group (-227 ± 27 and -275 ± 34 g for 6 and 24 h respectively), which did not occur in the group pre-treated with CARV (-221 ± 70 and -181 ± 35 g for 6 and 24 h respectively). These results demonstrated that BCD-CARV is a potential treatment for muscular inflammation and hyperalgesia. In addition, the complexation of this compound shows advantage for the treatment of muscle inflammation, when compared with the free compound. **Financial support**: Capes.

04.038

Complement activation on platelets in experimental cerebral malaria. Rodrigues FG[1], Campos MHA[1], Assis FA[1], Val CH[1], Brant F[1], Silva BC[2], Arifa RDN[3], Rachid MA[2], Machado FS[1], Teixeira AL[4], Teixeira MM[1] [1]ICB-UFMG – Biochemistry and Immunology, [2]ICB-UFMG – Pathology, [3]ICB-UFMG – Microbiology, [4]UFMG – Internal Medicine

Introduction: Cerebral malaria (CM) is a severe form of the disease that may result, in part, from an overt inflammatory response during infection by *Plasmodium falciparum*. The Complement System plays an important role in immune response, leading to inflammation, endothelial activation, opsonization and coagulation, processes which have been implicated in CM pathogenesis [Silver *et al*., *Microbiol*., 12 (8): 1036-45, 2010]. In addition, several studies have shown the thrombocytopenia in experimental cerebral malaria and in human severe malaria (Gérardin *et al*., *Am. J. Trop. Med. Hyg*., 66: 686-91, 2002; De Lacerda *et al*., *Mem. Inst. Oswaldo Cruz*, 106: 52-63, 2011). Platelet activating factor (PAF) is a mediator of inflammation shown to orchestrate inflammatory processes, including recruitment of leukocytes and increase of vascular permeability (Chao & Olson., *Biochem. J*., 292: 617–29, 1993). The aim of our study was to investigate the role of Complement System for the development of thrombocytopenia induced by *Plasmodium berghei* ANKA (PbA), in the experimental model of cerebral malaria (ECM). **Methods**: C57BL/6 (WT) and PAF receptor-deficient mice ($PAFR^{-/-}$) were infected with 5 x 10^5 PbA-parasitized erythrocytes, and the course of infection and survival were evaluated periodically. The thrombocytopenia was assessed by number of platelets in the blood of PbA infected mice on 3, and 6 days post infection (dpi). Brain homogenates and plasma of mice with CM were analyzed for C5a levels by ELISA. **Results and discussion:** In infected $PAFR^{-/-}$ mice, lethality was markedly delayed and brain inflammation was significantly reduced, as demonstrated by histology, as compared to infected WT mice. The platelets analysis confirmed the thrombocytopenia in PbA infected WT mice on 6 dpi. WT infected mice had higher brain C5a levels (2395 ± 1050 pg/100mg of tissue), particularly on day 6, as compared with non-infected WT mice (417.5 ± 35.85 pg/100mg of tissue). By FACs analysis using specific antibodies we demonstrated significant increase in Membrane Attack Complex (MAC) deposition on platelets from WT infected mice as compared to $PAFR^{-/-}$ infected mice. Taken together, the results suggest that PAFR signaling and the Complement activation on platelets is crucial for the development of thrombocytopenia induced by *Plasmodium berghei* infection. **Financial support:** CNPq Protocol CEUA/UFMG 93/2012

04.039

Intestinal mucositis induced by association of irinotecan and 5-fluorouracil in C57BL/6 mice: involvement of TNF-α and IL-6. Pereira VBM[1], Wong DVT[1], Melo AT[1], Assis-Júnior EM[1], Brito GAC[2], Ribeiro RA[1], Lima-Júnior RCP[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Morphology

Introduction: Intestinal mucositis (IM) is a common side effect induced by anticancer agents used for the treatment of colorectal cancer, which includes the protocol FOLFIRI (5-Fluorouracil [5-FU] + Irinotecan [IRI] + Leucovorin). The pathogenesis of IM has been largely investigated in animal models in which these drugs are injected alone, despite the use of polychemotherapy in clinical setting. However, the course of IM may vary according to the drug and regimens employed. Then, we aimed to develop a new experimental model of IM induced by the association of IRI and 5-FU and to investigate the involvement of pro-inflammatory cytokines. **Methods:** C57BL/6 mice (20-25g) were divided into experimental groups (n=6): 1) Saline (5 mL/kg, i.p); 2) IRI (30 or 45 mg/kg, i.p.); 3) 5-FU (25, 37.5 or 50 mg/kg, i.p.) or 4) IRI + 5-FU. All groups were treated for 4 days. Mortality rate and diarrhea score were evaluated daily for 7 days. On the 7th day, blood leukocyte count (cells/mm3) was obtained. Following animal euthanasia, ileum samples were collected for histopathological analysis, myeloperoxidase (MPO, neutrophil/mg protein) activity assay and TNF-α and IL-6 levels (pg/mg tissue). Kaplan-Mayer log rank test, Kruskal Wallis/Dunn's or ANOVA/Bonferroni's test were used for statistical analysis. $P<0.05$ was accepted. (CEPA protocol 76/11). Results: The best dose of associated drugs able to induce IM, without important mortality (100% survival) on the 7th day, was 5-FU (37.5 mg/kg) + IRI (45 mg/kg). These doses were used for subsequent studies. 5-FU + IRI induced diarrhea [2(0-3)], body weight loss (14.2%), leukopenia (7.3 ± 2.3x10^3), intestinal damage [4(3-4)] and inflammatory cells infiltration (MPO: 14641 ± 1598) and increased levels of pro-inflammatory cytokines (TNF-a: 3.2 ± 0.9; IL-6: 1.4 ± 0.5) when compared with ($P<0.05$) saline group (diarrhea: [0(0-1)]; leukocyte count: 215.5 ± 54.1x10^3; intestinal damage: [0(0-1)]; MPO: 5747 ± 1155; TNF-a: 0.7 ± 0.2; IL-6: 0.3 ± 0.1) or each drug given alone (5-FU: diarrhea [0(0-1)]; leukocyte count 30.4 ± 13.4x10^3; intestinal damage [2.5(2-3)]; MPO 3788 ± 121; TNF-a 0.7 ± 0.2; IL-6 0.2 ± 2.3 or IRI: diarrhea [0(0-1)]; leukocyte count 49.2 ± 5.5x10^3; intestinal damage [1(0-2)]; MPO 3580 ± 1613; TNF-a 0.4 ± 0.2; IL-6 0.07 ± 0.05). **Discussion:** We developed a new experimental model of IM induced by the association of 5-FU + IRI in mice which involves the production of pro-inflammatory cytokines (TNF-α and IL-6). **Financial support**: CNPq/Capes/FUNCAP.

04.040

5-FU increases the ligature-induced alveolar bone loss in rats. Calcia TBB[1], Araújo VMA[1], Melo IM[2], Guimarães MV[2], Nogueira GHC[1], Ribeiro RA[1], Lima V[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Clínica Odontológica

Introduction: 5-fluorouracil (5-FU) is a pyrimidine used on several protocols of cancer chemotherapy, including gastrointestinal cancers, head and neck cancer, and breast cancer (Malet-Martino, M, *Curr. Med. Chem. Anti-Cancer Agents*, v.2, p.267, 2002). Recently, its role on bone loss has been studied, since this drug showed a inhibitory effect on bone mineral density (BMD) (Nadhanan, Am *J Physiol* Endocrinol Metab,v. 302, p.E1440, 2012). We aimed to investigate whether 5-FU increases the ligature-induced alveolar bone loss (ABL) in rats. **Methods:** The ABL was induced in rats by a nylon-3.0 thread around the left upper 2nd molar, and contralateral hemiarcade was used as control. Male Wistar rats (180-220 g) were divided in 4 groups, with 4-7 animals each, which received 0.9% NaCl solution or 5-FU (37.5, 75 or 150 mg/kg) 1 hour prior the ligature. At the 11th day, they were killed and maxillae and gingival tissues were removed. The ABL was analyzed through macroscopy (mm2) and myeloperoxidase activity (MPO). Systemically, body mass variation, leucogram, hepatic transaminases serum dosages and indexes of liver, kidney and spleen were also analyzed. The data were presented as mean ± standard error of the mean. Ethical aspects: Ethics Committee for Animal Use-UFC no 85/13. Results: The ligature caused intense ABL (Control: 6.5 ± 0.7) when compared to normal maxillae, followed by a significant increasing of MPO (Normal= 1.3 ± 0.2; Control: 5.0 ± 0.5; $p<0.05$). Although the lower doses did not alter the ABL, the major dose of 5-FU caused an intense increasing of the ABL ligature-induced, when compared to Control (5-FU 150 mg/kg= 10.2 ± 0.8). 5-FU at any doses did not prevent the increasing of the MPO observed in Control gingival (5-FU 37.5 mg/kg= 6.3 ± 0.7; 75 mg/kg= 5.3 ± 0.3; 150 mg/kg= 4.0 ± 0.5; $p>0.05$). Systemically, it was observed that 5-FU did not alter the organ indexes and the hepatic transaminases ($p>0.05$). However, at 11th d 5-FU 150 mg/kg induced a significant neutropenia (Normal= 2.3 ± 0.1; Control= 4.5 ± 0.7; 5-FU 150 mg/kg= 0.4 ± 0.1; $p<0.05$) and important weight loss, when compared to control rats ($p<0.05$). **Discussion:** These findings corroborate with the previously studies that 5-FU showed inhibitory effect on growth of long bones (Xian CJ, J Cell Biochem, v.99, 1688, 2006). 5-FU reduce bone mineral density and increases the incidence of bone fractures (Xian CJ, Bone, v.35, p.379, 2004). In this model, ABL was not related with the increased MPO when compared with control group. These results taken together suggest that 5-FU can increase ligature-induced alveolar bone loss. Immunossupression, represented as neutropenia, can be related with this major bone loss. **Financial support**: CNPq.

04.041

Anti-inflammatory activity of Camphor on acute inflammatory response. Silva-Filho SE[1], Silva-Comar FMS[1], Rocha BA[1], Aguiar RP[1], Ames FQ[1], Freitag AF[1], Spironello RA[1], Rodrigues PJ[1], Cardia GFE[1], Bersani-Amado CA[1], Cuman RKN[1] [1]UEM – Farmacologia e Terapêutica

Introduction: Camphor ($C_{10}H_{16}O$) (CAM) is both a natural and synthetic terpenoid ketone compound presents in *Cinnamomum camphora* and other plant species. It is a popular household remedy that is believed to act as an aphrodisiac, contraceptive, abortifacient, and lactation suppressor. Many studies performed with plants extracts which contain camphor as constituent have showed biological activities, such as: anti-inflammatory and antioxidant. There are several reports of toxicity with CAM, but there are only few studies involving CAM activities. Several studies have been performed with extracts and essential oils containing CAM, but not as isolated compound. In this work, we evaluated the anti-inflammatory effect of isolated CAM by ear edema induced by croton oil and the enzyme Myeloperoxidase (MPO) activity. **Methods:** CAM was purchased from Sigma-Aldrich (St. Louis, MO, USA). Male Swiss mice were treated orally with CAM (100, 200 or 400 mg/kg), hydroalcoholic vehicle containing 2% ethyl alcohol and 1% Tween as negative control, or dexamethasone (1 mg/kg) as reference drug, 60 minutes before inflammatory stimuli (croton oil, 5% v/v, dilutes in acetone). The croton oil was applied in the inner surface of the mice right ear, the left ear received an equal volume of acetone. Eight animals were used for each group. Four hours after, the ear weight was determined (edema volume). MPO activity was evaluated in the ear's homogenates supernatant sections. The ear tissue was placed in potassium phosphate buffer, pH 6.0 containing 0.5% hexadeciltrimethyl ammonium bromide in a homogenizer. The supernatant was added to a 96-wells microplate, followed by addition of a potassium phosphate buffer solution containing o-dianisidine dihydrochloride and 1% H_2O_2. The MPO activity was determined by optical absorbance (460 nm) and measured in an ELISA reader. Results were statistically analyzed by using one-way variance analysis (ANOVA) followed by Tukey's test ($p<0.05$*) The experimental protocols were approved by the Ethical Committee on Animal Experimentation of the UEM (CEAE/UEM 070/2012). **Results:** CAM by orally administration promoted a reduction in the ear edema at doses of 100 and 200 mg/kg (59.4% and 49.8%, respectively, $p<0.01$), similar to that induced by dexamethasone (64.3%) ($p<0.01$). However, Camphor at a dose of 400 mg/kg did not showed an antiedematogenic effect, since croton oil application increased the ear edema by 21%. Additionally, the results demonstrated that oral treatment with camphor significantly reduced MPO activity, a marker of neutrophil infiltration, at doses of 100 and 200 mg/kg (51.5% and 60.6%, respectively; $p<0.01$) whereas, dexamethasone significantly inhibited MPO activity by 57.6% ($p<0.01$). Camphor at a dose of 400 mg/kg increased MPO activity by 21%. **Discussion:** CAM showed an anti-inflammatory effect after oral administration at lower doses. However, at higher doses an irritative effect was observed. Treatment with CAM decreased topic ear edema formation and also the neutrophils infiltration probably by inhibiting the release of inflammatory mediators. Data suggested that CAM has an antiedematogenic activity and an inhibitory effect on leukocyte infiltration to inflamed tissues. **Financial Support:** CNPq; Capes; Fundação Araucária.

04.042

***In vivo* assays of ibuprofen intercalated in layered double hydroxide carrier**. Lima AB[1], Dias D[1], Anicete M[2], Nascimento JLM[3], Bastos GNT[1] [1]UFPA – Neuroinflamação, [2]UFPA – Planejamento e Desenvolvimento de Fármacos, [3]UFPA – Neuroquímica Molecular e Celular

Layered double hydroxides (LDHs) compound of magnesium and aluminum cations with ibuprofen anti-inflammatory intercaled has been successfully prepared by coprecipitation Method: Usually, these materials are applied to controlled release of drugs. The aim of this study was to assess the cytotoxicity of LDHs in blood cells and to evaluate the biodisponibility of ibuprofen (IBU) incorporated into LDHs (IBU-LDHs) using models of nociception. The ibuprofen was adsorbed to calcined material resulting in reduction of specific surface area, volume and pore diameter. For the *in vivo* test we used male albine Swiss mice (6-8 weeks). The experimental protocols were approved by the Ethical Committee for Research with Experimental Animals (CEPAE) of UFPA (CEPAE-UFPA: 124-13). For the test hemolysis and nociception test. It was evaluated the hemolytic effect of LDHs. The erythrocytes were isolated and purifed by three successive washes with saline. The cells were incubated with LDHs at 250µg/ml. Controls were prepared in the same manner as the above erythrocytes samples except adding Triton-X (positive control) and DMSO (negative control) instead of the LDHs. After 1 h incubation at room temperature, the samples were spun down for the detection of hemoglobin released from hemolyzed erythrocytes. The results showed no hemolytic activity using our LDHs. IBU-LDHs, IBU, LDHs or vehicle, were administered by gavage at 200 mg/kg. The drugs were administered 2, 24 or 48 hours before the i.p. injection of 0,6% acetic acid. The IBU-LDHs decreased the number of writhing in 97.2%, 87.66% and 61.33%, to 2, 24 or 48 hours respectively. While the IBU decreased the number of writhing only in 2 hours. The results of *in vivo* drug release revealed that the LDH-IBU compound is a perspectival material for potential application as controlled drug delivery systems due it keeps the antinociceptive effect up to 48 hours with just one dose. **Acknowledgements:** The authors are grateful to Ministry of Science and Technology (MCT/CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and UFPA for financial support

04.043

Evaluation of 15-deoxy-delta-12,14 Prostaglandin J_2 treatment on asthma changes triggered by house dust mite in A/J mice. Coutinho DS[1], Anjos-Valotta EA[2], E Silva PMR[1], Martins MA[1] [1]IOC-Fiocruz – Inflamação, [2]UEZO

Introduction: Asthma is a serious health and socioeconomic issue all over the world affecting more than 300 million individuals, with approximately 250,000 annual deaths. The disease is characterized by pulmonary inflammation, bronchial remodeling and airway hyperresponsiveness to unspecific stimulus. These pathological changes are induced by environment aeroallergens inhalation, such as house dust mites (HDM), leading to symptoms such as wheezing, coughing and breathlessness in asthmatic patients. The majority of patients with asthma can be treated effectively with inhaled corticosteroids and short- or long-term-β2 agonists. However, this treatment is limited by side effects and refractoriness exhibited by some patients, justifying the search for new therapies. In this context, 15-deoxy-delta-12,14,Prostagandin J_2 (15d-PGJ_2) an peroxisome proliferator activated receptor gamma (PPAR-γ) endogenous ligand, can be thought as a promising alternative. 15d-PGJ_2 is a cyclopentenone prostaglandin which exerts potent anti-inflammatory activity, in part, by antagonizing the activities of pro-inflammatory transcription factors, such as nuclear factor kappa B (NF-κB). The aim of this study was to investigate the therapeutic potential of 15d-PGJ_2 in a murine model of HDM-induced asthma. **Methods:** All protocols and experimental procedures involving animals were approved by the Committee of Laboratory Animals Use of the Oswaldo Cruz Foundation (license number P-37/10.5). A/J mice were intranasally challenged with HDM extract 3 times per week (wk) for 3 wks. Subcutaneous administration of 15d-PGJ_2 (30 and 100 μg/kg) occurred 30 minutes before challenges at the last wk, and analyzes were performed 24 hours after the last HDM challenge. **Results and discussion:** We found that all doses of 15d-PGJ_2 reduced ($p<0.05$) pulmonary eosinophil numbers as well as mucus production and peribronchial deposit of extracellular matrix components. We also found a significant inhibition of interleukin (IL) -5, IL-13, IL-17 and TNF-α levels in lung homogenate. Moreover, 100 μg/kg of 15d-PGJ_2, but not the lower dose, inhibited airway hyper-reactivity as attested by inhibition of both increased lung resistance and elastance triggered by aerosolized methacholine. Finally, 15d-PGJ_2 reduced allergen-induced NF-kB expression in lung tissue lysates. **Conclusions:** Our findings show that 15d-PGJ_2 can attenuate critical pathological features in mice challenged by nasal instillation of HDM extract, suggesting that this eicosanoid should be further investigated as a therapeutic alternative for controlling asthma. **Financial Support:** Capes, CNPq, Faperj, Fiocruz

04.044

Imunnomodulatory effect of low-intensity exercise after traumatic injury of the sciatic nerve in mice. Bobinski F[1], Teixeira JM[2], Sluka KA[3], Santos ARS[1] – [1]UFSC – Neurobiology of Pain and Inflammation, [2]Unicamp – Structural and Functional Biology, [3]UIOWA –Physical Therapy and Rehabilitation Science

Introduction: After peripheral nerve injury the Schwann cells and immune reaction illustrates the extension of the neuroinflammatory reaction from the nerve fibers undergoing Wallerian degeneration to other compartments of the nervous system. It has been suggested that moderate exercise may modulate the immune response in a variety of diseases. Thereby, the aim of this work was to investigate the potential immunomodulatory effect of low-intensity exercise on local immune response after peripheral nerve injury. **Methods:** All protocols were approved by Ethics Committee on Animal Use from UFSC (PP00745) and UIOWA (11102229). Swiss, BALB/cJ and IL-4 knockout (IL-$4^{-/-}$) male mice under anesthesia were submitted to sciatic nerve crushing for 30 s. Mice performed low-intensity exercise (10m/min, 30 min daily) for 2 weeks, beginning on day 3 postoperative (PO). To study whether the exercise was be able to act on immune systems and modulate the neuropathic pain, first, a set of animals (Swiss) were pretreated with saline (10 mL/kg, i.p.) or fucoidin (100 µg/mouse, i.p., an inhibitor of leukocyte rolling), 20 minutes before each exercise session and the mechanical hypersensitivity was measured using von Frey filaments (0,4g). Second, we measured the anti-inflammatory cytokines IL-4, IL-5 and IL-1RA levels by ELISA, on sciatic nerve, in the 14^{th} day PO after exercise treatment. Because we found that exercise increased IL-4 levels, in another set of experiments, we used IL-$4^{-/-}$ or wild-type (WT) BALB/cJ mice to verify the importance of IL-4 on the mechanical anti-hypersensitivity effect of physical exercise after crushing. Since we know that IL-4 can modulate the phenotypic profile of macrophages inducing differentiation into 'alternative' anti-inflammatory M2 macrophages, after crushing and exercise treatment, the IL-$4^{-/-}$ or WT mice were euthanized, and the sciatic nerve was removed to immunohistochemistry of macrophages (M1 and M2). **Results and discussion:** Pre-administration of fucoidin prevented the anti-hypersensitivity produced by exercise from 7-14^{th} postoperative day ($p<0.001$). This result show that migrated leukocytes from blood circulation are require to anti-hypersensitivity effect of physical exercise. We also found that exercise was able to increase the levels of anti-inflammatory cytokines IL-4 and IL-1RA ($p<0.05$), but not IL-5. Besides, we show that IL-$4^{-/-}$ Exercised mice had no hyponociceptive effect when compared to WT Exercised mice ($p<0.01$). Then, we have shown that IL-4 can modulate the phenotypic profile of macrophages because, WT Exercised mice showed M2 macrophage staining, while IL-$4^{-/-}$ Exercised mice demonstrated an inverse profile, with more M1 macrophage staining ($p<0.001$). These findings provide new evidence that physical exercise produces a phenotypic switch in macrophages, increasing the release of IL-4, which may induce a Th2 response and reduce pain and inflammation. **Acknowledgments:** Capes, CNPq, UFSC and UIOWA.

04.045

Thymol isolated of *Thymus vulgaris* essential oil inhibit *in vivo* leukocyte behavior during acute inflammatory response. Aguiar RP[1], Bastos RL[1], Silva-Comar FMS[1], Silva-Filho SE[1], Wiirzler LAM[1], Rocha BA[1], Ames FQ[1], Bersani-Amado CA[1], Cuman RKN[1] [1]UEM – Farmacologia e Terapêutica

Introduction: *Thymus vulgaris* essential oil (TEO) is a mixture of monoterpenes. The main compounds of this oil are the natural terpenoid thymol and carvacrol which have antioxidative, antimicrobial, antitussive, expectorant, antispasmodic, and antibacterial effects. The aim of this study was to evaluate the effect of Thymol isolated of *Thymus vulgaris* essential oil on leukocyte behavior (rolling and adhesion) using an *in vivo* intravital microscopy model. **Methods:** The essential oil was extracted from fresh leaves by conventional steam distillation using a Clevenger-type apparatus. Thymol was isolated from TEO as fractions of hydrodistillated oil. Essential oil analysis and chemical identification were performed by Gas Chromatography-Mass Spectrometry (GC/MS) and Nuclear Magnetic Resonance (NMR). The leukocyte behavior was studied by *in vivo* intravital microscopy technique. Male balb/c mice were treated orally with Thymol (25, 50 or 100 mg/kg), vehicle (1%Tween 80 solution) as a negative control, or indomethacin (5 mg/kg) as a reference drug, 60 minutes before carrageenan intraperitoneal injection (500µg/cavity). Two hours after the injection the mice were anesthetized with an intramuscular injection of ketamine/xylazine (1:1 10 + 10µl/10g body weight) solution and the mesentery was exposed. The number of leukocytes rolling and adhered to the endothelium was determined at 10 minutes count in a post-capillary venule with 100µm length. The protocol was approved by the Ethic Committee for Animal Experimentation of the UEM (CAEA/UEM 066/2010). The results (mean ± SEM) were statistically analyzed using ANOVA followed by Turkey's test. Statistical significance was set at $P<0.05$ **Results and discussion:** The carrageenan injection significantly increased leukocytes rolling and leukocytes adhesion (58.22 ± 3.67 rolling/min and 0.5078 ± 0.0457 cells/100µm^2, respectively) to the vascular endothelium 2h after the stimulation, when compared to that of mice pretreated with an i.p. injection of saline (21.27 ± 1.12 rolling/min and 0.1917 ± 0.0332 cells/100µm^2, respectively). A significant reduction of leukocytes rolling was observed after the treatment with Thymol at doses of 25 and 50 mg/kg (24.64 ± 1.09 and 34.37 ± 1.65rolling/min, respectively) and indomethacin (26.76 ± 2.35 rolling/min). However, a leukocyte adhesion decreased was only observed at indomethacin group (0.3000 ± 0.0204 cells/100µm^2). Data showed that Thymol has an inhibitory effect on the initial stages of leukocyte migration, evaluated by the rolling behavior, but not leukocyte adhesion. Our data suggest an anti-inflammatory effect of Thymol by inhibition of leukocytes chemotaxis. **Sources of research support:** CNPQ; Capes; Fundação Araucária. **References:** Asbaghian S *et al*., *Nat. Prod. Comm*., 137, 6, 2011. Höferl M *et al*., *J of Essent. Oil. Res*., 459, 21, 2009.

04.046

The role of D6 receptor in pulmonary fungal infections: Insights in chemokine signalling in a model of aspergillosis. Moura TR[1], Machado ALC[1], Sucupira PHF[1], Rachid MA[2], Teixeira MM[3], Russo RC[4], Soriani FM[1] [1]UFMG – Biologia Geral, [2]UFMG – Patologia, [3]UFMG – Bioquímica e Imunologia, [4]UFMG – Fisiologia

Introduction: Chemokines form an important system for leukocyte recruitment and are regulated by decoy receptors which scavenge CC-type chemokines. D6 is an important atypical receptor during inflammatory diseases, but its role during fungal infections is poorly understood. The aim of this study is to characterize the role of D6 receptor during *Aspergillus fumigatus* (*Afu*) lung infection. **Methods:** Wild type (C57BL/6) and *D6*$^{-/-}$ mice were intranasally infected with 10^8 spores of *Afu* wild type strain. Lungs and BALFs were collected after 1 and 2 days post infection (dpi) (Ethics: 62/2011). **Results:** Results show that *D6*$^{-/-}$ mice had an increased mortality compared to WT mice, associated with higher fungal load in lungs after 1 dpi compared to WT mice (2.4 ± 0.6x10^6 vs 1.2 ± 0.1x10^6 UFC/left lung). This was associated with higher cellular infiltrates to the lungs of *D6*$^{-/-}$ mice, with high levels of neutrophils (4.2 ± 0.1 vs 1.7 ± 0.2x10^6 cells/BALF in WT mice) and macrophages (9.8 ± 0.8 vs 3.5 ± 0.4x10^5 cells/BALF in WT mice) after 1 dpi. Analysis of airways levels of CC chemokines in *D6*$^{-/-}$ mice show a possible kinetics of chemokine signaling during *Afu* infection with an earlier 2-fold increase of CCL2/MCP-1 followed by 3-fold increase of CCL5/RANTES and a 37-fold increase of CCL3/MIP-1α after 2 dpi. In order to characterize the role of CCL5/RANTES in *Afu* infection, we treated D6$^{-/-}$ mice with the CCR1/CCR5 antagonist met-RANTES. Results show that the antagonism of CCL5 signaling totally reversed the lethality phenotype. Moreover, this reversion is associated with lower cellular infiltrates in the airways (0.99 ± 0.15x10^6 cells/BALF in 8 mg/kg/day met-RANTES vs 3.05 ± 0.61x10^6 in untreated D6$^{-/-}$ group), especially represented by neutrophils (0.47 ± 0.20x10^6 cells/BALF in 8 mg/kg/day met-RANTES vs 2.66 ± 0.54x10^6 in untreated D6$^{-/-}$ group). These findings suggest that, during *Afu* lung infection, chemokine signaling is important for cell influx and D6 receptor plays important role controlling chemokine levels, especially, CCL5/RANTES, and preventing inflammation tissue damage. **Financial Support:** FAPEMIG, CNPq, Capes.

04.047

Staphylococcal enterotoxin type A (SEA) and B (SEB) exhibits an inhibitory effect on mice bone marrow neutrophils adhesion *in vitro.* Ferreira Duarte AP[1], Pinheiro Torres AS[1], De Souza IA[1], Mello GC[2], Antunes E[2], Anhê F G[2] [1]FMJ – Biologia e Fisiologia, [2]Unicamp – Farmacologia

Background: We have described that SEA and SEB mice airways exposition aggravate the pulmonary inflammation by exacerbation of lung eosinophils and neutrophils infiltration with increased bone marrow granulopoesis. These results are in accordance with several clinical evidences for a strong correlation between SEA and SEB and human respiratory disease exacerbation. In the present study we evaluated the effect of the incubation of bone marrow neutrophils from naïve animals with SEA and SEB on *in vitro* adhesion induced by FMLp and interleukin-8 (IL-8). **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 x 10^6 cells/ml. Bone marrow neutrophils were incubated *in vitro* with different doses of SEA or SEB and by different time intervals before the adhesion assays. Adhesion assays were carried out in 96-well plates pre-coated with recombinant mouse ICAM-1 (2.5 µg/ml) for 30 min in the presence of FMLp (10^{-8} M) or IL-8 (200 ng/ml). The bone marrow neutrophils adhesion was calculated by measuring neutrophil myeloperoxidase activity (MPO) on adherent cells. **Results:** Bone marrow neutrophils from naïve mice incubated *in vitro* with SEA or SEB for 2 h exhibited reduced adhesive response when stimulated by FMLp or IL-8 (Spontaneous Adhesion: 1.9 ± 0.2; FMLp: 3.2 ± 0.1*; SEA + FMLp: 2.5 ± 0.1*#; SEB + FMLp: 2.7 ± 0.1*#; IL-8: 3.3 ± 0.3*; SEA + IL-8: 2.2 ± 0.3*#; SEB + IL-8: 1.7 ± 0.3*# OD/NE× 10^6 cells). **Conclusion:** The inhibitory effect of SEA and SEB on bone marrow neutrophils *in vitro* adhesion suggests a role of these toxins on *downregulation* of bone marrow neutrophils surface adhesion molecules and can represent an important advance to clarify the mechanisms involved on the association between *Staphylococcus aureus* infections and respiratory diseases exacerbation. Ethical Committee for Animal Experimentation Approvment: 357-1; **Financial Support:** Fundação de Amparo a Pesquisa do Estado de São Paulo (2009/16522-0; 2012/05561-8).

04.048

Closed-spring placement promotes induced tooth movement with maximum at fourth day. Araújo VMA[1], Soares KA[2], Melo IM[3], Guimarães MV[3], Calcia TBB[1], Kurita BM[3], Lima V[1] [1]UFC – Physiology and Pharmacology, [2]UFC / UFRJ – Morphology, [3]UFC –Pharmacy, Dentistry and Nursing

Introduction: Closed-spring induced tooth movement (ITM) involves inflammation, and both bone resorption and formation, where, the side of compression is characterized by constriction of the periodontal ligament (PL) with areas of hyalinization, while the side of tension is characterized as osteogenic areas, respectively (Wise, GE; J Dent Res, v.87, p.414, 2008), varying according to the time course. The aim of this study was to identify the time of onset of both processes of bone resorption and formation. **Methods:** ITM was induced in 36 male *Wistar* rats (180-220 g) by a nickel-titanium closed-spring fixed with wire ligature in the upper left first molar and upper incisors, with contralateral right side as control. Groups of 6 rats were killed on the days 1, 4, 7, 11, 14 and 21. In these times, measurements of the distance between the palatal surface of the upper incisors and the mesial surface of the left and the right upper molars. In each time, the maxillae were removed for histometric analysis, degree of the reduction or increase in the PL, and formation of hyaline areas. Ethical aspects: Ethics Committee for Animal Use-UFC nº 21/14. **Results:** In the macroscopic analysis, a significant reduction of the distance between the left upper molars and incisors was observed from the 4th up to 21thd (Normal=0.0 ± 0.0; 1d=0.6 ± 0.6; 4d=3.9 ± 0.8; 7d=5.8 ± 0.7; 11d=7.3 ± 1.3; 14d=7.9 ± 1.0; 21d=8.9 ± 0.7). Corroborating this findings, the histometric study of the PL showed a reduction ($p<0.05$) in the area in the compression side in the 1st and 4th days, when compared to the Normal group (Normal=32.2 ± 0.9; 1d=26.5 ± 1.7; 4d=23.7 ± 1.2), while from the day 7 up to day 21 the areas were similar to Normal (7d=29.6 ± 1.5; 11d=29.3 ± 2.8; 14d=28.3 ± 1.7; 21d=28.7 ± 2.0, $p>0.05$). On the other hand, on tension side there was a significant increase of PL area from 1std up to 7thd (Normal=35.2 ± 0.9; 1d=44.1 ± 2.1; 4d=46.8 ± 4.6; 7d=47.3 ± 5.3, $p<0.05$). The 11thd forward, there was no difference when compared to Normal (11d=37.3 ± 3.4; 14d=34.6 ± 3.3; 21d=34.1 ± 1.7, $p>0.05$). About the hyaline areas, ITM promoted increasing from the 1std, reaching a maximum on 4thd (1d=7.9 ± 1.0; 4d=11.8 ± 1.4, $p<0.05$). Comparing to the 4thd, from the 7thd up to 21thd these areas were reduced ($p<0.05$) (7d=5.6 ± 1.4; 11d=4.7 ± 1.7; 14d=2.1 ± 0.2; 21d=1.9 ± 0.2). **Discussion:** These results are in agreement with others findings, which was observed that hyaline areas had maximum expression up to 5thd (Fracalossi, ANC; Dental Press, v.43, p. 143, 2009). The smallest area on the compression side opposite to the side of larger area corresponded to the tensile force made by the spring, and the changes observed after 7 and 11 days corresponded to the course bone remodeling. These results taken together indicate that the 4thd is the time of onset of both bone resorption and formation. **Financial support**: CNPq.

04.049

Protective effect of rutin on experimental acute pancreatitis in mice. Abreu FA[1], Santana MS[1], Souza ACA[1], Oliveira JP[1], Teixeira SA[2], Muscará M[2], Costa SKP[2], Camargo EA[1] [1]UFS – Physiology, [2]ICB-USP

Introduction: Acute pancreatitis (AP) is a severe disease that comes to be about 20% of patients who develop, causing hospitalization and death. The treatment of this condition is still insufficient to control the intrinsic inflammatory process and is focused on managing the complications and symptoms of patients. Among the many factors involved in AP, the inflammatory response and oxidative stress can be highlighted. In this context, rutin is a natural flavonoid with potential to treat AP, by considering its anti-inflammatory and antioxidant activities. The aim of this study is to investigate the possible protective effects of rutin on experimental AP induced by L-arginine administration in mice. **Methods and Results:** Adult male Swiss mice (n=6-7), were used in this study and all experiments were approved by this institution's Ethics Committee in Animal Research (43/2012). For the induction of AP, mice received 2 injections of L-arginine (8%, 4 g/kg, i.p., with an interval of 1 h). The control group received the same volume of saline (0.9%) instead of L-arginine. Mice submitted to AP induction were treated with rutin (75 mg/kg, p.o.) or vehicle (saline) after 24, 36, 48 and 60 h of the first injection of L-arginine. The control group received vehicle at the same time points. After 72 hours of the first L-arginine injection, the serum concentrations of amylase and IL-6, as well as pancreatic myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were measured. Pancreatic of 3-nitrotyrosine immunoexpression was also determined. Data were expressed as mean ± SEM and analyzed by one-way ANOVA/Bonferroni's test. Injection of L-arginine induced acute pancreatitis in mice, characterized by increased serum amylase ($p<0.001$) and IL-6 ($p<0.001$) concentration and pancreatic MPO ($p<0.001$) in comparison with saline-injected group. Treatment with rutin reduced ($p<0.001$) the serum concentration of amylase (403.80 ± 29.92 U/L) and IL-6 (9.29 ± 0.24 pg/mL), when compared with vehicle-treated group (1223.00 ± 153.80 U/L and 36.04 ± 4.33 pg/mL, respectively). The pancreatic MPO activity was also inhibited ($p<0.001$) by the treatment with rutin (0.18 ± 0.06 UMPO/mg of tissue), when compared with the control group (1.84 ± 0.26 UMPO/mg of tissue). The administration of rutin (75 mg/kg) increased pancreatic CAT (20.52 ± 1.43 UCAT/mg of protein; $p<0.001$), SOD (20.13 ± 0.94 mUSOD/mg of protein; $p<0.01$) and GSH-Px (15.63 ± 1.42 umolGSH/min/mg of protein; $p<0.05$) activities, when compared with vehicle-treated group (6.04 ± 1.11 UCAT/mg of protein; 13.75 ± 0.49 mUSOD/mg of protein and 11.01 ± 0.67 umol GSH/min/mg of protein, respectively), while decreased ($p<0.05$) the of 3-nitrotyrosine immunoexpression (174.20 ± 11.21) in comparison to vehicle-treated group (249.80 ± 29.87). **Conclusion:** These results show that rutin exerts anti-inflammatory and antioxidant effects during PA induced by L-arginine, which are suggestive that this flavonoid is of interest for developing future studies or approaches focused on new alternatives to treat AP in humans. **Financial support:** FAPITEC/SE, FUNTEC and CNPq.

04.050

Friedelin attenuates allergic airway inflammation in allergen-induced mouse asthma. Ferro JNS, Aquino FLT, Silva JPN, Santos SL, Conserva LM, Barreto EO UFAL

Introduction: Friedelin, a pentacyclic triterpene, has been reported to exhibit antinociceptive and antioxidant properties *in vivo* and *in vitro*. Previously, we found that friedelin suppressed LPS-induced pleural inflammation (Ferro *et al*., 2013). However, the effect of friedelin in the allergic inflammation remains unknown. The aim of the present study was to investigate the effects of friedelin on airway allergic inflammation in ovalbumin-induced mice asthma. **Methods**: Male Swiss mice were sensitized with ovalbumin (OVA, 50 µg) (day 0) and boosted (on days 7 and 14) subcutaneously with OVA (50 µg). At day 21, 22 and 23, mice were challenged with instillation OVA (1% in PBS) being the inflammatory parameters evaluated 48 h after the last OVA-challenge. Friedelin was administered by intraperitoneal route at a dose of 1, 10, and 50 mg/kg body weight 1h before each OVA challenge. At 48 h after the last challenge, bronchoalveolar lavage (BAL) was performed to analyze the counts of total and differential cells present in the airways. The amount of interleukin-5 (IL-5) in the BAL fluid was quantified by ELISA. In order to identify the changes induced by the antigenic provocation, lung segments were processed for H&E and PAS staining being histology was evaluated by light optical microscopy. This study was approved by Ethics Committee on Animal Use of UFAL (CEUA, License no. 43/2103). The results were statistically analyzed using one-way ANOVA followed by Neuman-Keuls-Student test. The difference were considered significant at $P<0.05$. **Results and discussion:** The results revealed that pretreatment with friedelin inhibited recruitment of total cells and eosinophils into airway, and decreased the levels of IL-5 in BALF. Moreover, pathologic changes of lung tissue induced by OVA such as infiltrates of inflammatory cells and goblet cell hyperplasia were inhibited by the pretreatment of friedelina. Thus, these results suggest that friedelin may be effective as alternative treatment for allergic airway inflammation by virtue of its anti-inflammatory activity. **Financial support**: CNPq, Capes and FAPEAL. **References:** Ferro JNS *et al*., In 11º World Congress Inflammation, 2013.

04.051

Free heme activates mice mesothelial cells: role of NADPHox in inflammatory response.

Candea ALP[1], Simões RL[2], Vergara FMF[1], Pereira-Ribeiro C[2], Arruda MA[2], Barja-Fidalgo TC[2], Henriques MGMO[1] [1]Farmanguinhos-Fiocruz – Farmacologia Aplicada, [2]UERJ – Farmacologia Celular e Molecular

Heme, a ubiquitous iron-containing compound, is present in large amounts in many cell types and is particularly dangerous when it escapes from intracellular compartment. The release of heme from damaged cells and tissues is supposed to be higher in diseases such as hemolytic anemia or in trauma, hemorrhage and pleural effusion. Pleural effusion is a common clinical manifestation that is associated with the presence of a variety of mediators in pleural fluid and neutrophilia. This neutrophilia is mediated by many chemokines/cytokines such as TNFα and (CXCL1) KC. Mesothelial cells (MC) are metabolically active cells that line the pleural cavity as a continuous monolayer, and are able to release several chemokines after inflammatory stimuli. Heme is a proinflammatory molecule that causes release of many cytokines/chemokines from mesothelial cells, an effect that likely contributes to chronic inflammation associated with hemolytic diseases. In this study, we aimed to determine the role of heme-induced NADPHox activity in mesothelial cells and their cytokine production.

All procedures were approved by the Committee for Animal Care and Use (CEUA-Fiocruz) under L0052/12 license. The mice pleural mesothelial cells were incubated or not with NADPHox inhibitors, like Apocinin (10μM) and DPI (10μM) for 15 minutes, then challenged with heme (10μM) for 24h thereafter. The cell-free supernatant has been collected for analysis of cytokine/chemokine production by ELISA and the cells were harvested for the RT-PCR analysis. Heme significantly increased KC (700% $p \geq 0,05$) and TNF-alpha (120% $p \geq 0,05$) production 24h after stimulus. Pre-treatment with Apocinin or DPI was able to decrease KC and TNF-alpha release as observed after ELISA. The RT-PCR analysis confirmed an increase in TNF-alpha and KC expression in heme-stimulated mesothelial cells, as well as a significant KC reduction in DPI pretreated cells (13 ± 0.9 to 0.2 ± 0.01 ΔCt related to control) whereas the expression of TNF-alpha mRNA presented only a slight decrease ($2,45 \pm 0.3$ to 1.89 ± 0.1 ΔCt related to control).

Our data showed that heme induces KC and TNF-alpha release in a NADPHox-dependent manner, which is corroborated by the mRNA cytokine expression. Thus, heme-stimulated mesothelial cells may contribute to neutrophil migration in pleural inflammatory process.

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04.052

The role of *Aspergillus fumigatus* phosphatase-calcineurin in the modulation of immune response in a model of aspergillosis. Machado ALC[1], Rocha WV[1], Moura TR[1], Teixeira MM[2], Rachid MA[3], Soriani FM[1] [1]UFMG – Biologia Geral, [2]UFMG – Bioquímica e Imunologia, [3]UFMG – Patologia

Introduction: In *Aspergillus fumigatus* (*Afu*), calcium homeostasis has been described as a key event in the control of biological processes and survival of this fungus, especially during infection. Calcium signaling is mediated by the phosphatase calcineurin that controls the expression of different genes involved in stress adaptation. In this sense, *Afu* calcineurin is critical for hyphal growth, tissue invasion and pathogenicity. Moreover, the null mutant (*ΔcalA*) showed changes in cell wall composition, regarding to galactomannan levels. These phenotypes, together with evidences that C-type lectin receptors are important for *Afu* recognition and immune response, put calcineurin signaling as one of the key factors modulating host-pathogen interactions, during Aspergillosis. The aim of this study is to characterize host-pathogen interactions settled by *ΔcalA* strain during *Afu* lung infection. **Methods:** Balb/C mice were intranasally infected with 10^8 spores of *Afu* wild type (WT) and *ΔcalA* strains. Lungs and BALFs were collected after 1 and 3 days post infection (dpi). (Ethics: 62/2011). Results: Results show that *ΔcalA* infected mice had increased survival associated with higher inflammatory scores. This phenotype is accompanied by higher cellular infiltrates in the airways at 1 dpi (3.79 ± 0.36 vs 1.77 ± 0.35x10^6 cells/BALF in WT *Afu* infected mice), mainly represented by neutrophils (2.9 ± 0.28 vs 0.6 ± 0.08x10^6 cells/BALF in WT *Afu* infected mice). Analysis of airways levels of chemokines and cytokines revealed, despite the cellularity, a low activation of inflammatory response in *ΔcalA* infected mice with 28, 2 and 1.6 times lower levels of IL-1β, TNF-α and CXCL1, respectively, after 1 dpi; and a 4.3-fold decrease in IFN-γ levels after 3 dpi. Conclusion: These findings corroborate previous results of lower virulence of *ΔcalA* mutant and demonstrate that *Afu* cell wall composition, controlled by calcineurin activity, represented by different PAMPs responsible for pathogen recognition, is important for the inset of immune response during lung infection. These results can also contribute for the understanding of immune responses in fungal lung infections. **Financial support**: FAPEMIG, CNPq, Capes.

04.053

Effect anti-inflammatory of crude extract *Cecropia obtusa* in a rheumatoid arthritis model in mice. Beck VR[1], Fiuza TL[2], Hamann F[3], Rigo F[4], Rubin M[2], Sauzem PD[5] [1]UFSM – Farmacologia, [2]UFSM – Bioquímica Toxicologica, [3]UFSM – Farmácia, [4]Santa Casa BH, [5]Unipampa

Previous studies have shown that the genus Cecropia has effect anti-inflammatory, analgesic and antiulcer. In Brazilian traditional medicine that genre is used as healing, analgesic and anti-inflammatory. Considering the high incidence of inflammatory and painful processes as well as the difficulty of finding effective drugs with fewer adverse effects, it becomes important to investigate new therapeutic alternatives. The objective was to evaluate anti-inflammatory and analgesic effect of *Cecropia obtusa* in a model of rheumatoid arthritis induced by administration of complete Freund's adjuvant (CFA) in mice. **Methods:** The experiments were conducted on adult male Swiss mice (25-35g). Model of rheumatoid arthritis induced by intra-articular injection of CFA in the ankle (tibio-tarsal) of mice. We injected 20 µl of vehicle (saline) or 20 µl of CFA in the ankle of mice anesthetized with isoflurane. To evaluate the behavior of hyperalgesia, animals were acclimated and mechanical threshold was determined before and after intra-articular CFA/salina injection, through the von Frey filaments using the Up-and- Down Method: The animals were treated with the crude extract of *C. obtusa* (30, 100, 300 mg/kg) in a single administration (acute treatment) or treated (300 mg/kg) for 7, 14 or 28, once a day, to verify the effect of cumulative treatment. To investigate the possibility of the development of effects of muscle relaxants and sedative of treatment, all animals were assessed in the open field test. After acute or cumulative treatment, mice were euthanized and joint tissue was removed for measurement of inflammatory and anti-inflammatory cytokines. Serum was collected for renal and hepatic biochemical measurements. This project was approved by the ethics of animal use committee – UFSM: n° protocol 116/2013. **Results:** The acute treatment with single dose of 300 mg/kg was able to reverse nociception caused by intra-articular injection of CFA at 1, 2 and 4 hours after treatment, a single dose of 100 mg/kg was able attenuate nociception at 1 and 2 hours, but the dose of 30 mg/kg wasn't able to reverse the mechanical hyperalgesia. The treatment of mice for 7 days at dose of 300 mg/kg was able to reverse the nociception CFA-induced at 1, 2 and 4 hours. Furthermore, the cumulative treatment during 14 or 28 days was able to reverse the mechanical hyperalgesia at 1, 2, 4 and 6 hours. The cumulative treatment for 7, 14 and 28 days was able to reverse the increase of inflammatory cytokines (IL-1, IL-6, TNF-alfa, INF-gama) induced by CFA. Furthermore, these treatments reversed the decline of IL-10 (anti-inflammatory) caused by the CFA. The extract did not cause changes in hepatic and renal parameters. In the open field test was not observed changes in locomotor activity. **Discussion:** The crude extract of *C. obtusa* at a dose of 300 mg/kg was able to reverse nociception, both in the acute treatment as the cumulative. Moreover showed anti-inflammatory activity, due to decreased levels of inflammatory cytokines and increased levels of anti-inflammatory cytokine. The data found in this study are in agreement with the popular use, being important to carry out further studies with this plant. **Financial Support:**Capes, CNPQ and FAPERGS

04.054

Dyspeptic symptoms, gastric emptying, ghrelin and leptin serum levels in inflammatory bowel diseases patients. Sales KMO[1], Cavalcanti RF[1], Santos AA[1], Braga LLBCB[1], Oliveira RB[3], Castro M[2], Souza MHLP[1] [1]UFC – Physiology and Pharmacology, [2]FMRP-USP

Background: Animals and humans studies have demonstrated that the inflammation may trigger gastrointestinal motility disorders. We demonstrated that delayed gastric emptying in inactive Crohn disease was associated with dyspeptic symptoms. Serum ghrelin levels are elevated in active Crohńs patients and could modify the gastrintestinal motility. However, the association with serum ghrelin and leptin levels, dyspeptic symptoms and gastric emptying was not totally defined.AIM: To investigate the correlation between the dyspeptic symptoms, gastric emptying, ghrelin and leptin in inflammatory bowel disease. **Methods:** Fourteen patients with Crohńs disease (mean age: 42, 43% female) and thirteen patients with ulcerative colitis(mean age: 45, 77% female) were evaluated. The presence of dyspeptic symptoms was evaluated by The Porto Alegre Dyspeptic Symptoms Questionnaire (PADYQ), the gastric emptying was measured by breath test using 13C octanoic acid coupled to a solid meal, and ghrelin or leptin fasting serum levels were determined by a commercially RIA kit. Results: There were notstatistical differences between age, gender and mesalazine use in the patients with Crohn and ulcerative colitis. Crohn patients have more (p= 0.0214) dyspeptic symptoms (12.86 ± 3.176) then patients with ulcerative colitis (4.769 ± 1.939), however there were not statistical difference in gastric emptying between patients with Crohn's disease (t1/2=256.7 ± 19.44, t lag= 144.9 ± 8.713) and ulcerative colitis (t1/2= 232.1 ± 20.65, tlag= 151.9 ± 10.21). Ghrelin, but not leptin, levels were increased (p=0.0006) in ulcerative colitis (39.85 ± 4.888) then in Crohn (19.90 ± 2.725). It was observed the linear correlation between the upper abdominal bloating and dyspeptic symptoms in Crohńs patients (p=0.0421; r=0,228). Conclusion: Patients with Crohńs disease have more dyspeptic symptoms then ulcerative colitis patients. These symptoms did not correlate with a delay in gastric emptying, but was associated with lower serum ghrelin levels. **Financial support**: Capes, CNPq.
CÓDIGO CEP: 045.04.11

04.055

Plasmin induces macrophage recruitment and reprogramming. Ribeiro ALC[1], Carmo AAF[2], Costa BRC[1], Vago JP[2], Garcia CC[3], Teixeira MM[3], Sousa LP[1] [1]UFMG – Análises Clínicas e Toxicológicas, [2]UFMG – Biologia Celular, [3]UFMG – Bioquímica e Imunologia

Introduction: Besides acting in fibrinolysis, the plasminogen/plasmin (Plg/Pla) system components have been shown to play a central role in cell migration, and therefore could regulate the inflammatory response. Although the capacity of Pla to induce cell migration is well known, the profiles of recruited cells are undefined. In this study, we investigate the ability of Pla and Plg to induce leukocyte migration to the pleural cavity and the profile of recruited cells, as well the capacity to enhance the efferocytotic process, which is essential in the resolution of inflammation. **Methods:** BALB/C mice were challenged by intrapleural injection of Pla or Plg (2µg/cavity) or PBS. Pleural cavity cells were harvested at 24 and 48h after injection and processed for total and differential leukocyte counts. Flow cytometry analysis was performed to detect the profile of mononuclear recruited cells. An *in vitro* efferocytosis assay was performed with intraperitoneal murine macrophages challenged with Pla, Plg or PBS and apoptotic human neutrophils, and the efferocytosis index was obtained. This study was approved by the Ethics Committee in Animal Experimentation (CETEA/UFMG), protocol number 19/2011 **Results:** Intrapleural injection of Pla induced a time-dependent influx of leukocytes into the pleural cavity of mice that was increased at 24 and 48 h. The recruited cells were almost entirely mononuclear cells without any significant modification in neutrophil numbers. Interestingly, Plg was also able to promote leukocyte recruitment to the pleural cavity in the same manner as Pla. Flow cytometry analysis using several cell markers, including CD45, F4/80, GR1 and CD11b, to determine the profile of the recruited cells, showed that the recruited macrophages are of anti-inflammatory (M2 – $Gr1^{-}$ $F480^{high}$ e $CD11b^{high}$) and resolving profiles (Mres-$F4/80^{med}$ $CD11b^{low}$). Furthermore, Plg and Pla increased the efferocytic capability of murine macrophages. **Discussion:** Pla injection promotes recruitment of M2 and Mres macrophages into the pleural cavity and increases effeocytosis. Therefore, Pla can induce migration of macrophages and enhance it efferocytic capability, involved in an anti-inflammatory and resolving process, which might promote the resolution of the inflammatory response. **Key-words:** Plasminogen system, leukocyte migration, inflammation. **Financial Support:** CNPq, PRPq-UFMG and FAPEMIG

04.056

Anti-inflammatory effects of cinnamaldehyde treatment in sepsis. Mendes SJF[1], Sousa FIAB[1], Ferro TAF[1], Silva BLR[1], Pereira ICP[1], Falcai A[1], Grisotto MAG[1], Brain SD[2], Fernandes ES[1,2] [1]Ceuma, [2]King's College

Introduction: Sepsis is a potentially fatal condition and affects thousands of people annually (Rittirsch *et al.*, 2007). We have been studying the effects of natural products on sepsis. Indeed, cinnamon bark has been used by the popular medicine as anti-inflammatory, analgesic and antipyretic; and evidences have suggested an antimicrobial effect for cinnamaldehyde (CINN), a compound present in cinnamon bark (Chang *et al.*, 2001). Recently, a protective role for Transient Receptor Vanilloid 1 (TRPV1) was suggested in bacteria-induced sepsis. Cinnamaldehyde was shown to activate transient receptor potential ankyrin 1 (TRPA1), a non-selective cation channel found on neuronal and non-neuronal cells co-localized to TRPV1 (Fernandes *et al.*, 2012). Herein, we evaluated the anti-inflammatory properties of CINN in a murine model of sepsis induced by LPS. **Methods and Materials:** All procedures were conducted in accordance with the Brazilian laws and were approved by the Ethics Committee of the University CEUMA (Protocol 273/12). Sepsis was induced in Swiss mice (2-month old), by an intraperitoneal injection of LPS (*E. coli*, serotype 111:B4; 11.25 millions EU/kg). CINN was given orally (250 mg/kg, n=8), 1h prior to sepsis induction. Vehicle-treated animals were used as controls (n=8). Mice were culled 4h following LPS-injection and the peritoneal lavage and plasma were collected for analysis. Total and differential cell counting was evaluated and organ damage markers were quantified. Statistical comparisons of the data were performed by unpaired t test. The p values <0.05 were considered significant. The results are presented as the mean ± standard deviation (SEM). The percentages of inhibition are reported as mean ± SEM of inhibitions obtained in each individual experiment compared with control samples. **Results and discussion:** CINN was previously suggested to present anti-inflammatory properties. Indeed, CINN is able to reduce TNFa and nitric oxide release from macrophages stimulated by LPS *in vitro* (Kim *et al.*, 2010). We found that CINN significantly reduces lipase (21 ± 6%) and creatinine (59 ± 14%) levels (indicatives of liver and kidney failure, respectively) in LPS-treated animals. Also, septic mice treated with CINN presented with a 7-fold increase in the number of peritoneal macrophages when compared to vehicle-treated controls (mean ± SEM values are as follows: vehicle-treated group 0.04 ± 0.01 $x10^6$ cells; CINN-treated group 0.27 ± 0.08 $x10^6$ cells). Also, a 3-fold decrease was noticed in the number of PI^+ (propidium iodide-permeable cells) peritoneal $CD14^+$ -expressing cells obtained from CINN-treated when compared to vehicle-treated mice challenged with LPS, suggesting CINN prevents macrophage apoptosis. Overall, we show additional mechanisms underlying the anti-inflammatory actions of CINN and newer evidence on its potential to prevent organ failure in sepsis. This research was funded by FAPEMA, CNPq and Capes. **References**: Chang ST, Chen PF, Chang SC.J *Ethnopharmacol* 2001; 77(1):123-127. Fernandes, *et al.*, *Br J Pharmacol*, 166: 510, 2012. Kim, *et al.*, 2010; *Mediators Inflamm*, doi: 10.1155/2010/529359. Rittirsch *et al.*, 2007; *J Leukoc Biol*, 81: 137-413.

04.057

TRPV1 antagonism by capsazepine modulates innate immune response in mice infected with *Plasmodium berghei* Anka. Fernandes ES[1,2], Brito CXL[1], Teixeira SA[3], Barboza R[4], dos Reis AS[3], Azevedo-Santos APS[5], Muscará M[3], Costa SKP[3], Marinho CRF[3], Brain SD[2], Grisotto MAG[1,6] [1]Ceuma, [2]King's College – Cardiovascular Division, [3]USP, [4]Unifesp, [5]UFMA, [6]Instituto Florence

Introduction: Thousands of people die of severe malaria every year. Innate immune response plays a determinant role in host's defence to malaria (WHO, 2013). TRPV1 is suggested to modulate macrophage-mediated responses in sepsis (Fernandes *et al.*, 2012), but its role in other pathogenic diseases has never been addressed. We investigated the effects of capsazepine, a TRPV1 antagonist, in malaria. **Methods:** All procedures were conducted in accordance with the Brazilian laws and were approved by the Ethics Committee of the University of São Paulo (Protocol nº003 page 98 book 2). C57BL/6 mice (2-month old) received 10^5 red blood cells infected with *Plasmodium berghei* ANKA intraperitoneally (n=8) (Elias *et al.*, 2013). Non-infected mice were used as controls (n=5). Capsazepine or vehicle were given intraperitoneally for 6 days. Mice were culled on day 7 post-infection and blood and spleen cell phenotype and activation were evaluated. Statistical comparisons of the data were performed by ANOVA followed by Bonferroni and unpaired t test when appropriate. The p values <0.05 were considered significant. The results are presented as the mean ± standard deviation (SD). The percentages of inhibition are reported as mean ± SD of inhibitions obtained in each individual experiment compared with control samples. **Results:** Capsazepine decreased circulating $F4/80^+$ $Ly6G^+$ cell numbers (25 ± 5%) as well as activation of both $F4/80^+$ (75 ± 23%) and $F4/80^+$ $Ly6G^+$ (90 ± 7%) cells in infected-animals without affecting spleen $F4/80^+$ or $F4/80^+$ $Ly6G^+$ cells. In addition, capsazepine increased circulating (2.5-fold increase for $GR1^+$ and 1.5-fold increase for NKT cells) but not spleen $GR1^+$ and NK population, without interfering with NKT cell number and blood NK and NKT activation. However, capsazepine diminished CD69 expression in spleen NKT (46 ± 18%) but not NK cells. Increased levels of lipid peroxidation (1.3-fold increase), TNFα (31-fold increase) and IFNγ (12-fold increase) were found in infected mice, with capsazepine-treated group exhibiting lower levels of lipid peroxidation (20 ± 6%) and TNFα (71 ± 15%). Capsazepine treatment did not affect parasitaemia. Our study provides the first evidences that TRPV1 modulates malaria by mediating innate immune response It is possible that blocking TRPV1 may be either beneficial, as a reduction of oxidative stress may reflect on reduced vascular dysfunction; or deleterious, as impairment of innate response may lead to an inefficient acquired immune response to malaria. However, the impact TRPV1 antagonism may have on severe malaria outcome is of importance and remains to be investigated. This research was funded by FAPEMA, CNPq, Capes and Fapesp. Elias, *et al.* Plos One, 7: e44004, 2012. Fernandes, *et al. J Immunol*, 188: 5741, 2012. World Health Organization (WHO), p. 1, 2013.

04.058

***Matricaria recutita* reduces ligature-induced alveolar bone loss via TNF-alpha inhibition.**

Lassance Cunha E, Guimarães MV, Melo IM, Araujo VMA, Wong DVT, Ribeiro RA, Brasil T, Lima V UFC – Fisiologia e Farmacologa

Periodontitis is an immunoinflammatory disease where the involvement of mediators induces alveolar bone loss (ABL) (Yucel-Lindberg, Expert Rev Mol Med, 15:1, 2013). Although of knowledge about its pathogenesis, some patients do not respond satisfactorily to conventional treatments (Matuliene, J Clin Periodontol, 37:191, 2010). So, medicinal plants are highlighted in the alternative therapy for periodontitis. The Matricaria recutita (MTR), or chamomile, and its isolated constituents stands out in literature for its anti-inflammatory effect (Mazokopakis, Phytomedicine, 12:25, 2005; Bandyopadhyay, *Biochem Pharmacol*, 72:184, 2006; Lee, Arch Pharm Res, 30:1318, 2007; Duarte, Int J Mol Sci, 14:17664, 2013). We used dry extract of MTR content 128.5 ± 0.99 mg/g of apigenin. The ABL was induced in 48 Wistar rats (220.4 ± 3.9 g) by ligature (nylon 3.0) of 2° upper left molar, and the contralateral hemiarcada was used as control. The rats received Tween 80 (TW) or MTR (10, 30 and 90 mg/kg/day by gavage) and at 11 d were killed. We evaluated the gingival tissue by determination ELISA of tumor necrosis factor alpha (TNFa) (pg/mg) and analysis of mieloperoxidase activity (MPO; mg/g). The ABL was analyzed by macroscopic (mm2) and serum bone alkaline phosphatase (BALP, U/l). We considered $p<0.05$; (#) for normal and (*) to TW. Ethics committee for animal use-UFC 70/13. It was found that ligature has caused significant ABL (TW=5.1 ± 0.2), increased of MPO (Normal=3,0 ± 0,5; TW=8,7 ± 1,2#) and TNFa (Normal=0.2 ± 0; TW=1.2 ± 0.2#), and reduction of BALP (Normal=131 ± 8.3; TW=56.4 ± 17.2#). Although MTR has not prevented the reduction of BALP induced by ligature [MTR (10)=75.7 ± 6.9; (30)=74.5 ± 5.7; (90)=78.8 ± 9.2); #,*$p<0.05$], it prevented the ABL induced by periodontitis [MTR (10)=4.4 ± 0.1*; (30)=2.8 ± 0.1*; (90)=2.8 ± 0*], increased MPO activity [MTR (10)=10.2 ± 3.3; (30)=4.5 ± 0.8*; (90)=4,3 ± 0,8*] and TNFa [MTR (10)=0.4 ± 0.2*; (30)=0.2 ± 0.1*; (90)=0.1 ± 0*]. The bone protective effect demonstrated by MTR in our study is consistent with previous reports in which the MTR and its flavonoid apigenin appears to interfere in process of bone resorption via modulation of inflammatory response (Siddiqui, Mol Cell Endocrinol, 323:256, 2010). Considering that the plasma concentration of BALP is used as a biochemical indicator of bone formation (Christenson, Clin Biochem. 1997, 30:573) and that the MPO activity is related to infiltration of neutrophils in tissues (Krawisz, Gastroenterology, 87:1344, 1984), the antiresorptive effect demonstrated by MTR probably does conducted by its anti-inflammatory effect. In fact, our results indicate the reduction of TNFa as one of the possible mechanisms by which MTR produces antiresorptive effect, since the TNFa increases the osteoclastogenesis via cooperative mechanisms with the ligand for receptor activator of NF-©§B (RANKL) (Zhang, *J Biol Chem*, 276:563, 2001), acting in intracellular pathways in response to RANKL (Yarilina, *Proc Natl Acad Sci USA*, 108:1573, 2011), and has antiapoptotic effect on osteoclast (Lee, *J Biol Chem*, 276:49343, 2001). In short, MTR was able to prevent the ABL via reduction of TNFa, without interfering with bone anabolism. Financing: Capes; CNPq.

04.059

Suppression by the flavonol quercetin of chronic lung inflammatory response caused by silica particles in mice. Guimarães FV, Ferreira TPT, Arantes ACS, Azevedo RB, Martins MA, Silva PMR IOC-Fiocruz

Introduction: Among a wide range of occupational diseases, silicosis is a dysfunction caused by a long-term inhalation of silica crystalline particles and is characterized by an intense inflammation and fibrosis, including the presence of granulomas dispersed in the lungs. There is no effective treatment currently available. Quercetin is a flavonoid present in several plants including fruits, vegetables and some grains, which was shown to have important antioxidant and anti-inflammatory properties. In this study we investigated the potential therapeutic effect of quercetin on the experimental model silicosis in mice. **Methods**: Male Swiss-Webster mice were instilled with intranasal silica (10 mg/50 µL) and quercetin was administered orally (2.5 – 10 mg/kg), once a day, for 7 consecutive days, starting 21 days post-silica. The analyses were performed 24 h after the last administration and included: i) leukocyte infiltration, collagen deposition and granuloma formation evaluated in the lung tissue by classical histological techniques (H&E and Picrus sirius); ii) chemokine and cytokine generation quantification by ELISA; iii) pulmonary mechanics and airways hyper-reactivity to methacholine measured by whole body invasive plethismography (Finepointe, Buxco System). All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (L-034/09). **Results**: We noted that silicotic mice exhibited a significant increase in basal levels of lung resistance and elastance as well as airways hyper-reactivity to aerolization with methacholine, as compared to control mice. A marked inflammatory response in the lungs was also detected, characterized by leukocyte infiltration, intense collagen deposition and granuloma formation. The oral therapeutic administration of quercetin reduced the inflammatory leukocyte infiltration, fibrogenesis and granuloma formation as well as collagen deposition in the lungs of silicotic mice. Production of cytokines (IL1beta, IL6 and TNF-alpha) and chemokines (KC, MIP1alpha and MCP-1), in the lung tissue of silica-challenged mice, was inhibited by treatment with quercetin. The compound also suppressed the increased lung resistance and elastance as well as airways hyperreactivity to methacholine in the silicotic mice. **Discussion:** Our findings show that oral administration of quercetin is effective to inhibit the decrease in lung function and hyperreactivity observed in silicotic mice in association with blockade of inflammation and fibrotic responses. Quercetin seems to be potential pharmacological tool to be used in the therapeutic treatment of fibrotic lung diseases such as silicosis. **Financial Support:** Fiocruz, CNPq, Faperj – Brazil and TIMER (EU).

04.060

The role of FcγR2b and FcγR3a in the gut inflammation after intestinal ischemia and reperfusion. Brito CB, Arifa RDN, Lima RL, Menezes-Garcia Z, Souza DG UFMG – Microbiologia

Introduction: Intestinal ischemia is a serious abdominal emergency associated with a high mortality rate. Ischemia leads to cell death and tissue damage, and reperfusion is required for restoration of tissue functions. However, reperfusion results in intense inflammatory process characterized by increased influx of leukocytes. It has been shown which Fcγ receptors (FcyR) are expressed by leukocytes and are involved in the promotion and regulation of inflammatory immune response and several others pathogenic processes. The FcyRs are for IgG receptors formed by different types, such as FcγR2b and FcγR3a. It has been demonstrated that FcγR2b is involved in negative regulation in various inflammatory diseases and FcγR3 plays an important role in the development of autoimmune diseases. However, there are no data published demonstrating the role of these receptors in intestinal ischemia and reperfusion. The aim of this work is to elucidate the role of FcγR2b and FcγR3a in acute intestinal inflammation after ischemia and reperfusion by occlusion of the superior mesenteric artery. **Methods:** Intestinal ischemia was performed for 30 minutes in C57BL / 6 WT, FcγR2b$^{-/-}$ and FcγR3a $-/-$ mice, followed by three hours of reperfusion. Then, the mice were euthanized and their intestines and blood were collected for analysis of MPO, histology, Evans Blue and total and differential count in blood. The experiments were performed in accordance with the ethics committee and animal experimentation of the UFMG. **Results:** After ischemia and reperfusion (IRI) WT mice presented 90% of lethality while FcγR3a$^{-/-}$ mice presented 100% of survival. Interestingly, FcγR3a $^{-/-}$ showed less intestinal injury compared to WT mice, which it was associated with decreased neutrophils influx to gut in these mice. In addition, there was increased in vascular permeability, as showed by higher hematocrit and Evans Blue in intestine of WT mice, these parameters were diminished in FcγR3a $-/-$ mice. Furthermore, there was increased in neutrophils number in blood of WT mice after IRI compared to sham group, this increased was abolished in FcγR3a $-/-$ mice. In other hand, FcγR2b$^{-/-}$ mice showed 100% lethality between 2 and 4 hours while WT mice presented 90% between 4 and 15 hours. The earlier mortality FcγR2b$^{-/-}$ group was associated with increased in neutrophils influx to intestine and intestinal injury. In addition, this group also showed increased in number of total leukocytes in blood, with a predominance of mononuclear when compared to WT group, what suggest a higher systemic inflammation. ($P<0,05$) **Conclusion:** Our results demonstrated that activation of FcγR3a is involved in exacerbation of intestinal inflammation after IRI, once absence of this receptor resulted in decrease in influx of neutrophils and less intestinal injury. In the other hand, the absence of FcγR2b was associated with increased inflammatory response after IRI, characterized by increased influx of neutrophils and higher intestinal injury, which indicated that FcγR2b exerts down-regulation role after IRI. Thus, these receptors are potential therapeutic targets for the treatment of intestinal inflammation. Cetea n° 134 / 2014. **Financial support:** CNPq and Fapemig.

04.061

Punica granatum controls inflammation in arthritic chronic assay in rats. Almeida EKC, Monteiro Silva JF, Oliveira LMS, Viana MDM, Silva Neto GJ, Vieira ACS, Campesatto EA
UFAL – Physiology and Pharmacology

Introduction: Despite several pharmacological alternatives, a growing number of researchers are interested to discovery new drugs because the currently treatments have adverse effects that limit their use. Therefore, is necessary effort in the search for agents more effective. One source alternative is the use of medicinal plants, including the *Punica granatum* (pomegranate) reported in literature as anti-infective and anti-inflammatory. Considering the previous results of *Punica granatum* and the necessity to amplify the knowledge about the use of *P. granatum*, this study aimed to evaluate the anti-inflammatory potential of fruit's peel ethanolic extract of *P. granatum* (EEPG) in arthritic chronic assay. **Methods:** Peels, from Maragogi-AL, were lyophilized, triturated, macerated in ethanol PA, filtered and the final product rotaevaporated, obtaining the EEPG that in the previous phytochemical analysis revealed the presence of flavonoids, tannins and saponins. Adults *Wistar* rats (150 – 200 g), both genres, were used. The syndrome chronic arthritis assay was induced by Freund's complete adjuvant (1 mg/mL, intradermal route) on the dorsal surface of the rat paw (Newbould, *Br J Clin Pharmacol*, 21, 127, 1963). On day 0 the animals were weighed, paws were measured with a caliper (mm) and made to induce arthritis. From the 14th day the animals were treated with vehicle (10 mL/kg, p.o.); dexamethasone (2 mg/kg, p.o.); or EEPG (100, 300 and 500 mg/kg, p.o.) and maintained until day 21. On 22nd day, the animals were euthanized and spleens were removed and weighed to analyze a possible immunosuppression. All experiments were approved by the Ethics Committee for Animal Research of UFAL (protocol number 03/2013). **Results and discussion:** Treatment with the three tested doses of EEPG inhibited the increase in paw volume compared to the positive control from the 17th day; and at end of the assay, the doses of 100, 300 and 500 mg/kg, reduced the paw volume in 18.5%, 26.8% and 18.46%, respectively. The dexamethasone inhibited paw volume significantly from 2nd day of treatment reaching a reduction of 45% at the end of the test. None of the three tested doses of EEPG was able to reduce the mass of the spleen, such as is seen when this parameter was significant for the group treated with dexamethasone (p<0.001), already described the immunosuppressive action of glucocorticoids. Further studies with longer duration are needed, since these results showed a satisfatory anti-inflammatory activity in seven days of treatment. In addition, it is necessary to continue these studies to prove and define the mechanisms of action of this EEPG anti-inflammatory activity, because the results provide support not only research with natural products, as well as research for new substances with anti-inflammatory activity. **Financial Agencies**: Capes and INCT-INOFAR.

04.062

Nanoencapsulation improves the anti-inflammatory activity of curcumin in rats

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Introduction: *Curcuma longa* L., commonly known as turmeric, has been popularly used to treat some diseases such as gastric ulcers, skin and eyes infections and inflammatory diseases. However, studies have shown that the curcumin (Cur) has low water solubility and bioavailability hampered. The aim of this study was to obtain a new formulation of curcumin using biodegradable polymers (CurNano) and compare their effectiveness with that of curcumin *in natura* (Cur) on acute inflammatory response in rats. **Methods:** Technique miniemulsificação/solvent evaporation was used to obtain nanoparticles of poly (L-lactic acid) containing Cur. Anti-inflammatory activity was evaluated by paw edema model induced by carrageenan (Cg – 200 µg/paw). The same volume of vehicle (0.9% saline) was injected into the contralateral paw. The volume of paws was determined at times of 60, 120 and 240 minutes after Cg application, with a plethysmograph apparatus. The increase of final volume of the paw was calculated by subtracting the volume of the paw injected with saline (control paw) by volume of the paw injected with Cg. The rats (n=6) were treated orally, one hour before Cg injection with Cur at doses of 50, 100, 200 and 400 mg/kg or CurNano at doses of 25 and 50 mg/kg or indomethacin (Indo) at a dose 5 mg/kg. The results were statistically analyzed using ANOVA followed by Tukey's test. Differences were considered significant at $P<0,05$. The experimental protocol were approved by the Ethics Committee on Animal Experimentation of the State University of Maringa (CEAE/UEM 025/2013). **Results and discussion:** The Cg injection increased paw edema at 1st, 2nd and 4th hours (23.7 ± 1,6; 50,0 ± 2.2 and 62,5 ± 2,2, respectively). Cur treatment at doses of 100 and 200 mg/kg caused a significant reduction at 4th hours after Cg injection (Cur100=23.6%; Cur200=30.8%). Cur treatment at a dose of 400 mg/kg caused a significant reduction at 2nd and 4th hours after the Cg injection (Cur400=25.6% and 36.2%, respectively). On the other hand, Cur treatment at the dose of 50 mg/kg no alter the development of inflammatory process. Treatment of rats with CurNano at doses of 25 and 50 mg/kg also significantly reduced the intensity of edema at 4th hour after the Cg injection (CurNano25=34.9%, CurNano50=50,2%, respectively). Indo treatment at the dose of 5 mg/kg caused a significant reduction at 2nd and 4th hours after Cg injection (Indo5=55.7% and 63,7%, respectively). The results showed that treatment with CurNano in dose 8-fold smaller than Cur, obtained similar inhibitory effects on the development of paw edema. Thus, the data provide evidence that the process of nanoencapsulation improved the bioavailabity of curcumin.

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04.063

Anastrozole increased inflammatory response without enhance alveolar bone loss in ovariectomized rats submitted to periodontitis. Melo IM[1], Araújo VMA[2], Guimarães MV[1], Calcia TBB[2], Forte TCM[1], Ribeiro RA[2], Lima V[2] [1]UFC – [1]FFOE-UFC, [2]UFC – Physiology and Pharmacology

Introduction: Biosynthesis of estrogen is catalyzed by aromatase enzyme and its inhibition is important for breast cancer therapy. Patients who use aromatase inhibitors as Anastrozole (ANA) show a higher number of fractures and lower bone density (Baum M, Lancet, v.359, p.2131, 2002). Periodontitis is characterized by inflammatory response and alveolar bone loss (ABL) (Page RC, Periodontol 2000, v.14, p.9, 1997). The aim of this study was to investigate whether ANA affects the periodontitis in ovariectomized rats (OVX). **Methods:** Initially, rats were divided into 3 groups, Normal (NOR; n=7), OVX (n=28) and Sham-OVX (S-OVX; n=7). After 14d, the OVX group was divided into 4 groups (n=7), submitted to ligature around the upper 2nd molar and receiving vo saline (SAL) and ANA (0.02, 0.1 or 0.5 mg/kg). After 11d, periodontitis was analyzed by myeloperoxidase activity [MPO (U/mg of gingival)], macroscopy (mm^2), histometry (mm) and histology (scores). Serum dosages of estrogen, leukogram, weight variation and liver, renal and spleen analysis were also performed. The data were presented as mean ± standard error of the mean or median (and range). Ethical aspects: Ethics committee for animal use-UFC nº 69/11. **Results** 14d of ovariectomy reduced estrogen and lasted until 11thd after ligature (d-14=47.5 ± 3.4; d0=32.7 ± 3.6; d11=31.7 ± 4.6 pg/ml; $p<0.05$). Ligature caused an increase ($p<0.05$) in MPO when compared to NOR tissue (NOR=0.76 ± 0.15; S-OVX=3.9 ± 0.7). Although OVX only did not increase ($p>0.05$) MPO when compared to S-OVX, ANA0.5 demonstrated an important ($p<0.05$) increase in MPO when compared to SAL (SAL=3.9 ± 1.2; ANA 0.5=14.35 ± 3.8). Ligature caused intense ABL (NOR=0.0 ± 0.0; S-OVX=5.3 ± 0.4; $p<0.05$), being corroborated by histometry (NOR=0.08 ± 0.01; S-OVX=0.3 ± 0.05). OVX only or associated with ANA did not increase ABL ($p>0.05$) after 25d of OVX [(macroscopy: SAL=4.68 ± 0.37; ANA0.02=5.15 ± 0.2; ANA0.1=5.4 ± 0.4; ANA0.5=5.08 ± 0.4); (histometry: SAL=0.3 ± 0.03; ANA0.5=0.3 ± 0.03)]. The ligature also caused cell infiltration and intense destruction of alveolar bone, cementum and periodontal ligament in S-OVX ($p<0.05$) [NOR=0(0-1); S-OVX=3(1-3)], but these parameters were not worse ($p>0.05$) in SAL or ANA [SAL=2(1-3); ANA 0.5=2(1-3)]. On the 11thd, OVX combined or not with ANA caused leukocytosis, but did not cause any changes in liver, kidney or spleen. SAL group showed more gain of weigth compared to S-OVX, but similarly to ANA weight curve ($p<0.05$). **Discussion:** Considering the role of neutrophil in pathogenesis of periodontitis, the presence of this cell in gingival tissue was assessed by MPO assay, since this enzyme is an important marker of neutrophil presence in inflamed tissues (Lima V, Eur J Oral Sci, v. 113, p. 210, 2005). Therefore, it was believed that the increased observed with ANA could be correlated with greater ABL, however this was not found. Thus, although the reduction of estrogen-induced 25 days of OVX with the last ones 11 days of administration of ANA, have increased this early inflammatory response, it was not sufficient to increase ABL in short term periodontitis. **Financial support:** Capes; CNPq.

04.064

Characterization of leukocytes in adipose tissue of mice after acute and chronic consumption of refined carbohydrate-containing diet. Silveira ALM[1], Oliveira MC[1,2], Nunes LR[1,2], Rodrigues DF[1,2], Lana JP[1,2], Batista NV[1], Ferreira AVM[1], Teixeira MM[1] [1]ICB-UFMG – Biochemistry and Immunology, [2]EECC-UFMG – Nutritionl

Introduction: The consumption of diets containing high fat or high refined carbohydrate (HC) content causes adipose tissue expansion. This expansion has been associated to an increase in the recruitment of immune cells and an inflammatory response in adipose tissue. This inflammatory response may cause metabolic dysfunction associated with obesity, but it may also act as a homeostatic mechanism to control exaggerated fat pad expansion. Metabolic dysfunction occurs already from day 3 and is maintained till 12 wks after HC diet administration. At wk 2, adipose tissue mass reaches its maximal and this is associated with a trough in pro-inflammatory cytokine expression and maximal expression of anti-inflammatory molecules. Thereafter, there is an increase in expression of pro-inflammatory molecules but without further increase in adipose tissue mass (Oliveira *et al. Obesity*. 21: 396, 2013). The aim of the present study was to describe leukocyte profile in adipose tissue after HC diet to determine some leukocytes subtypes associated with fat pad expansion and metabolic dysfunction. **Methods and Results:** Mice were fed chow (C) or HC diet for 3 days (3D), 2 (2wk) or 8 wks (8wk) and the epididymal adipose tissue removed and subject to further assays (Animal Ethics Committee: 060/2010). Animals showed an increase in adiposity index already at 3 days after consumption of a HC diet and this fat expansion was maintained through the observation period (means ± SEM, C:1.9 ± 0.1; 3D:3.3 ± 0.2; 2wk:4.4 ± 0.2; 8wk:3.2 ± 0.2). Along with the adipose tissue expansion, there was an increase in the infiltration of leukocytes, in epididymal adipose tissue, as evaluated by flow cytometry. There was an increase in neutrophils after consumption of the HC diet for 3 days (C:6.2 ± 1.1; 3D:34.7 ± 4.6; 2wk:21.7 ± 2.1; 8wk:17.6 ± 3.8 cellsx10^4/tissue). Number of classically activated macrophage (M1) was higher at 3 days, 2 and 8 wks after HC diet (C:0.8 ± 0.3; 3D:3.1 ± 0.4; 2wk:3.0 ± 0.3; 8wk:2.1 ± 0.3), while number of alternatively activated macrophages (M2) was higher only after 2 wks of HC diet consumption, compared with control mice (C:0.2 ± 0.1; 3D:1.9 ± 0.3; 2wk:3.2 ± 0.6; 8wk:1.7 ± 0.4). Similar to the M2 macrophages, regulatory T cells (Treg) were just increased at 2 wks of HC diet consumption (C:14.5 ± 4.2; 3D:31.6 ± 5.6; 2wk:40.8 ± 8.2; 8wk:17.2 ± 3.2). **Discussion:** The consumption of a high refined carbohydrate-containing diet causes adipose tissue expansion that is associated with an increase in the number of leukocytes in the adipose tissue. M1 macrophages are enhanced throughout the observation period whereas M2 macrophages and Tregs enhance only at 2 wks when adipose tissue mass reaches its maximal and pro-inflammatory cytokine expression in tissues is at lowest. Continuous administration of diet is associated with increased expression of pro-inflammatory molecules and decreased number of M2 cells and Tregs. **Financial support**: Capes, CNPq and Fapemig.

04.065

Effect of c-Jun NH_2-terminal kinase (JNK) inhibitor SP600125 on experimental silicosis in mice. Leite MLM[1], Arantes ACS[1], Lagente V[2], Cordeiro RSB[1], Martins MA[1], Silva PMR[1], Ferreira TPT[1] [1]IOC-Fiocruz, [2]Université de Rennes

Introduction: Environmental and occupational lung disorders are important aspects of clinical medicine and the worldwide economic costs are staggering. Silicosis is a dysfunction caused by long-term inhalation of crystalline silica particles, characterized by intense inflammation and fibrosis, including the presence of granulomas dispersed in the lung parenchyma. In spite of the therapeutic arsenal currently available, there is no specific treatment for this disease. JNK is a member of the MAPK group of signaling proteins whose role in inflammatory responses is widely accepted. In this study, we investigated the potential effect of the JNK inhibitor, SP600125, on the fibrotic component of the experimental model of silicosis in mice. **Methods:** Swiss-Webster mice were nasally instilled with silica particles (13 mg/50 µL) and the treatment with SP600125 (1.25, 2 and 5 mg/kg) was administered orally (p.o), daily, starting on day 21 up to day 27 post silica provocation. Twenty four hours later, the analyses were performed. All animals were killed and whole lung samples were prepared for biochemical and histological analyses. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (licence LO34/09). The analyses included i) leukocyte infiltration, collagen deposition and granuloma formation evaluated in the lung tissue by classical histological techniques (H&E and Picrus sirius); ii) expression of extracellular matrix components by immunohistochemistry and iii) expression of pJNK by Western Blot. **Results:** The compound SP600125 (1.25, 2 and 5 mg/kg, p.o) markedly inhibited the lung fibrotic responses as attested by the decrease of granuloma area (11%, 13% and 15%, respectively) as compared to silica group (48%). A significant reduction of collagen deposition was evidenced by Picrus sirius staining and Sircol technique. Coherently, the expression of extracellular matrix components (laminin and fibronectin) was sensitive to SP600125. We did not observe significant difference between the treatment groups (SP600125 1.25, 2 and 5 mg/kg) as attested by histological analyses. In parallel, we showed that SP600125 decreased the number of silica particles in the lungs. At last, we noted that the increased levels of phosphor-JNK were sensitive to treatment with the compound. **Discussion:** Our findings indicate that JNK inhibitor SP600125 effectively suppressed the fibrotic phase in silicosis in mice, which is clearly reflected by the impairment of lung tissue alterations, strongly supporting the therapeutic potential of orally available JNK inhibitor for the treatment of pulmonary fibrosis. **Financial support:** Fiocruz, CNPq, Faperj.

04.066

Effect of a protein fraction isolated from the latex *Calotropis procera* (Ait.) R. Br in the inflammatory response *in vitro*. Rangel PFG[1], Rabelo LFA[1], Figueiredo TSI[1], Souza GFT[1], Alencar NMN[1], Ramos VM[2] [1]UFC – Physiology and Pharmacology, [2]UFC – Biochemistry and Molecular Biology

Introduction: In chronic inflammatory diseases, there is an inefficiency in leukocyte migration that affect the restoration of homeostasis. Substances able to recompose the inflammatory response have been investigated for these purposes, among them, laticifers proteins extracted from the latex of *Calotropis procera* (LP), which display important immunomodulatory actions as described in the literature. **Methods**: Neutrophils were isolated from mouse bone marrow by Percoll discontinuous gradient. A Boyden chamber was used for evaluation of neutrophil migration. A suspension of cells (1 x 10^6 / ml) was added in the upper compartment and in the lower compartment were added LP (100 ug /well), RPMI (negative control group) or KC – positive control group (20 ng / well) in a final volume of 28.6 uL/well. Both compartments were separated by a membrane and incubated for 1 h. Neutrophil migration was quantified by optical microscopy (1000 X). Neutrophils (1 x 10^6 / ml) was incubated (37 °C/CO_2-2%) with LP (100 ug / well), LPS (5 ug / ml) and RPMI for 24 hours. The supernatant was collected to determinate nitrite levels by Griess reaction and IL1-β, TNF-α and PGE_2 by ELISA. All results were expressed as mean ± S.E.M. Statistical significance was assessed by ANOVA followed by Bonferroni's test. The level of significance was determined as $p<0.05$. The protocols used in this study are consistent with the ethical standards established by the Ethics Committee on Animal Research of the UFC (protocol 24/09). **Results**: Neutrophils stimulated by laticifers proteins (LP) showed significant migration (19,6 ± 1,6) compared to RPMI (2.0 ± 1.0) and similar to KC (26.2 ± 6.9) ($P<0.05$). Neutrophils incubated with LPS were able to release TNF-α (1218.5 ± 154.5 pg / ml) IL- 1β (4498 ± 766 pg / ml), NO_2 (41, 2 ± 8.8 pg / ml) and PGE_2 452.6 ± 73.5 pg / ml significantly ($p<0.05$) when compared to the levels obtained in response to RPMI: TNF-α (60.7 ± 267.3 pg / ml) IL- 1β (1234 ± 168 pg / ml), NO_2 (10.2 ± 1.4 pg / ml) and PGE_2 (104.5 ± 26.4 pg / ml). Significant levels of TNF-α (834.2 ± 138.5 pg / ml) IL-1β (3226 ± 383 pg / ml), NO_2 (33.2 ± 4.9 pg / ml) and PGE_2 (346.6 ± 28.5 pg / ml) were obtained in the supernatants from neutrophil culture stimulated with LP, when compared with RPMI ($P<0.05$). **Discussion:** The migration of leukocytes to the inflammatory site aims to combat the infectious agent and restore tissue homeostasis. Our results demonstrate the ability of laticifers proteins (LP) in stimulate directly neutrophils by increased migration and effective release of pro-inflammatory mediators. The pro-inflammatory effect demonstrated by LP suggests its ability to restore the immunological response in clinical situations characterized by the failure of neutrophil migration. **Financial support**: CNPq, Capes AND FUNCAP .

04.067

Anti-inflammatory activity of *Citrus limon* (L.) Burm. f. essential oil in rodents. Oliveira LMS[1], Monteiro-Silva JF[1], Almeida EKC[1], Silva NKGT[1], Viana MDM[1], Silva-Neto GJ[1], Vieira ACS[1], Sant'Ana AEG[2], Alexandre-Moreira MS[1], Campesatto EA[1] [1]UFAL – Physiology and Pharmacology, [2]UFAL – Natural Resources

Introduction: The *Citrus* genus presents high levels of essential oils (EO). *Citrus limon* species has been used in folk medicine in inflammatory conditions such as bronchitis and rheumatism. Thus, we assessed fruit's peel *Citrus limon* essential oil (CLEO) in inflammatory murine assays. **Methods**: The CLEO (Ferquima, São Paulo) was analyzed by the Gas Chromatography – Mass Spectrum and identified six monoterpenes. Adults *Wistar* rats (150 – 200 g), both genres, were used. The syndrome chronic arthritis assay was induced by Freund's complete adjuvant (1 mg/mL intradermal route) in the dorsal paw of the rat (Newbould, *Br J Clin Pharmacol*, 21, 127, 1963) .From de 14th day the animals were treated with vehicle (10 mL/kg, p.o.); or dexamethasone (2 mg/kg, p.o.) or CLEO (300 mg/kg, p.o.) and maintained until day 21. On 22nd day, the animals were euthanized and spleens were removed and weighed to analyze a possible immunosuppression. In addition to the mentioned test, zymosan- induced peritonitis test was carried out with *Swiss* mice (20-30g), adults, which were pre-treated with indomethacin (35,7 mg/kg p.o.), standard drug; or CLEO (100 mg/kg or 300 mg/kg, p.o.); or vehicle (10 mL/kg p.o), and after all groups were submitted to administration of 500 µL of zymosan (2,0 mg/mL). The animals were sacrificed and the peritoneal fluid collected for cell count. All the experiments were approved by the Ethics Committee for Animal Research of UFAL protocol number nº 02/2014. **Results and discussion:** In the peritonitis assay, all doses of CLEO reduced cell migration induced by zymosan (p<0,001) when compared to control, indicating that the CLEO possibly contains biologically active substances that act in order to reduce the inflammatory activity. In arthritis chronic assay, CLEO (300 mg/kg) inhibited (p<0.001) the increase in paw volume compared to vehicle-treated group. Dexamethasone inhibited (p<0.001) the increase in paw volume as compared to the group treated with vehicle, demonstrated to be effective in all days of experiments. The results showed that tested dose of CLEO were effective to induce inhibition of edema characteristic of adjuvant-induced arthritis. This work contributes to the partial knowledge of pharmacotherapeutic potential of this species, whose depth research may lead to the discovery of a new therapeutic approach for inflammatory conditions. **Financial Agencies**: Capes, FAPEAL and INCT-INOFAR. **Acknowledgments:** Prof. Dr. Antônio Euzébio Goulart Sant'Ana for technical assistance.

04.068

NO and alpha-TNF aggravate the effects of pilocarpine in animal seizure model. Rios ERV[1,2], Rocha NFM[1], Vasconcelos LF[1], Carvalho AMR[1], Dias ML[1], Fonteles MMF[1,3] [1]UFC – Fisiologia e Farmacologia, [2]FAMETRO – Farmácia, [3]UFC – Farmácia

Introduction: The inflammatory process is involved in neurological disorders such as Parkinson's and Alzheimer's disease. The control and knowledge of these changes can help treat these and other diseases. The aim of this study was to investigate the neuroinflammatory dependence on pilocarpine-induced seizure model in mice. **Methods**: Mice (28-32g) were pretreated with thalidomide (25, 100 and 200 mg/kg, ip), an inhibitor of the release of TNFα, Infliximab (2, 5 and 10 mg/kg, sc), an antibody anti-TNFα, Aminoguanidine (25 and 100 mg/kg, ip), an inhibitor of inducible nitric oxide synthase, or ODQ (2.5, 10 and 20 mg/kg, ip), an inhibitor of guanylate cyclase, and 30 minutes after was induced the seizure with pilocarpine (400 mg/kg, sc). In the behavioral assessment was observed following parameters: Latency for a first seizure and latency to death. For statistical analysis, we used analysis of variance (ANOVA) and Student-Newman-Keuls as post hoc test. This work was approved by the local ethics committee (protocol number 41/10). **Results and discussion:** Our results show the attenuation of the behavioral parameters (increased latency to first seizure and decreased of mortality) at higher doses of the groups pretreated with thalidomide and infliximab. In groups pretreated with the higher dose of Aminoguanidine and in a dose of 10 mg/kg of ODQ showed the increased latency and survival of animals' death. **Conclusion**: We demonstrate the neuroprotective activity of thalidomide and infliximab, which may be related to the decreased amount of TNF, while, aminoguanidine and ODQ to increase the survival rate can be related to an aggressor effect of nitric oxide via cGMP. **Financial support**: CNPq and Capes

04.069

Protective effect of complex metallic cis-[RuCl(qui)(bpy)2]PF6 against naproxen- induced gastric damage in mice. Albuquerque-Teixeira AE[1], Santana APM[1], Pires FG[1], Silva FON[2], Loples LGF[2], Souza MHLP[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Organic and Inorganic Chemistry

Introduction: Gastric lesions associated to excessive consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) have an important role in the clinical gastroenterology (Hernández-Díaz S, *Arch Intern Med*, v.160, p. 2093, 2000). The nitric oxide (NO) signaling pathway is well established (JB Cerqueira. *Int Braz J Urol*, v.34, p.638, 2008), which is the main activator of the enzyme soluble guanylate cyclase (sGC) in mediating relaxation through elevations in the intracellular cGMP concentration. However, due to clinical problems with nitrate therapy such as the development of tolerance and cGMP-independent effects induced the interest in direct stimulators of sGC. In this context, was synthetized the novel metallopharmaceutical cis-[RuCl(qui)$(bpy)_2$]$PF_{6,}$ called Quinoline (QUI), which succeeds directly stimulating sGC, promoting vasodilatador effect. The aim of this study was therefore to investigate the protective effect of QUI in experimental model of gastric damage induced by naproxen in mice and the involvement of soluble guanylate cyclase (sGC) and ATP-sensitive potassium (K_{ATP}) channels in this effect. **Methods**: Swiss mice (20-23 g) were pre-treated with QUI (0,3; 3 or 30 mg kg^{-1}, p.o). In another experimental group, the animals were pretreated with ODQ, a sGC inhibitor (10mg kg^{-1}, p.o) or glibenclamide, a K_{ATP} inhibitor (10mg kg^{-1}, i.p) thirty minutes or 1 hour, respectively, before QUI administration (3mg kg^{-1}). After 30 min, the animals received NPX (300 mg kg^{-1}) by gavage. After 6h, the animals were sacrificed and the stomachs removed for evaluation of gastric lesions using a digital caliper. Samples of gastric mucosa were removed for measurement of MPO activity. Local ethics committee protocol NH12/14. **Results:** NPX induced gastric damage (10.6 ± 1.5 mm), when compared to the negative control group (0.1 ± 0 mm). However, pretreatment with QUI protected, in a dose dependent manner, the naproxen-induced gastric damage with the maximum effect in the dose of 3 mg kg^{-1} (1.9 ± 0.7 mm). On the other hand, pretreatment with ODQ (10.49 ± 2.0 mm) or glibenclamide (11.8 ± 2.5 mm) reverted the protective effect of the QUI. **Discussion**: We can infer that QUI prevented the gastric damage through an activation of the sGC and KATP channels. However, further studies are required to determine other possible mechanisms involved. **Financial Support**: CNPq, Capes, FUNCAP.

04.070

Role of the aryl hydrocarbon receptor in the pathogenesis of sepsis. Freitas A, Donate PB, Castanheira FVS, Borges VF, Nascimento DC, Talbot J, Alves-Filho JC, Cunha FQ
FMRP-USP – Pharmacology

Introduction: Sepsis is a systemic inflammatory response resulting from the inability of the host to restrict the infection locally. Studies realized in our laboratory demonstrated that the high mortality observed in severe sepsis correlates with the failure of the neutrophil migration to infectious focus and dissemination of infection. However, animals subjected to a model of non-severe sepsis present an efficient neutrophil recruitment by which hosts are able to constrain the spreading of infection. In this context, recently we demonstrated a direct action of IL-17 mediating the neutrophil recruitment to infection site (1). Recent reports support that activation of aryl hydrocarbon receptor (AhR), a transcription factor, has a role in immune response. The AHR is expressed by Th17 cells and also by cells from innate immune system and is important for their effectors functions, including IL-17 and IL-22 production (2). Herein, we investigate the role of AhR in polymicrobial sepsis induced by cecal ligation and puncture (CLP). **Methods:** Adult C57BL/6 mice were subjected to non-severe (NS-CLP) or severe (S-CLP) sepsis. The protein expressions of AhR and CYP1A1 (indicator of AhR activation) were determined in the spleen, liver and lung by *Western blot* methodology, 18 hours after sepsis induction. The AhR and CYP1A1 were also evaluated, 6 hours after surgery, by PCR in mononuclear cells obtained from blood. Moreover, to determine the role of AhR in the pathogenesis of sepsis, mice were pretreated with vehicle or 30 ug/kg of 6-formylindolo [3,2-b] carbazole (FICZ), high affinity agonist of AhR, 12 and 1 hour before the induction of moderate model of sepsis (M-CLP). Intraperitoneal neutrophil migration, bacteremia, kidney function and AhR activation were determined 6 hours after sepsis induction. The survival rate of animals was assessed every 12 hours up to 120 hours after surgery. The means of different treatments were compared by ANOVA followed by Bonferroni test and the survival rate by the Mantel-Cox log rank test. All experiments was approved by the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo – Ethical Commission of Ethics in Animal Research, protocol number 047/2012. **Results and discussion:** It was observed reduced expression in AhR and CYP1A1 in lung, liver and spleen of mice subjected to S-CLP model as compared with NS-CLP mice. Moreover, AhR and CYP1A1 were not detected in mononuclear cells after S-CLP. Our results also demonstrated that FICZ pretreatment increases the neutrophil recruitment to the peritoneal cavity of mice subjected to M-CLP. As the consequence, these animals presented reduced bacteremia and kidney injury, resulting in increase of survival rate. We also confirmed through *Western blot* that FICZ activated AhR in our system through the increase of CYP1A1 expression. These data suggested that during severe infection the AhR expression is reduced and can contribute to the mortality observed in this process. **References:** **1**) Freitas A. *J Immunol*, 182, 7846, 2009; **2**) Esser C. *Trends in Immunology*, 30, 447, 2009. **Financial Support**: Grant #2012/04076-9 and # 2013/08216-2 from Sao Paulo Research Foundation (Fapesp).

04.071

Evaluation of antiedematogenic activity of limonene epoxide in mice. Almeida AAC[1], Brito TV[2], Reis AL[2], Freitas RM[1] [1]UFPI – Neuroquímica Experimental, [2]UFPI – Fisiofarmacologia Experimental

Introduction: Inflammation is a dynamic and complex process that arises in response towards cellular injury. It has an important role in tissue repair, yet can cause undesirable effects such as tissue damage and function loss. Inflammation process is characterized by production of a mediator's cascade that regulate important factors of inflammatory response, as well as an increase in vascular permeability and recruitment of leukocytes in blood (Rodriguez-Vita J; Lawrence T. *Cytokine Growth Factor Rev*. v.21, p. 61, 2010). Currently several analgesics and anti-inflammatory drugs can be associated with important side effects, low efficacy and specificity. For this reason, studies are conducted to identify novel therapeutic tools to develop and introduce new drugs with greater safety and efficacy (Damasceno, S.R.B., *Life Sci*. v. 94, p. 58, 2014). **Methods:** Male *Swiss* mice (25–30 g) were housed at temperature of 25 ± 2 ˚C under a 12/12-h light/dark cycle with food and water ad libitum. All experiments were approved by Ethics Committee in Research of the Federal University of Piaui (protocol number. 013/11). The animals were randomly divided into five groups (n = 5), and edema was induced by injection of 50 µl of carrageenan suspension (500 µg/paw) in 0.9% saline into the right hind paw (group I). Mice were pretreated intraperitoneally (i.p.) with either 0.05% saline (group II untreated control); indomethacin 10 mg/kg (group III reference control); or limonene epoxide, 50 or 75 mg/kg (groups IV and V, respectively). Paw volume was measured immediately before, and at 1, 2, 3, and 4 h after carrageenan treatment, using a plethysmometer (Panlab, Barcelona, Spain). The effect of pretreatment was calculated as percent inhibition of edema relative to paw volume of the saline treated (controls) (Winter, C.A. *Proc Soc Exp Biol Med*. v.111, p. 544, 1962). **Results and Discussion**: Subplantar injection of carrageenan promoted an increase in paw volume dependent on time; this increase was maintained until the fourth hour, getting the maximum value at the third hour. Paw edema was significantly decreased by indomethacin ($P<0.05$), throughout the experimental period, with 78.08 % inhibition of third hour. The limonene epoxide was evaluated at the doses of 50 and 75 mg/kg, where was observed significant inhibition of edema being at all times evaluated. The dose of 75 mg/kg granting the maximum of inhibition at third hour, 93.42% ($P<0.05$), when compared with carrageenan group. Suggesting that this dose (75 mg/kg) afforded most of protection against the inflammatory effects caused by carrageenan, this dose was selected for clarified of limonene epoxide anti-inflammatory action mechanism. Considering the presented results establish the limonene epoxide is antiedematogenic, with the dose of 75 mg/kg to more efficient. **Financial Suport**: Capes, FAPEPI and CNPq.

04.072

Effect of low-level laser on mRNA expression of inflammatory mediators and kinin receptors in the subplantar muscle of mice paw subjected to *Bothrops moojeni* venom.

Nadur-Andrade N[1], Silva Jr JA[2], Dale CS[3], Zamuner SR[2] [1]Uninove – Ciências da Reabilitação, [2]Uninove – Medicina, [3]USP – Morfology

Introduction: *Bothrops* snake venom induces severe local response such as pain, edema, hemorrhage and necrosis. It has been shown that low level laser (LLL) decreased local inflammatory response caused by bothropic venoms. However, the mechanism involved in this effect is not known. In this study, we evaluated if LLL alters the levels of mRNA expression of the cytokines IL-1β, IL-6, IL-10 and TNF-α, as well as the Kinin B1 and B2 receptors in the plantar muscle of mice after *Bothrops moojeni* venom (BmV) injection. **Methodology:** Male Swiss mice (22-25 g) were used. Animals received a subplantar injection of crude BmV (1.0 µg diluted in 50 µl of sterile saline). LLL was applied at 30 min and 3 h after BmV injection (15 sec, 660 nm, 2.2 J/cm2, irradiated area of 0.2 cm^2). The effect of LLL was evaluated when applied alone or in combination with the antivenom. mRNA expression of IL-1β, IL-6, IL-10 and TNF-α, as well as Kinin B1 and B2 receptors was analyzed in the subplantar muscle of mice, 6 h after the BmV injection, by real-time polymerase chain reaction (RT-PCR). Ethics Committee: AN0021/2011. **Results:** IL-1β, IL-6 and TNF-α, as well as kinin B1 and B2 receptors mRNA presented a significantly increase ($p<0.001$) in the paw of mice 6 h after venom injection. IL-10 mRNA expression was significantly reduced ($p<0.001$) by the venom. LLL was effective in reducing levels of mRNA expression of IL-1β, IL-6, TNF-α, kinin B1 and B2 receptors by 66%, 67%, 41%, 66% and 67%, respectively, evaluated in the subplantar muscle of the animals. In addition, treatment with LLL was able to increase expression of the anti-inflammatory cytokine IL-10 (31%) in the subplantar muscle of mice. Laser treatment in combination with antivenom treatment showed the same result as the laser used alone. **Conclusion:** The results obtained in this study suggest that expression of inflammatory mediators as well as kinin receptors is modulated by LLL, possibly contributing to its anti-inflammatory effect. Also, LLL in the parameters used in this work can be an alternative for the treatment of the local effects caused by bothropic venom accidents as well as an important tool for studying the mechanisms involved in the inflammatory process induced by venom. **Financial support:** Fapesp, 2012/09710-8.

04.073

Effect of fish oil on the acute inflammatory response in rats. Ames FQ, Arruda LLM, Rocha BA, Gil APM, Aguiar RP, Silva-Comar FMS, Silva-Filho SE, Cuman RKN, Bersani-Amado CA UEM – Pharmacology and Therapeutics

Introduction: Currently, many studies point to the important roles of the essential polyunsaturated fatty acids (PUFAs) as the omega-3 (ω-3), whose the richest source is the oil extracted from certain cold water fish, in the prevention and treatment of coronary heart disease, arthritis, and other inflammatory and autoimmune diseases. However, few studies have been conducted to evaluate the activity of ω-3 in acute inflammatory diseases. The aim of this study was to investigate the effect of fish oil (FO) on acute inflammatory response evaluated by pleurisy and paw edema model. **Methods:** Pleurisy was induced by carrageenan (Cg-200 µg) in the intrapleural cavity in rats (n=6) treated with FO diluted in olive oil (OO) in a dose of 18,75; 37,5; 75; 150 and 300 mg/kg, or OO, or indomethacin (Indo) at a dose of 5 mg/kg, orally 30 minutes before Cg injection. Four hours after, the exudate of pleural cavity was collected and the volume of exudate and leucocytes number was determined. Paw edema was induced in rats (n=6) by Cg (200 µg/paw). The same volume of vehicle (0.9% saline) was injected into the contralateral paw. The volume of paws was determined at times of 60, 120 and 240 minutes after Cg application, with a plethysmograph apparatus. The increase of final volume of the paw was calculated by subtracting the volume of the paw injected with saline (control paw) by volume of the paw injected with Cg. The rats were treated orally, one hour before Cg injection with FO diluted in OO at doses of 30 and 300 mg/kg, or OO, or Indo at a dose 5 mg/kg. The results were statistically analyzed using ANOVA followed by Tukey's test. Differences were considered significant at $p<0.05$. The experimental protocol was approved by the Ethics Committee on Animal Experimentation of the State University of Maringa (CEAE/UEM 045/2012). **Results and discussion:** Intrapleural injection of Cg induced an acute inflammatory response, characterized by an increase of the exudate volume and the intense cell migration to the pleural cavity. The oral administration of FO in the amounts of 18.75; 37.5; 75; 150 and 300 mg/kg reduced both the volume of pleural inflammatory exudate (34.3%, 35.4%, 43.3%, 38.1% and 37.0%, respectively) and the number leukocytes recruited into the pleural cavity (24.4%, 17.3%, 20.8%, 18.9% and 20.3%, respectively) with the same effectiveness. Indo treatment only reduced the volume of pleural exudate (62.2%) and OO caused no effect on the inflammatory response. The Cg injection increased paw edema at 1^{st}, 2^{nd} and 4^{th} hours (21.6 ± 3.2; 57.0 ± 4.2 and 70.8 ± 4.0, respectively). FO treatment at doses of 30 and 300 mg/kg, and Indo treatment caused a significant reduction at 2^{nd} and 4^{th} hours after Cg injection (FO_{30}= 44.6% and 52,4%; FO_{300}= 39,9% and 50,8%; Indo= 58,0% and 73,0%, respectively). As a whole, the data provided evidence that FO has inhibitory activity on acute inflammatory response in animals. This activity can be attributed at least partially, to the direct inhibitory effect of FO on the production and/or release of active metabolites of arachidonic acid or other mediators involved in the inflammatory process. Support by Capes/CnpQ; Fundação Araucária-PR.

04.074

Effect of low level laser on C2C12 muscle cells subjected to injury by BthTX-I myotoxin islalated from Bothrops jararacussu snake venom. Santos AS[1], Zammuner SR[1], Hyslop S[2] [1]Uninove, [2]Unicamp

Introduction: Local myonecrose is a common consequence in envenoming caused by snakes of the genus Bothrops which occurs through the action of myotoxins that acts directly in the muscle cell membrane. Previous studies from our group showed that LLL causes an increase in the viability of C2C12 cells incubated with the *B. jararacussu* venom. However, it is not known the effect of LLL on muscle cells incubated with BthTX-I myotoxin isolated from the *Bothrops. jararacussu* venom. The present study was designed to investigate the effect of LLL on C2C12 muscle cells submitted to injury by BThTx I myotoxin isolated from *Bothrops jararacussu* venom. **Methodology:** C2C12 muscle cell line was used. The cells were grown in culture medium DMEM supplemented with 10% fetal bovine serum, incubated at 37˚C with 5% CO2 for 24 hours for cell attachment, after that, the cells received BthTx-I in the respective concentrations 10, 25, 50 and 75 µg/mL and incubated for 15, 30 and 60 min. The cell viability was analyzed by MTT. Cells were irradiated for 13 s immediately after BthTx-I administration with a semiconductor laser at 635 and 830 nm, dose of 4 J/cm2 and power of 100 mW. The cells that did not receive myotoxin and irradiation served as control. **Results:** Our results showed that BhTx-I caused a decrease in cell viability of C2C12 cells that was dose and time dependent. The venom caused a significant decrease in cell viability at doses of 25, 50 and 75 mg/mL by 37%, 41% and 66%, respectively at 60 min of incubation. LLL had no effect on cell viability caused by BthTx-I. **Conclusions:** BthTx-I is toxic to muscle cells and LLL parameters used in this study was not able to protect muscle cells against the effect of myotoxin. **Financial Support:** Fapesp – 2013/23757-0

04.075

Hypertension and periodontal disease are factors that change osteogenic gene expressions in rats. do-Amaral CCF, Brito VGB, Landim de Barros T, Chaves-Neto AH, Oliveira SHP FOA-Unesp – Ciências Básicas

This study aimed to evaluate the effect of experimentally induced periodontitis (PD) in alveolar bone *in vivo* and osteogenic differentiation *in vitro* from bone marrow-derived stromal cells (MSCs) from normotensive (Wistar) and hypertensive rats (SHR). Wistar and SHR rats (180–220 g) were divided into 2 groups each: Wistar control [WC] and SHR control [SC], and Wistar with PD [WPD] and SHR with PD [SPD] groups. Immunohistochemical analysis of RANKL, OPG, BALP, OC, MMP-2, MMP-9, and TRAP of the furcation region were assessed 15 days after PD. MSCs obtained from all groups were collected and osteogenic markers were assessed on day 10 by q PCR. Mineralization of matrix was analyzed at 17 day. RANKL was weakly expressed in both strains, but moderately in SPD animals. OPG was moderately expressed in animals without PD, but strongly in those with PD. The expression of BALP was higher in SPD than in WPD animals. Immunolabeling for OC was absent or weak in both strains and exhibited a tendency to increase in the presence of PD. MMP-2 expression was absent or weak in animals without PD, but it increased slightly in WPD animals. MMP-9 was moderately expressed in animals without PD, but strongly after PD, especially in the SPD group. The numbers of TRAP-positive cells observed in the WC, SC, WPD, and SPD groups were 16.25 ± 6.24; 48 ± 9.83; 69.25 ± 6.75; and 95 ± 13.34, respectively. Preliminary data showed proliferation rate, total protein content, ALP activity, and bone-like nodule formation were increased in osteoblasts from Wistar rats compared with those from SHR. The presence of PD tended to increase proliferation rate and total protein content but decrease ALP activity in both strains. In relation of osteogenic markers, osteoblast obtained from WC MSCs expressed higher BMP-2, Col-I, OCN and BSP but not OPN. The presence of local (PD) or systemic (hypertension) inflammatory condition promotes higher OPN mRNA expression. On the other hand, in both conditions the OPN expression is decreased. Bone regulatory transcription factor RUNX-2 is enhanced in WC, whereas ⇓-CAT is increased in groups with local and systemic inflammation. Taken together, our results demonstrated that PD evokes different responses in alveolar bone for SHR and Wistar rats. Additionally, our data indicate that, while essential hypertension in rats causes increased bone turnover even after PD, important changes in the osteoblast phenotype were observed in both conditions hypertension and PD. By the other hand, *in vitro*, bone markers and transcriptional factors were similar in PD or in hypertension condition, suggesting that inflammation plays an important role in the osteoimmunology, osteoporosis and bone development. Financial support: Fapesp.

04.076

Phytocannabinoid, beta-caryophyllene, modulates inflammatory response induced by *Mycobacterium bovis bacillus* Calmette-Guérin in pulmonary and pleurisy model of infection. Andrade-Silva M, Correa L, Candé A, Rosas EC, Henriques MG
[1]Farmanguinhos-Fiocruz – Farmacologia Aplicada

Introduction: Inflammatory process has a pivotal role in host defense against *Mycobacterium* infection. However, some reports have demonstrated that inflammatory events may favor intracellular survival and/or replication of *Mycobacterium.* Thus continued research to identify new strategies for modulation of inflammatory response is crucial. Therefore, we investigated the effects of the plant metabolite, β-caryophyllene (BCP), a natural agonist of cannabinoid 2 receptors (Gertsch J. *et al. Proc Natl Acad Sci USA*. v. 105, p. 9099, 2008) in the inflammatory reaction induced by *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) in pulmonary and pleurisy animal model of infection. **Methods:** To evaluate the effects of BCP on the inflammatory reaction in pleural cavity, mice received an intrathoracic injection (*i.t.*) of BCG, $4x10^5$ UFC/cavity (100 µL) 1 hour after the oral pretreatment with BCP (50 mg/kg) and after 24 hours the thoracic cavity fluid was collected. In the lung inflammatory reaction, C57BL/6 mice were treated orally with BCP (50 mg/kg) 1h before and 24h after BCG intranasal instillation (BCG, $1x10^6$ UFC; *i.n*) and the effects were evaluated 48 hours after infection from bronchoalveolar lavage fluid (BALF).In both models was evaluated total and differential leukocytes migration using May Grunwald-Giemsa staining and/or flow cytometry analyses and cytokine production. All protocols were approved by the Fiocruz animal Welfare Committee (P62/13). The results were expressed as mean ± SEM using from 6 to 8 animals. **Results:** BCG triggered an inflammatory reaction characterized by leukocyte accumulation in the lung and pleural cavity. The oral pretreatment with BCP decrease significantly total leukocyte (from 9.1 ± 0.30 to 6.3 ± 0.31 $x10^6$/cavity), neutrophil (from 6.0 ± 0.3 to 3.3 ± 0.3 $x10^6$/cavity) and eosinophil (from 0.8 ± 0.1 to 0.6 ± 0.0 $x10^6$/cavity), but not mononuclear cells accumulation in pleural cavity. Previous studies related that activated neutrophil increased CD11b expression on cell membrane, thus we verified whether BCP could modulate this event on $Ly6G^+$ $CD11b^+$ cells. Pre-treatment with BCP was able to reduce CD11b expression as demonstrated by the mean fluorescence intensity (230 ± 7.2 to 165 ± 8.2). Flow cytometry analyses showed that BCG induces an increase in CD3 lymphocytes accumulation in pleural cavity (50% CD4; 58% CD8 and 75% TCRγδ) however the treatment with BCP did not reduce lymphocytes numbers. In the lung, the pretreatment with BCP decreased total leukocyte (from 6.6 ± 1.4 to 1.6 ± 0.2 $x10^5$/lung), mononuclear cells (from 2.4 ± 0.3 to 1.5 ± 0.2 $x10^5$/lung), neutrophil (from 4.0 ± 1.1 to 0.1 ± 0.0 $x10^5$/lung) and eosinophil (from 0.1 ± 0.0 to 0.03 ± 0.0 $x10^5$/lung) accumulation in BCG-induced inflammatory lung. In addiction BCP was able to reduce production of IL-10 (99%), MCP-1 (100%), IFN-γ (98%), and IL-12 (97%). **Discussion:** Our results show that BCP inhibit the inflammatory reaction induced by *Mycobacterium bovis* BCG, however, further studies are needed to investigate its mechanism of action. **Financial Support:** Capes; Faperj; CNPq

04.077

Local administration of gold nanoparticles prevents pivotal pathological changes in murine models of atopic asthma. Serra MF[1], Gomes LC[1], Barreto E[2], Santos RV[2], Santos CEA[2], Hickmann J[2], Cotias AC[1], Pão CRR[1], Trindade SG[3], Schimidt V[3], Giacomelli C[3], Carvalho VF[1], Silva PMR[1], Cordeiro RSB[1], Martins MA[1] [1]Fiocruz – Inflamação, [2]UFAL, [3]UFSM – Química

Introduction: Although gold nanoparticles have been shown to exhibit a range of beneficial biological properties, including anti-inflammatory and anti-oxidant effects, their putative impact on allergic asthma has not been addressed. In this study, we evaluated the potential of nasal-instilled gold nanoparticles to prevent allergen-induced asthma in distinct murine models of this disease. **Methods:** Swiss-Webster (outbred -License no 014512/2011-65) and A/J (inbred -License no LW-23/10) mice were sensitized with ovalbumin and then treated with intranasal injections of gold nanoparticles (6 and 60 μg/kg), 1 h before ovalbumin challenges. Lung function, leukocyte infiltration, mucus exacerbation, extracellular matrix deposition, cytokine generation and oxidative stress were evaluated 24 h after the last challenge. In both mice strains, gold nanoparticles clearly inhibited (70-100%) allergen-induced accumulation of inflammatory cells as well as the production of both pro-inflammatory cytokines and reactive oxygen species. In A/J mice, recognized as genetic asthma prone animals, instilled gold nanoparticles clearly prevented mucus production, peribronchiolar fibrosis and airway hyper-reactivity triggered by allergen provocation. **Results and discussion:** In conclusion, these findings demonstrate that gold nanoparticles prevented pivotal features of asthma, including airway hyper-reactivity, inflammation and lung remodelling. Such protective effects are accounted for by reduction in lung tissue generation of pro-inflammatory cytokines and chemokines, in a mechanism probably related to down-regulation in the levels of oxidative stress. **Financial Support:** Capes and CNPq.

04.078

Methyl gallate inhibits inflammatory mediators and neutrophils migration in zymosan-induced arthritis. Correa LB, Pádua TA, Andrade-Silva M, Seito LN, Henriques MG, Rosas EC CDTS-Fiocruz – Farmacologia Aplicada

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease morphologically characterized by infiltration of inflammatory cells and hyperplasia of synovial tissue (Firestein, *Nature*. 423:356, 2003). Neutrophils are the most abundant cells present in the synovial fluid from inflamed joints and they are abundant at the pannus/cartilage interface. These cells are responsible for production cytokines, chemokines, reactive oxygen species, lysosomal enzymes, and metalloproteinases (Németh, *Immunol Lett* 143:9, 2012). Therapy for arthritis involves NSAIDs and/or glucocorticoids, but they are not satisfactory. Plants with anti-inflammatory action have potential against arthritis (Lama, IJPSR. 2:1116, 2011). Methyl gallate (MG) has been found in wide variety of plants and its anti-inflammatory activity has been demonstrated in different models. Our previous results showed that oral treatment with MG inhibited paw edema and pleurisy induced by zymosan (ZYM) in mice. In this work we studied the effect of MG on inflammatory mediators and neutrophils migration in ZYM-induced arthritis. **Methods:** All protocols were approved by Animal Ethics Committees from Oswaldo Cruz Foundation (P62/13). Mice were pre-treated with MG (7 mg/kg 1 hour before the stimulus) and the anti-inflammatory effects were evaluated in murine model of ZYM-induced arthritis (500 µg/cavity). The MG anti-inflammatory activity was assessed on the knee joint oedema; neutrophil accumulation in synovial cavities; and levels of TNF-α, CXCL-1/KC and prostaglandin E_2 (PGE_2). The direct effect of MG in neutrophils chemotaxis and adhesion was evaluated by Boyden chamber assays and cell adhesion assay, respectively. The effect of MG in the expression of COX-2 in macrophages J774A.1 stimulated with ZYM was measured by immunoblotting. **Results:** Oral administration of MG inhibited the synovial joint edema (6 h – 23%; 24 h – 49%), and neutrophils migration (6 h – 58%; 24 h – 70%) after i.a. injection of ZYM. In addition, pretreatment with MG was able to reduce significantly the production of TNF-α (6 – 46%; 24 h – 61%), CXCL-1/KC (6 h – 48%; 24 h – 77%), and the levels of PGE_2 (6 h – 65%; 24 h – 52%) in synovial fluid 6 and 24 hours after the ZYM reaction. *in vitro* pretreatment of neutrophils with MG (0,1µM 1 hour before the stimulus) markedly inhibited neutrophil chemotaxis toward the CXCL-1/KC. *in vitro* pre-treatment with MG was also able to inhibit neutrophil adhesion to mTNF-α-primed endothelial cells. It was also observed a decreased expression of COX-2 in macrophages stimulated with ZYM after incubation with MG. **Discussion:** Taken together, our results demonstrate that MG has an anti-inflammatory effect in arthritis experimental induced by ZYM in mice due to its action on neutrophils migration and production of inflammatory mediators. **Supported by**: CNPQ, Faperj, Capes, PDTIS/Fiocruz.

04.079

Evaluation of anti-inflammatory activity of essential oil *Hyptis martiusii* Benth (cidreira brava) by peritonitis carrageenan-induced model. Rodrigues LB[1], Ramos AGB[1], Lacerda Neto LJ[1], Cesário FRAS[1], Oliveira CDM[1], Tintino SR[1], Sales VS[1], Pereira AOB[2], Menezes IRA[1], Rodrigues CKS[1] [1]URCA – Biological Chemistry, [2]UFPE – Fisiologia e Farmacologia

Introduction: Pain is a reaction in this species of higher animals, whose physiological role works as an alert to detect threats to the physical integrity of the body. The anti-inflammatory therapeutic potential of many medicinal plants demonstrated by deriving preclinical trials, among others, ethnodirected studies. Several species of the genus Hyptis have proven anti-inflammatory activities. This species has been used by communities of Chapada of Araripe for the treatment of inflammatory and gastrointestinal diseases. However, the species *Hyptis martiusii* Benth (cidreira-brava) not presents studies demonstrating anti-inflammatory actions. The essential oil of *Hyptis martiusii* (OEHM) having as major constituent the 1,8-cineole (24.3%)[1]. **Objectives**: This study was carried out with the purpose of investigate the anti-inflammatory potential of the essential oil of *Hyptis martiusii* Benth (OEHM) assessed by the reduction in the migration of leukocytes and neutrophils in models of carrageenan-induced peritonitis. **Methods:** The project was approved in the Ethics Committee on Animal Research (CEUA) of the Regional University Cariri under number 18/2012.2. Mice (n = 6) *Mus musculus* of both sexes, with body mass index between 25-30g were used in this study. All animals is orally treated with saline 0.1 mL/10g (control group), dexamethasone 5 mg/kg (positive group) and OEHM 100 mg/kg (test group). After 60 minutes from treatment the animals received an intraperitoneal injection of carrageenan. After 4 hours, the migration of leukocytes and neutrophils into the peritoneal cavity was evaluated. For this, the animals were euthanized by cervical dislocation, next 3 ml heparinized PBS was injected into the peritoneal cavity. A sample of peritoneal fluid was diluted 1:20 and total cell counts in the lavage fluid were performed in a Neubauer chamber. Statistical analysis was one-way ANOVA using the Student Newman Keuls test using Prism software. **Results and discussion:** Animals treated with dexamethasone or OEHM show a significant decrease in the total number of leukocytes (2025 ± 69.5 and 1700 ± 87.6, respectively) compared to control group (3680 ± 184.5). A significant reduction in neutrophil infiltration was also observed in the dexamethasone or OEHM treatment (1086 ± 27.7 and 903.5 ± 22.8, respectively) in comparison with control group (2582 ± 87.65). These findings demonstrate an inhibition of migrations of cells involved in the inflammatory process promoted by carrageenan. Others studies using essential oil from other species of the genus Hyptis also showed significant anti-inflammatory activity in peritonitis model. The 1,8-cineole, also presented results in an inhibition of leukocyte migration into the synovial cavity, confirming the results on the anti-inflammatory activity suggesting that the component 1,8-cineole may be responsible for anti-inflammatory activity observed[2,3]. **References:** 1- Araújo *et al*, J. *Agric Food Chem*, 51, 3760, 2003. 2- Hajhashemi *et al*, J. Ethnopharmacology, 89, 67, 2003. 3- Takaki *et al*, *J Medic Food*, 11, 741, 2008. **Financial support:** CNPQ, Capes.

04.080

Evaluation of anti-inflammatory and antinociceptive activity of the ethanol extract from *Psychotria carrascoana*. Aguiar MA[1], Filho JMSR[1], Freitas LBN[1], Araújo BQ[1], Nascimento RRG[2], Pimenta ATA[2], Lima MAS[2], Leal LKAM[1] [1]UFC – Estudos Farmacêuticos e Cosméticos, [2]UFC – Química Orgânica e Inorgânica

Introduction: *Psychotria carrascoana* (Rubiaceae) can be easily found in the State of Ceará, being used in traditional medicine against dizziness, dementia and pain disorders. This species containing alkaloids, have not been sufficiently studied so far. Thus, the aim of the present study was to investigate the effect of ethanol extract (EE) obtained from leaves of the *Psychotria carrascoana* in experimental models of nociception and inflammation in rodents. **Methods:** Animal handling and experimental protocols were registered on the Ethics Committee under number NS29. Wistar rats (150-200 g) were pretreated with EE (50 and 100 mg/kg, p.o.), water (10mL\kg p.o. vehicle) or indomethacin (20 mg/kg, p.o.) 60 min before receiving the injection of carrageenan (Cg, 1% w/v, 100 µl/ paw) into the subplantar area of the right hind paw. The paw volume was determined with a pletismometer as described by Levy *et al.* (1969). The antinociceptive effect of EE was investigated through the Formalin test (Hunskaar *et al*., 1985) and hot plate test (Woolfe e MacDonald, 1944). Swiss mice (25-30g) were treated with the EE (50 and 100 mg/kg p.o.), water (10ml/kg p.o. control), morphine (5 mg/kg, s.c.), 60 min before the formalin (2%, 20 µL/paw) administration. In the formalin test, the time the animal remained licking the injected paw with formalin was measured within the first 5 minutes (phase 1) and 20-30 minutes (phase 2). In the hot plate test, the reaction time of the animals treated or not treated (control) with EE was monitored at 55 ˚C for 0, 30, 60, 90, 120 min. To investigate the involvement of opioid receptors, mice treated with EE (50 and 100 mg/kg, p.o.) were also pretreated with naloxone (2 mg/kg, s.c.), before heat stimulus. Statistical analysis: ANOVA followed by Newman-Keuls Test, $p<0.05$. **Results and discussion:** The EE at the highest dose (100 mg/kg, p.o.), caused significant inhibitions of paw edema since from the 2nd until the 4th h after carrageenan injection (46, 47 and 56 %, respectively). In the formalin test, EE at higher dose significantly inhibited with a similar potency the first and second phase (69 and 61 %, respectively). This antinociceptive effect of EE (100 mg/kg) significantly reduced response in the 1st phase (21,36 ± 4,99, 69,44% inhibition) and in the 2nd of the test (44,27 ± 10,75; 60,96% inhibition) as well as morphine (19,0 ± 4,65; 68,82% and 3,57 ± 2,15; 96,85% inhibition in the 1st and 2nd phase, respectively). On the other hand, in the hot plate test, the EE did not alter significantly the reaction time of the animals when compared to control group, showing that the antinociceptive effect of EE appears to not be related to supraspinal sites. The results showed that EE of *P. carrascoana* has antiedematogenic and antinociceptive effects in rodents. Its antinociceptive effect may be peripherally mediated. However, additional studies are necessary for the identification of the active principles of plant and the elucidation of its mechanism of action. **Acknowledgements**: FUNCAP, CNPq

04.081

Skin pre-exposition with Staphyloccoccal enterotoxin A (SEA) potentiates mice allergic dermatitis. Cabral-Melo A[1], Trabulsi V[1], Ferreira-Duarte AP[1], Pinheiro-Torres SA[1], Mello GC[2], Antunes E[2], DeSouza IA[1] [1]FMJ – Biologia e Fisiologia, [2]FCM-UNICAMP – Farmacologia

Background: Staphylococcal enterotoxins (SEs) are proteins produced and secreted by gram-positive bacterium *Staphylococcus aureus*. This pathogenic bacterium can be found at a natural microflora of the skin, respiratory and intestinal tract. Several clinical investigations have shown that approximately 80% of human allergic dermatitis episodes are correlated with increased skin *Staphylococcus aureus* colonization. In addition, the severity of dermatitis in humans displays a strong correlation with SEs specific IgE production. Thus, the aim of the present study was investigated the role of mice skin pre-exposition with Staphylococcal enterotoxin type A (SEA) on the Ovalbumin (OVA)-induced allergic dermatitis in mice. **Method:** BALB/C mice were actively sensitized with a subcutaneous injection (0.4 ml) of 100 µg of OVA (grade V) mixed with 1.6 mg of $Al(OH)_3$ in 0.9% NaCl (*day 0*). One week later (*day 7*), mice received a second subcutaneous injection of OVA (100 µg in 0.4 ml). At day 14, sensitized mice were submitted to allergic dermatitis assays. Briefly, mice received an intravenous injection of Evans Blue (20 mg/kg) before the subcutaneous (s.c.) administration of Tyrode solution (Control) or SEA (300 ng/100 µl) at randomized sites of skin. After 1 h, the skin sites were submitted to OVA (10 µg/sites) s.c. injection. 30 min later, animals were sacrificed and circulating blood and the skin sites removed and placed to Evans Blue extraction in formamide for 24 h at 37° C. The allergic skin inflammation was expressed as µg of Evans Blue/skin site. **Results:** Our preliminary results shows that mice skin SEA pre-exposition induce an expressive exacerbation on allergic dermatitis induced by OVA (Tyrode + Tyrode: 1.66 ± 0.50, Tyrode + OVA: 3.30 ± 0.32*, SEA + Tyrode: 1.76 ± 0.17, SEA + OVA: 6.03 ± 1.74*# µg of Evans Blue/skin site). **Conclusion**: The present results shows that mice skin SEA pre-exposition are able to induce an expressive aggravation on allergic dermatitis conditions. These results suggests that this toxins can be responsible for severe allergic dermatitis episodes correlated with increased skin *Staphylococcus aureus* colonization and may represent an important advance to found therapeutics alternatives for allergic dermatitis, especially when preceded by *Staphylococcus aureus* infections. Ethical Committee for Animal Experimentation Approvment: 357-1; **Financial Support:** Fundação de Amparo a Pesquisa do Estado de São Paulo (2009/16522-0; 2013/14547-1).

04.082

Pharmacological evaluation of a new series of sulfonamide derivatives in the control of LPS-induced lung injury in mice. Souza ET[1], Carvalho VF[1], Ferreira TPT[1], Nunes IKC[2], Ciambarella BT[1], Barreiro EJL[2], Martins MA[1], Lima LM[2], Silva PMR[1] [1]IOC-Fiocruz – Inflammation [2]LASSBio-UFRJ

Introduction: The design and synthesis of a new series of sulphonamide derivatives planned by structural modification of the prototype LASSBio-448 have been described. LASSBio-448 was shown to be a phosphodiesterase (PDE) 4 inhibitor with therapeutic index and potency higher than of the standard inhibitor (R,S)-rolipram. In this study we evaluated the pharmacological profile of a new series of sulfonamide derivatives both *in vitro* and *in vivo* systems. **Methods:** A series of 13 derivatives was first screened for anti-PDE 4 isoenzyme activity by IMAPTM TR-FRET system. The standard PDE4 inhibitors rolipram and cilomilast were used as control. The compounds were screened on LPS-activated alveolar macrophages (AMJ2C11) and TNF levels quantified by ELISA. For *in vivo* system, A/J mice (18-20g) were instilled with LPS (25 µg/25 µL) (i.n.) and treated with compounds by oral route (6.25 – 25 µmol/kg), 1 h before challenge. Analyses were made 24 h after LPS and included: i) lung function (resistance and elastance) and airways hyperreactivity to methacholine; ii) determination of myeloperoxidase activity (MPO); iii) histology (H&E stain). Potential emetic effects were analysed indirectly by means of duration of xylazine/ketamine-induced anesthesia in mice. All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license L034/09). **Results**: We showed that at the concentration of 10 µM, only LASSBio-1612, LASSBio-1628, LASSBio-1631 and LASSBio-1632 inhibited PDE41A and PDE4D3 activity, but not PDE4B1 and PDE4C. Incubation of macrophages with rolipram and the compounds (1-100 µM), 1 h before LPS, reduced TNF release. The LASSBio-1612, LASSBio-1628, LASSBio-1631 and LASSBio-1632 inhibited reduced lung function (increase in resistance and elastance) as well as methacholine-triggered hyper-reactivity in LPS-stimulated mice. Leukocyte infiltration was also sensitive to the compounds as attested by decreased MPO levels in the lung tissue. We also noted that, similarly to PDE4 inhibitors rolipram and LASSBio-448, all the compounds reduced the duration of anaesthesia in mice. **Conclusion**: Our results show that 4 compounds (LASSBio-1612, LASSBio-1628, LASSBio-1631 and LASSBio-1632) of a new series (13) of sulfonamide derivatives showed inhibitory activity against PDE4 isotypes (PDE4A1A and PDE4D3), which were then selected for biological testing. These compounds reduced the activation of macrophages *in vitro* and differentially inhibited changes in lung function, as well as inflammatory component associated to stimulation with LPS in mice. They also demonstrated that these compounds showed some central effect as compared to LASSBio-448 and rolipram. Such compounds show promise and studies are underway to better characterize the pharmacological effect of these derivatives. **Financial support:** Fiocruz, INCT-INOFAR, CNPq, Faperj.

04.083

Obesity increases CD16 expression in monocytes: The role of adipose tissue secreted molecules. Martins MR[1], Matheus ME[2], Andrade IR[1], Silva SV[1], Reis MC[1], Souza AAP[2], Silva CC[2], Bouskela E[1], Barja-Fidalgo TC[1] [1]UERJ, [2]UFRJ

Introduction: Obesity is associated with a low-grade inflammation in which macrophages play an important role. In this context the adipose tissue functions as a pro-inflammatory (M1) macrophages reservoir while in healthy conditions anti-inflammatory (M2) macrophages are predominant. In humans three subpopulations of monocytes have been described: $CD14^+$ $CD16^-$, $CD14^+$ $CD16^+$ and $CD14^{dim}CD16^+$ monocytes. It was previously shown that the percentages and numbers of $CD16^+$ monocytes are increased in obese subjects. Since macrophages are derived from blood monocytes it becomes important to look into monocytes behaviour during obesity in order to better understand their differentiation into macrophages. So, we asked if obese adipose tissue can interfere in monocytes polarization into macrophages toward M1 phenotype. **Methods** Human monocytes were obtained by density Ficoll gradient and analyzed through flow cytometry. Subpopulations of monocytes were distinguished by their surface expression pattern of CD14 and CD16. In order to analyse the adipose's tissue secreted molecules effect over CD16 acquirence monocytes were plated for 24 hours in presence of lean and obese conditioned media (CM) obtained from lean and obeses subjects, respectively. Also, monocytes were differentiated into macrophages, for seven days, in the presence of adipose tissue CM obtained from lean and obese subjects. After differentiation, M1 and M2 macrophages were distinguished by their surface expression pattern of CD86 and CD206 receptors by flow cytometry and iNOS and arginase mRNA expression by qPCR. Monocytes migration toward adipose tissue-conditioned media were performed in boyden chamber for 2 hours (Protocol Nº 074/2012). **Results:** Obese CM incubation for 24 hours increased in 50% the CD16 surface expression in monocytes. Next, we investigated the effect of the adipose tissue CM incubation during 7 days over macrophages and we observed that obese conditioned media increased in 80% the CD16 expression in macrophages derived from monocytes as well as 90% increase in CD86 and a 60% increase in CD206, respectively M1 and M2 markers. Also, CM from obese subjects induced a 5-fold increase in iNOS and a 1.35-fold decrease in arginase mRNA in macrophages obtained from healthy subjects. Furthermore we observed that intermediary (CD14 + CD16) monocytes presented a 6-fold-increase migration toward to obese CM, thus indicating their migratory potential in obese conditions. **Conclusion**: Molecules secreted from obese adipose tissue can alter monocyte and macrophage CD16 expression, as well as its migration towards the adipose tissue increasing M1 macrophage polarization. **Financial support**: Capes, CNPq, Faperj

04.084

Differencial effect of lidocaine on acute and late phases of silicosis in mice. Lacerda L, Ciambarella BT, Ferreira TPT, Martins MA, Silva PMR Fiocruz – Farmacodinâmica

Introduction: Silicosis is an occupational disease caused by crystalline silica particles inhalation, being characterized by an inflammatory process followed by an intense granulomatous fibrosis. Macrophages have been identified as pivotal regulators of fibrosis progression by functioning as phagocytic cells. Lidocaine is a local anaesthetic, which has significant anti-inflammatory properties as attested in the case of som administration in the experimental model of silicosis in mice. **Methods:** Male Swiss-Webster mice were injected intranasally with silica particles (10 mg) and 3 different protocols of nebulized lidocaine (1 and 2%) were used: Protocol 1 – during 7 days, starting 6 h post-silica and analysis on day 8; protocol 2 – during 7 days, starting 6 h post-silica and analysis on day 21 after the last dose, and protocol 3 – from day 21 to 27 post-silica and analysis on day 28. Parameters included F4/80 positive cells (macrophages) by immunohistochemistry; lung function and airway hyper-reactivity to methacholine by invasive plethysmography (Buxco System, UK) and granuloma formation by morphometry (H&E stain). All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (L-034/09). **Results:** Silicotic mice showed a reduction in the lung function (increased resistance and elastance) and methacholine-induced airways hyper-reactivity. Morphometric analysis revealed a time-dependent increase in fibrosis and a high number of F4/80 + macrophages found in the granuloma area. Treatment of silica-challenged mice with nebulized lidocaine restored lung function and inhibited granuloma formation and F4/80 + macrophage infiltration following protocol 1. Although to a lesser extent, lidocaine effectively suppressed both lung and tissue alterations in the silicotic mice following protocol 2. No changes were evidenced in the case of lidocaine according to protocol 3. **Discussion:** Our findings show that lidocaine though able to inhibit silicosis installation, in association with its anti-inflammatory properties, did not show benefits when silicosis was already established. Moreover, they indicate that there is a correlative link between acute inflammation and fibrosis, and also suggest that more than one kind of macrophage may be involved in both phases of silicosis, exhibiting different sensitivity to lidocaine. More studies are needed in order to clarify this point. **Financial support:** Fiocruz, PDTIS, CNPq, Faperj.

04.085

Platelet-activating factor receptor deficient mice develop more severe experimental colitis. Lima R L[1], Arifa RDN[1], Menezes-Garcia Z[1], Brito CB[1], Dourado LPA[1], Teixeira M M[2], Souza D G[1] – [1]ICB-UFMG – Microbiologia, [2]ICB-UFMG – Imunologia e Bioquímica

Introduction: The incidence of inflammatory bowel diseases (IBD) has increased in recent decades in developing countries, and affect about 0.1% of the population of developed countries. This is attributed to the multiple and complex factors, such as changes in eating habits, loss of homeostasis between antigens from the intestinal lumen and mucosal immunity. Platelet-activating factor (PAF), a potent pro-inflammatory phospholipid mediator, has been implicated in inducing intestinal inflammation in diseases such as inflammatory bowel disease (IBD). The aim of this work was to investigate the response of PAFR deficient mice to in experimental colitis induced by DSS. **Methods:** The use of animals in this study was approved by the Ethics Committee on Animal Experimentation of UFMG (CEUA – 138/2012). $PAFR^{-/-}$ C57BL6 and WT C57BL6 mice were used and the colitis was induced by dextran sulphate sodium (DSS) 3% in water from day 0 to day 7. We analyzed the weight loss, the consistency and the presence of blood in the stool, the clinical signs of disease and the colon length. We also evaluated the neutrophils influx into the colon through the activity of MPO. In addition, we measured hematocrit and leukocyte count in the blood and the colon concentration of IL-1β, CXCL-1, IL-6 and TGF-β by ELISA. **Results and discussion:** The WT colitis group has shown higher weight loss and clinical score than WT control group. These mice also have shown shortening of the colon, lower hematocrit, higher total number of leukocytes in the blood and higher activity of MPO when compared to WT control group. Furthermore, the WT colitis group has shown higher IL-1β, IL-6 and CXCL1. Interestingly, the $PAFR^{-/-}$ colitis group has shown all the clinical and inflammatory parameters of colitis very exacerbated. The $PAFR^{-/-}$ colitis group has shown higher weight loss and higher clinical score than WT colitis group. These mice also have shown shortening of the colon, lower hematocrit, higher total number of leukocytes in the blood and greater activity of MPO when compared to WT colitis group. Furthermore, $PAFR^{-/-}$ colitis group has induced higher IL-1β, IL-6 and CXCL1 concentration in the colon when compared to WT colitis group. There was no difference between control groups. **Conclusion:** We conclude that, in this work, the absence of PAFR aggravate the clinical sings and increase the inflammatory response in experimental colitis induced by DSS. **Financial support:** CNPq, FAPEMIG e Capes

04.086

Gedunin impairs toll-like receptor 4/MD-2 signaling. Borges P[1], Moret K[1], Monteiro C[2], Batista P[3], Caffarena E[3], Henriques MG[1], Penido C[1] [1]Farmanguinhos-Fiocruz – Farmacologia Aplicada, [2]IOC-Fiocruz – Imunofarmacologia; [3]Fiocruz – Biofísica Computacional e Modelagem Molecular, [4]Fiocruz – Farmacologia Aplicada

Introduction: Recognition of bacterial lipopolysaccharide (LPS) by innate immune system is mediated by TLR4/MD-2 complex. Additional receptors, such as heat shock proteins (Hsp), have been suggested to be part of this activation cluster (*Triantafilou*, *M. et al.*, *Trends of Immunol. 23:301*, *2002*). Previous data from our group and others have demonstrated that gedunin, a Hsp90 inhibitor, present marked antiinflammatory effects (*Ferraris*, *F.et al.*, *IntImmunopharmacol. 14:82*, *2012*; *Matts*, *RL. et al. Bioorg. & Med. Chem. 19:684*, *2011*). Here, we have investigated the role of gedunin in LPS-induced inflammatory response in macrophages (Mø). **Methods and Results:** The incubation of immortalized (Mø) with gedunin (0.01-100 μM) did not induced citotoxicity (>90% viability), as assessed by resazurin reduction Method: The pretreatment of Mø with gedunin (10 μM), inhibited LPS (50 ng/ml)-induced cytokine production, similar to dexamethasone (50 nM) and 17AAG (Hsp90 inhibitor, 1 μM), as assessed by ELISA: IL-6 (DEXA: 99%; 17AAG: 98%; GED 93% of inhibition) and TNF-α (DEXA: 90%; 17AAG: 62%;GED: 54% of inhibition).The production of nitric oxide (NO) by macrophages stimulated with LPS plus interferon-γ (IFN-γ; 20 IU) was also inhibited by gedunin (30% of inhibition), as determined by Griess Method: Pretreatment of Mø with gedunin impaired LPS-induced NFκB activation and cyclooxygenase-2 (COX-2) expression, whereas upregulated Hsp70 expression, as revealed by western blot. To investigate the effect of gedunin on adaptor proteins, the production of proinflammatory cytokines triggered by LPS was investigated in Mal- and TRIF-deficient Mø. Gedunin pre-treatment inhibited LPS-induced cytokine production in $Mal^{-/-}$Mø: TNF-α (24h, DEXA: 52%; 17AAG: 72%; GED: 70% of inhibition); $TRIF^{-/-}$Mø: TNF-α (6h, DEXA: 54%; 17AAG: 68%;GED: 72% of inhibition) and IL-6 (6h, DEXA: 54%; 17AAG: 31%; GED: 32% of inhibition). Computational modeling studies, through docking structure calculations, demonstrated that gedunin efficiently docked into the MD-2 LPS binding site, suggesting that it might act as a competitive inhibitor of LPS, blocking the formation of TLR4/MD-2/LPS complex. **Discussion:** Gedunin suppresses LPS-induced macrophage activation, inhibiting the production of inflammatory mediators and upregulating Hsp70, which presents anti-inflammatory effects (Borges, T.J. *et al.*, *Front Immunol.* 3: 95, 2012). The inhibition of Hsp90 activity, as observed by 17AAG treatment, is capable to impair LPS-induced inflammatory responses; however, it seems that gedunin, in addition to inhibit Hsp90, regulates upstream LPS signaling events by blocking the complex TLR4/MD-2/LPS. **Financial support:** Fiocruz/ CNPQ/Faperj.

04.087

Role of nitric oxide in hypoxia signaling during colonic inflammation. Caria CRP, Moscato CH, Tomé RBG, Pedrazzoli Jr J, Rocha T, Ribeiro ML, Gambero A USF – Clinical Pharmacology and Gastroenterology

Introduction: Intestinal inflammation develops due to disruption of intestinal epithelial barrier, with local recruitment of inflammatory cells. Consequently, a reduction in the availability of oxygen occurs, leading to hypoxia. Nitric oxide (NO), an inflammatory mediator, may interfere with the signaling of hypoxia and it could result in alterations in inflammatory response and wound healing. Thus, we aimed to evaluate the role of NO in hypoxia signaling during colonic inflammation and its ability to modify the expression of genes/proteins induced by hypoxia. **Method:** The experiments were received approval from the Ethics Committee of São Francisco University, Bragança Paulista, SP, Brazil (Protocol 002.09.09). Colitis was induced by single (acute) or repeated (reactivated colitis) trinitrobenzenosulfonic acid (TNBS) administration in rats. Rats with colitis were also treated with L-NAME to block NO synthase. Colitis was assessed by macroscopic score and myeloperoxidase activity in the colon samples. To assess colonic hypoxia during colitis induced TNBS administration, rats were treated intraperitoneally with pimonidazole 1 h prior to sacrifice. The expression of HIF-1α and HIF-induced factors (VEGF and apelin) was assessed using Western blotting. **Results:** The single or repeated administration of TNBS to rats induced colitis which was characterized by a high macroscopic score (0.6 ± 0.2 and 7 ± 0.7 for acute colitis, and 0.2 ± 0.2 and 4.8 ± 0.6 for reactivated colitis) and myeloperoxidase activity (1 ± 0.3 and 47 ± 18 U/g tissue for acute colitis, and 3.8 ± 2.6 and 91 ± 0.2 U/g tissue for reactivated colitis). Hypoxia was observed with both protocols due pimonidazole induced adducts deposition. During acute colitis, HIF-1α expression was not increased, but VEGF and apelin were increased. HIF-1α expression was inhibited during reactivated colitis, and VEGF and apelin were not increased. L-NAME treatment during the final 7 days of the experiment (after the final TNBS administration in the reactivation protocol) reduced the number of macroscopically observed lesions (0.2 ± 0.2 for control, 5.7 ± 0.8 for for colitis, and 3.5 ± 0.2 for colitis/L-NAME). MPO activity was not significantly reduced (1.6 ± 0.5 for control, 6.7 ± 2.3 for for colitis, and 3.5 ± 2.4 for colitis/L-NAME). However, L-NAME blockade during reactivated colitis restored HIF-1α, VEGF and apelin expression. **Discussion:** NO could interfere with hypoxia signaling during reactivated colitis inflammation modifying the expression of proteins regulated by HIF-1α. **Sources of research support:** Fapesp.

04.088

Proteinase-activated receptor (PAR)-2 plays a role in the recruitment of leukocytes in experimental lung inflammation. Matos NA, Klein A ICB-UFMG – Farmacologia

Introduction: Proteinase-activated receptors (PARs) have been shown to be implicated in inflammation. PAR-2 is a subtype of this receptor expressed on leukocytes and activated by trypsin and mast cell tryptase (TRY). We have demonstrated that PAR-2 plays a role in eosinophil (Eo) recruitment in TRY- or allergen-induced pleurisy in mice supporting additional evidences for a role for PAR-2 in allergic diseases. The role of PAR-2 in lung inflammation has not been fully understood. Our goal is to investigate the role of PAR-2 in neutrophil (No) and Eo recruitment in ovabulmin (OVA)-, PAR-2 agonist SLIGRL-NH_2 (SLIG)- or TRY-induced experimental asthma model, as well as to investigate the role of cysteinyl leucotrienes (CysLT) in No recruitment induced by PAR-2 activation. **Methods:** BALB/c mice (20-25g) were sensitized intraperitoneally (i.p.) with 100µg of OVA in 2% aluminum hydroxide gel adjuvant on day 0, then challenged intranasally (i.n.) on days 8–10 with 10µg of OVA or PBS. Bronchoalveolar lavage fluids (BALF) were obtained 4, 24, 48 and 72 h after challenging. Furthermore, mice were treated i.p. with the PAR-2 antagonist ENMD1068 (11µg) 1h before i.n. instillation of OVA on days 8-10 and the number of infiltrating leukocytes to the lung was evaluated 30 min to 24 h after. Pretreated mice with CysLT antagonist montelukast (MK, 5 mg/kg) 30 min before i.n. instillation of SLIG (30µg) or TRY (300ng) and infiltrating leukocytes were analysed 24h after. Statistical analyses were performed using One-Way ANOVA followed by Newman-Keuls post-test. Experimental procedures were approved by the local animal ethics committee (protocol number 168/2014). **Results:** Intranasal challenge OVA in immunized mice induces Eo recruitment in BALF 4 to 72 h peaking 48 and 72 h after OVA challenging when compared to PBS-treated mice (48h: PBS, 0.08 ± 0.08; OVA, 3.9 ± 1.5*; 72h: PBS, 0.0 ± 0.0; OVA, 3.9 ± 1.3** Eox10^3, *P<0.01; **P<0.001). ENMD abolished the neutrophilia induced by OVA 2, 4, 8, 12 h after challenge (2h: PBS, 0.0 ± 0.0; PBS + OVA, 3.6 ± 0.6*; ENMD + OVA, 1.7 ± 0.01*), (4h: PBS, 0.0 ± 0.0; PBS + OVA, 6.8 ± 0.5*; ENMD + OVA, 1.3 ± 0.02*), (8h: PBS, 0.08 ± 0.04; PBS + OVA, 3.3 ± 0.2*; ENMD + OVA, 1.8 ± 0.2*), (12h: PBS, 0.0 ± 0.0; PBS + OVA, 2.9 ± 0.2*; ENMD + OVA, 1.0 ± 0.01*) Nox10^3, *p<0.001. ENMD also inhibited eosinophilia induced by OVA 8, 12, 24 h after (8h: PBS, 0.0 ± 0.0; PBS + OVA, 0.9 ± 0.07**; ENMD + OVA, 0.5 ± 0.1*), (12h: PBS, 0.0 ± 0.0; PBS + OVA, 0.9 ± 0.1**; ENMD + OVA, 0.2 ± 0.03**), (24h: PBS, 0.0 ± 0.0; PBS + OVA, 3.5 ± 0.4**; ENMD + OVA, 0.6 ± 0.06**) Eox10^3, *p<0.01, **p<0.001. Indeed SLIG or TRY induced a neutrophil recruitment in the BALF in naïve mice 24h after when compared to PBS-treated mice and MK reduced the number of No induced by SLIG or TRY (PBS, 0,1 ± 0.06; PBS + SLIG, 3.7 ± 0.4*; MK+ SLIG, 1.9 ± 0.1*); (PBS, 0.0 ± 0.0; PBS + TRY, 6.5 ± 1.0*; MK+ TRY, 1.8 ± 0.1*) Nox10^3, *P<0.001. **Discussion:** Allergen-induced airway inflammation significantly increased levels of No in earlier times and increasing of Eo in longer times through a PAR-2-dependent activation. Montelukast inhibited No migration induced by PAR-2 activating peptide or the endogenous agonist TRY. We suggest that PAR-2 plays a role in leukocyte migration in experimental lung inflammation. **Financial support:** FAPEMIG/CNPq.

04.089

Effect of low level laser therapy combined with physical training in experimental arthritis. Silva MP, Sanches IC, Angelis KD, Zamuner SR Uninove – Rehabilitation Sciences

Introduction: Arthritis is associated with changes in the extracellular matrix and increased immune system activity. Also, several studies detected abnormalities in the autonomic nervous system correlated with cardiovascular morbidity in arthritis patients. Non-steroidal anti-inflammatory agents and selective cyclooxygenase inhibitors are commonly used as analgesic and anti-inflammatory agent in arthritis, but this treatment is related with side effects on gastrointestinal system. These considerations have prompted the search for alternative non-drug treatments for arthritis. Our hypothesis is that low level laser therapy (LLLT) associated with physical training (PT) improves inflammatory process. **Aim:** To evaluate the action of combined non-invasive therapies LLLT and PT in arthritis induced by zymosan in the rat knee. **Methods:** Wistar rats (250-280g) were used. The arthritis was induced by injection of zymosan (1 mg/50µL of sterile saline) intra-articular in the knee joint. The animals were divided in 4 groups: Control-saline (C); Arthritis (A); Arthritis-PT (AP) and Arthritis-PT-LLLT (APL). The treatment protocol was conducted using a semiconductor laser InGaAlP (660 nm, 05 mW, 2.5 J/cm^2, 20s) associated with PT of moderate intensity (40% to 70% of the maximal treadmill) for 40 days. After 40 days the hemodynamic and autonomic parameters [Mean Arterial Pressure (MAP); Heart rate (HR); variance of systolic blood pressure (SBP-VAR) and Low frequency band of systolic blood pressure (SBP-LF)] was evaluated. Also, analyzes of functional capacity, weight gain, local cellular influx and histological analysis was analyzed. Ethics committee: Protocol AN0017/2012. **Results**: Functional evaluation showed that AP and APL group had a better performance compared to C and A group (C: 9.1 ± 2.7min; A: 8.14 ± 0.3min; AP: 13.3 ± 0.3min; APL: 14.3 ± 1.2min). Animals in the AP and APL group showed a significant difference in the weight gain at the end of protocol compared to C and A group (C: 118.34 ± 13.8 g; A: 126.87 ± 10.8 g; AP: 98.85 ± 10.2 g; and APL: 90.71 ± 7.2 g). MAP showed no statistical difference among the studied groups (C: 112.83 ± 6.0 mmHg, A: 110.56 ± 4.4 mmHg, AP: 110.83 ± 2.8 mmHg and APL: 109.70 ± 1.7 mmHg). Also, HR was not different (C: 328.24 ± 3.8 bpm, A: 343.89 ± 14.4 bpm, AP: 321.07 ± 5.8 bpm and APL: 318.41 ± 3.8 bpm). However, the autonomic analyzes showed that arthritis increased SBP-VAR and AP and APL group maintained the same levels of control group (C: 11.31 ± 3.2 mmHg; A: 19.64 ± 5.8 mmHg; AP: 12.80 ± 4.9 mmHg; APL: 13.63 ± 3.3 mmHg). SBP-LF showed that APL group was significantly different from A group (C: 1.44 ± 0.6mmHg; A: 3.60 ± 1.6mmHg; AP: 3.13 ± 1.3mmHg; APL: 2.22 ± 0.8mmHg). Total leukocyte influx showed a significant increase in knee joint cavity in A and AP group compared to C (C: 141.6 ± 20.6 x 10^4/mL; A: 287.5 ± 44 x 10^4/mL; AP: 275 ± 29.8 x 10^4/mL). The APL group showed a reduction in the leukocyte influx (193.75 ± 27.4 x 10^4/mL). Histological images showed greater morphologic disorganization in group A compared to other groups. **Conclusion**: Together, these data demonstrated that the combined non-invasive treatment with LLLT and PT improves local and systemic effects arising from the deleterious events caused by local inflammation. **Financial support**: Capes/ PROSUP

04.090

Non-clinical evaluation of anti-inflammatory activity and toxicity of standardized dry extract and fractions from *Amburana cearensis* (Cumaru). Amaral HHS[1], Pierdoná TM[2], Araujo EVO[1], RIBEIRO RG[3], Santos GBM[3], Silveira ER[3], Viana GSB[2], Leal LKAM[1] [1]CEFAC-UFC – Farmácia, [2]UFC – Fisiologia e Farmacologia, [3]UFC – Química Orgânica e Inorgânica

Introduction: *Amburana cearensis* A.C. Smith (Fabaceae) is a wild medicinal plant popularly known as "cumaru" and extensively used in the Northeast of Brazil. Previous chemical studies of *A. cearensis* showed to the isolation of several bioactives molecules, such as coumarin (CM) and amburoside A (AMB). Other studies with the plant spray-drying powder (SDP) demonstrated its anti-inflammatory, antioxidant and neuroprotective potential in rodents. The aim of the present study was to evaluate the toxicity of SDP and the aqueous and the residual phenol fractions from *A. cearensis* (AFAC and RPFAC, respectively) in human neutrophils, also the antinociceptive potential on the formalin test in mice. **Methods**: The SDP was standardized through HPLC analysis (chemical markers – CM: 26.23 ± 1.20; – AMB: 74.54 ± 1.45 mg/g of SDP). The toxicity of the SDP, AFAC and RPFAC (50, 100 and 200 µg/mL) was evaluated through the measurement of lactate dehydrogenase (LDH) activity and the MTT test (MOSMAM, J. Immunol. Methods, v.65, p.55, 1983) in human polymorphonuclear cells ($2.5x10^6$ cells/mL), mainly neutrophils (80-90%), (Approval COMEP: 218/10), while the antinociceptive activity of SDP (100 and 200 mg/kg, p.o.) in Swiss mice (25 – 30 g) was investigated by the formalin test (Hunskaar, Pain v30, p103, 1987) (Approval CEPA: 03/2013). **Results and discussion:** Even at higher concentration both the extract (14.26 ± 2.54 U/L) and the fractions (AFAC: 15.14 ± 1.78 U/L; RPFAC: 14.94 ± 0.75U/L) did not increase significantly LDH activity compared to control (12.5 ± 3.6 U/L) (ANOVA, Tukey post hoc test, $p<0.05$). Similar results were observed on the MTT test, where the lower percentage of cell viability after the treatment of cells with the bioproducts from *A. cearensis* was 98.9 ± 2.9 %. In the formalin test the SDP 100 and 200 mg/kg reduced significantly only the phase II (79.8 ± 14.0s and 42.2 ± 13.0s, respectively) when compared to control group (142.0 ± 18.2s), while morphine (standard drug) inhibited both phases of test (phase I: 15.7 ± 6.57; phase II: 5.5 ± 3.57 s). The preliminary results suggest that SDP and fractions from *A. cearensis* do not appear to be toxic to human neutrophils affecting the plasma membrane or the human neutrophils metabolism. In addition, the SDP showed an antinociceptive effect possibly related to its anti-inflammatory potential established in previous studies developed by our laboratory. **Financial support:** CNPq

04.091

Role of enzyme 5-lipoxygenase in experimental model of septic arthritis. Boff D, Oliveira SLV, Martins MM, Amaral AF UFMG – Bioquimica e Imunologia

Introduction: Septic Arthritis is a disease that occurs when the pathogen invades the joint, causing inflammation and tissue damage. The main microorganism involved in that pathology is *Staphylococcus aureus*. This disease has high mortality rate (~50%) and results in irreversible joint damage. The enzyme 5-lipoxigenase (5-LO) leads to the synthesis of leukotrienes and lipoxin A4 (LXA4) from the substrate araquidonic acid, mediators that present pro-inflammatory and anti-inflammatory properties, respectively. Thus, these molecules could interfere in the course of *S. aureus*-induced inflammation, as seen in the pathogenesis of other non-infectious articular diseases. The aim of this study was investigate the role of 5-LO in articular inflammation following *S. aureus* infection. **Methods:** Experimental septic arthritis was induced by intra-articular injection of *S. aureus* (10^7 CFU; 10 µL) in WT (SV129) and mice deficient for 5-LO (5-LO$^{-/-}$) (5-6 mice/group). The inflammatory parameters were analyzed at day 7 post infection. Hypernociception was evaluated by electronic von Frey test. After euthanasia, the articular lavage was performed for cellular counting (optical microscope) and the articular tissue was collected to recovery bacteria, for measurement of cytokines and chemokines (ELISA) and for histopatological analysis. **Results:** 5-LO$^{-/-}$ mice presented a decreased number of infiltrated neutrophils to the joint (WT not infected (NI): 2.89 ± 2.70; WT infected (I): 94.56 ± 10.40; 5-LO$^{-/-}$ NI: 1.97 ± 2.08; 5-LO$^{-/-}$ I: 18.23 ± 4.45) and reduced levels of the cytokine IL-1β (WT NI: 97.98 ± 54.99; WT I: 317.61 ± 151.62; 5-LO$^{-/-}$ NI: 48.98 ± 15.50; 5-LO$^{-/-}$ I: 106.80 ± 25.44) and chemokine CXCL1 (WT NI: 101.26 ± 54.29; WT I: 449.95 ± 228.81; 5-LO$^{-/-}$ NI: 42.54 ± 32.63; 5-LO$^{-/-}$ I: 180.38 ± 20,30) when compared to WT littermates. This reduced inflammation in 5-LO$^{-/-}$ mice was accompanied by a higher paw withdrawal threshold (WT I: 5.11 ± 0.46; 5-LO$^{-/-}$ I: 6.46 ± 0.83), meaning a decreased hipernociception behavior. Interestingly, 5-LO$^{-/-}$ mice had decreased bacteria in the joint (WT I: 703 ± 360.78; 5-LO$^{-/-}$ I: 143.33 ± 195.13) compared to WT mice. Confirming these results, 5-LO$^{-/-}$ mice showed less articular damage accessed by histopatological score (WT NI: 0 ± 0; WT I: 3.75 ± 0.95; 5-LO$^{-/-}$ NI: 0.33 ± 0.57; 5-LO$^{-/-}$ I: 2.25 ± 0.5) and proteoglycan (WT NI: 2.43; WT I: 33.45 ± 4.46; 5-LO$^{-/-}$ NI: 5.10 ± 2.33; 5-LO$^{-/-}$ I: 17.43 ± 2.80). **Discussion:** The absence of two antagonistic inflammatory mediators, LXA4 and leukotrienes, favors to a better control of *S. aureus* and consequently limit the articular inflammation in this model. LXA4, a potent anti-inflammatory molecule, limits cellular activation. Thus, the lack of lipoxin A4 could result in a better clearance of *S. aureus* by resident and infiltrated cells. **Financial support**: Capes, CNPq and FAPEMIG. Protocol CEUA/UFMG 236/2012

04.092

Strontium ranelate does not interfere on the process of induced tooth movement in rats. Soares KA[1], Araújo VMA[2], Guimarães MV[2], Melo IM[2], Silva SRL[2], Farina M[1], Lima V[2]
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Introduction: The induced tooth movement (ITM) is characterized by alveolar bone resorption on the compression side and bone deposition on the traction side of periodontal ligament (PL) of a tooth subjected to force (Krishnan *et al.* Am J Orthod Dentofacial Orthop. v129. p469. 2006). Sodium alendronate (SA) induces apoptosis of osteoclasts and reduction of bone resorption, but it has been associated with osteonecrosis of the jaws and reduction of tooth movement (Karras JC *et al.* Am J Orthod Dentofacial Orthop. v136. p.843. 2009). Strontium ranelate (SR) is a newer drug that has a double effect, both by reducing bone resorption, as well as inducing bone formation (Chen B *et al.* Osteoporos Int. v24. p2115. 2013). The aim of this study was to evaluate the acute effect of SR on ITM in rats. **Methods:** 24 male Wistar rats (± 200 g) were submitted to ITM using a nickel-titanium closed spring fixed between the left 1st molar and incisor teeth with wire ligature. The contralateral hemiarcade was used as control. During 4 d groups of animals received by gavage 0.9% NaCl solution (2 ml/kg, Control Group) or SR 630 mg/kg, 30 min before ITM, and daily until the sacrifice. Additional group received a single dose of SA 7 mg/kg 1h before insertion of the spring. Areas of the PL on the traction and compression sides, and hyaline areas were evaluated. The inflammation by measuring myeloperoxidase activity (MPO) was also evaluated. Ethics committee for animal use-UFC nº 100/11. **Results:** In the cross-sectional roots of animals on the 4th day, ITM increased the PL area on the traction side, when related to normal periodontium (Normal=33.5 ± 1.4; Control=45.5 ± 1.9), as well as SA group (48.1 ± 2.1) and SR group (45.3 ± 2.3) ($p<0.05$). On the other hand, in the compression side, ITM reduced the PL area (Normal=32 ± 1.4; Control=19.3 ± 0.6), as well as SA group (20.3 ± 1.5) and SR group (20.6 ± 1.2) ($p<0.05$). On this same side of compression, ITM increased the percentage of hyaline areas (Control=41.9 ± 4.1%), when compared to normal periodontium (0%), while SA group reduced hyaline areas (SA=22.3 ± 4.6%) ($p<0.05$). On the other hand, SR, at a dose of 630 mg/kg, significantly increased hyaline areas (SR=55.3 ± 3.5%), when compared to control or to the positive control SA. At the same time, ITM altered MPO, an indirectly marker of polymorphonuclear neutrophils presence (Marcaccini AM *et al.* Am J Orthod Dentofacial Orthop. v138. p613. 2010), increasing its activity in the gingival (8.7 ± 1.3), when related to the control group (0.9 ± 0.1). SA (1.5 ± 0.3) and SR (4.4 ± 0.8) were both able to prevent the increase of MPO activity ($p<0.05$). **Discussion:** In this study, acutely SR has allowed the rise of hyaline areas and reduced MPO without affecting the onset of tooth movement. This fact can be explained, in part, because strontium ions are readily incorporated into the calcified matrix of these tissues, and its content in bone increases significantly during SR treatment, especially in the newly formed bone observed in the development or in the remodeling process, what can be quite useful for the process of bone remodeling needed for orthodontic tooth movement. **Financial support:** CNPq; Capes.

04.093

Involvement of central ETA and CB1 receptors and arginine-vasopressin release in sepsis induced by cecal ligation and puncture in rats. Leite MCG[1], Lomba LA[1], Brito HO[1], Bastos-Pereira AL[1], Fraga D[2], Zampronio AR[1] [1]UFPR – Inflamação, [2]UFMS

Introduction: We have showed before that endothelins (ET), in particular ET-1, reduced the frequency of spontaneous excitatory currents in vasopressinergic magnocellular cells (MNCs) of rats through activation of ET_A receptors. This effect was abolished by the CB_1 receptor antagonist suggesting the involvement of endogenous cannabinoids (eCB). This study was aimed to verify if this mechanism could be evidenced *in vivo* using a model of severe sepsis, a condition where ET-1 levels are high and arginine-vasopressin levels are diminished. **Methods** Sepsis was induced in Male Wistar rats by cecal ligation and puncture (CLP). All procedures were approved by the institution's Ethical Committee for Animal Use (# 629). Animals were treated with CB_1 receptor antagonist rimonabant (Rim, 10 or 20 mg/kg, p.o.) 4h after CLP (3 punctures), or with BQ123 (100 pmol, i.c.v) 2 h and 4 h, 4 and 8 h, or 8 h or with BQ788 (100pmol, i.c.v.) 4 and 8 h after CLP. Body temperature was measured using SubCue data loggers. The neutrophil migration to the peritoneal cavity, leucopenia, plasma IL-6 and AVP levels were also evaluated. **Results**: Animals treated with CB_1 Rim, 10 or 20 mg/kg, p.o. after CLP (3 punctures) showed a survival rate of 73% at both doses, significantly different from CLP/vehicle group (34%). The treatment of the animals with BQ123 2 h and 4 h, or 8 h after CLP did not significantly improve 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 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 improve survival rate (40% for BQ788-treated group compared to 22% for CLP group). 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. Rim treatment did not change the neutrophil migration to the peritoneal cavity, leucopenia and plasma IL-6 levels induced by CLP. CLP reduced AVP levels at 8 h (38%). The treatment of the animals with Rim, reduced the AVP levels at 6 h (46.8%) and increased AVP levels 12 h (128%) in relation to CLP/vehicle group. BQ123 treatment also increased AVP levels 12 h (72.6%) after CLP. **Discussion**: These results show that the blockade of CB_1 receptors and central ET_A receptors prevented the mortality induced by CLP. The blockade of CB_1 receptors did not affect peripheral responses such as neutrophil migration, leukocytosis and circulating IL-6 levels but reduced the febrile response and changed the AVP blood levels suggesting a central action of this antagonist. The effect of Rim and BQ123 in the survival rate after CLP may be related to an increase in the circulating levels of AVP. Financial support: CNPq and Capes.

04.094

Adenosine A2A receptor role upon adipose tissue inflammation control in an experimental model of obesity. de Oliveira CC[1], Gotardo EMF[2], Caria CRP[2], Nakamitsu PFZ[1], Gambero A[2] [1]Unicamp – Farmacologia, [2]USF – Imunofarmacologia e Biologia Molecular

Introduction: Macrophage infiltration and production of mediators with pro-inflammatory activity by adipose tissue play a role in the establishment and maintenance of a framework of chronic inflammation in obese individuals, an event that precedes insulin resistance in peripheral tissues associated with obesity. Adenosine levels increase during inflammation and modulate inflammatory responses. A2A receptor mediates anti-inflammatory actions while activation of A1 receptors has pro-inflammatory effects. A better understanding of the actions mediated by these receptors may contribute to increase the knowledge about the role of adenosinergic system during obesity. **Methods and Results:** Swiss mice were fed with commercial chow (CN) or high fat diet (HFD) for 12 weeks. In the last two weeks, mice were treated with N^6-cyclopentyladenosine (CPA, selective A1 agonist, 0.05 and 0.1 mg/kg/day), CGS 21680 (CGS, selective A2A agonist, 0.1 and 0.5 mg/kg/day) or 5'-N-ethylcarboxamidoadenosine (NECA, non-selective agonist, 0.05 and 0.1 mg/kg/day). (CEA/USF Protocol 007.09.11). Body weight, adiposity and glucose basal levels were evaluated. Adipose tissue expression of vascular endothelial growth factor (VEGF) was assessed by Western blot. Adipokines were measured by ELISA. Macrophage infiltration in adipose tissue was evaluated by immunochemistry (F4/80 + cells). CPA treated mice presented no reduction in body weight, adipositu or glucose levels. Monocyte chemoattractant protein-1 (MCP-1) levels were reduced (0.96 ± 0.25, 16.49 ± 2.33, 6.13 ± 0.80 for CN, HFD and CPA 0.1, respectively). No changes were observed in Interleukin-10 (IL-10), Tumor necrosis factor alpha (TNF-α) and leptin in adipose tissue. VEGF and F4/80 expression were not modified. After CGS administration, mice presented no alterations in body weight or adiposity. Glucose blood levels were reduced after CGS treatment (123.40 ± 10.80, 224.20 ± 38.37, 144.50 ± 6.02 mg/dL glucose for CN, HFD and CGS 0.1, respectively) as well as, mice was sensible to insulin (3.46 ± 1.21, 1.85 ± 0.68, 3.80 ± 0.43 for CN, HFD and CGS 0.5, respectively). IL-10 and leptin levels were not modified but TNF-α levels in adipose tissue were reduced (0.40 ± 0.09, 0.69 ± 0.11, 0.29 ± 0.08 for CN, HFD and CGS 0.1, respectively) and MCP-1 levels in both doses (0.96 ± 0.25, 16.49 ± 2.33, 7.06 ± 1.82, 6.42 ± 2.68 for CN, HFD, CGS 0.1 and CGS 0.5, respectively). VEGF expression was increased in both doses but F4/80 + cells were reduced. After NECA administration, there were no modifications in the metabolic parameters evaluated. MCP-1 levels were reduced (0.96 ± 0.25, 16.49 ± 2.33, 5.45 ± 1.17 for CN, HFD, NECA 0.05, respectively). **Conclusions:** Activation of A2A receptor mediates anti-inflammatory actions in adipose tissue during obesity probably through macrophage infiltration inhibition and it could contribute to the improvement in the metabolic parameters. **Financial support:** Capes and Fapesp.

04.095

Polysaccharides *Caesalpinia ferrea* decreases acute inflammation in 5-fluorouracil-induced intestinal mucositis in mice. Alcântara JR[1], Morais JAV[2], Sampaio LP[2], Franco AX[2], Costa DVS[2], Martins CS[2], Morais PAF[2], Soares PMG[2], Souza EP[2] – [1]UECE, [2]UFC – Morfologia

Introduction: Intestinal mucositis is a disorder that appears as an adverse effect of radiotherapy and chemotherapy treatments. Among the anticancer drugs widely used in chemotherapy treatment is 5 -fluorouracil (5FU). *Caesalpinia ferrea* (*C. ferrea*), also popularly known as Juca, is a large tree that has been widely used for the treatment of organic disorders due to the antioxidant and anti-inflammatory activities. The present study aimed to examine the anti-inflammatory response of total polysaccharides extracted from the bark of this plant, by analyzing the activity of myeloperoxidase (MPO), an enzyme localized in intracellular granules of neutrophils and used as an indicator of acute inflammation. **Methods:** Polysaccharides (PLT) from the bark of *C. ferrea*, extracted and purified, were used for treatment in 5 – fluorouracil-induced intestinal mucositis in mice. All animals were randomly divided into five groups (n = 8 per group): saline (control), saline + 5FU, 5FU + PLT (1 mg/kg), 5FU + PLT (5 mg/kg) or 5FU + PLT (15 mg/kg). In frist day, was induced intestinal mucositis (5FU: 450 mg/kg, single dose, intraperitoneally), immediately after the animals were treated with PLT (1, 5 or 15 mg/kg, single dose, gavage) during four days. In fifth day, all animals were sacrificed and jejunum and ileum segments were removed for analysis of MPO. Data were presented as mean ± standard error of the mean (SEM) with application of analysis of variance (ANOVA) followed by Bonferroni test. Results: PLT *C. ferrea* statiscally reduced MPO levels, expressed as absorbance at 450nm, in both segments ($p<0.05$), whereas the jejunal portion only significant difference with the PLT intermediate dose (5 mg/kg) in relation to 5FU group (mean MPO levels in the control group: 0.134, 5FU: 0.160; 1 mg/kg dose: 0.144; 5 mg/kg dose: 0.131; 15 mg/kg dose: 0.154); already in the ileal portion of all three doses had significant difference compared to 5FU (mean MPO levels of the control group: 0.157; 5FU: 0.186; 1 mg/kg dose: 0.136; 5 mg/kg dose: 0.145; 15 mg/kg dose: 0.159). Conclusion: Our results suggest that the bark PLT *C. ferrea* provided a protective effect against acute inflammation in the intestinal mucositis induced by 5FU, reducing the neutrophil infiltration. Number of the Animal Ethics Committees: protocol nº 66/13. Department of Physiology and Pharmacology, Federal University of Ceará.

04.096

Experimental alcoholic pancreatitis: new therapeutic approaches from natural products.

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Acute pancreatitis is a common debilitating disease caused predominantly by gallstones and alcohol. This disease is characterized by severe abdominal pain and elevated serum amylase and lipase, then the increase in inflammatory markers, such as TNF – α, IL-1β and IL-6 and production of reactive oxygen species, leading to lipid peroxidation, which results in a severe form of the disease associated with the syndrome of multiple organ failure and death. Based on *in vitro* and *in vivo* studies that demonstrate changes caused by alcohol and one of its metabolites by a non-oxidative pathway, the palmitoleic acid (POA), this study aimed to validate a model of acute alcoholic experimental pancreatitis in mice *Swiss* and to test the effect anti-inflammatory of total extract (TPE) non-sulfated polysaccharides from *Caesalpinia ferrea*, and a sulfated polysaccharide, the Fucoidin, in the proposed model. Male animals were divided into 6 groups, one group received ethanol (1.35 g/kg), another received palmitoleic acid (150 mg/kg) diluted with ethanol (in the ratio 2:3), another which received saline, and other three receiving the same substances above, but that were pre-treated with either the TPE of *C. ferrea* (1 mg/kg) 30 min before induction of pancreatitis or fucoidin (25 mg/kg) 30min before and 30min after the induction of pancreatitis. After 24 hours, serum levels of amylase, lipase, malondialdehyde (MDA) and level of glutathione (GSH) and activity of myeloperoxidase (MPO) in the pancreas were measured. In addition, it was performed histological analysis to determine the capability of these natural products in modulating inflammation, being evaluated edema, infiltration of inflammatory cells and necrosis. This study was approved by CEPA – UFC, with 98/2013 number protocol. Parametric data were expressed as Mean ± SEM and statistical analysis by ANOVA and Bonferroni test. Non-parametric data were expressed as median (minimum and maximum) and statistical analysis by Kruskall-Wallis test. In all trials n=6-8, $p<0.05$. The model used by us was effective in inducing pancreatic changes similar to those found in the clinic. There was an increase of serum amylase in use isolated of alcohol (4694 ± 2694 µL/dL) or combined with POA (6160 ± 2453 µL/dL) versus control (3336 ± 1898 µL/dL) and MPO activity (control 3,153 ± 2,167 UMPO/mg of tissue; alcohol 17,20 ± 16,46 UMPO/mg of tissue and POA, 19,57 ± 12,5 UMPO/mg of tissue). However, lipase and serum MDA were increased only in the group with POA (278,2 ± 15,85 UI/L versus 258,1 ± 32,87 UI/L). Both alcohol (17,02 ± 6,098 GSH/g of tissue) and POA (14,26 ± 5,72 GSH/g of tissue) were able to decrease serum levels of GSH compared to control (31,90 ± 5,470 GSH/g of tissue). Treatment with sulfated polysaccharide of *C. ferrea* did not attenuate inflammatory parameters analyzed, however treatment with fucoidin reduced myeloperoxidase activity and histological parameters to similar levels to the control in the pancreas, in groups, the alcohol and the POA. This polysaccharide did not interfered in serum levels of MDA nor GSH level in the pancreas. We conclude, therefore, that the model of intraperitoneal injections of alcohol combined or not with POA, is able to induce experimental pancreatitis in *Swiss* mice and fucoidin exerts anti-inflammatory effect, despite not having shown antioxidant effects in the models evaluated. **Financial support**: PPSUS-CE (FUNCAP/SESA/MS/CNPq).

04.097

Acute pancreatitis induced by taurolithocholic acid cause inflammatory and functional changes in the lung. Oliveira TB[1], Morais CM[1], Justino PFC[1], Fiorio BC[1], Damasceno SRB[1], Morais PAF[1], Menezes KLS[1], Loiola AN[1], Morais JAV[1], Mendonça VA[1], Xavier AF[1], Ribeiro RA[1], Souza MHLP[1], Barbalho JVM[1], Girão DKFB[1], Criddle DN[2], Soares PMG[3] [1]UFC – Farmacologia, [2]University of Liverpool, [3]UFC – Morfologia

Introduction: Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are a severe complication to acute pancreatitis (AP) and a significant health problem associated with a considerable mortality. **Objectives:** To assess the inflammatory and functional alterations of the lung in the course of AP induced by retrograde infusion of taurolithocholic acid sulfate (TLC-S) into the pancreatic duct. **Methods:** Male Swiss mice (weight, 25-30g) were assigned to saline (S), sham (SH), TLC-S (T) groups. TLC-S (50 µL, 3%) was retrogradely infused into the mouse pancreatic duct. In the saline group, the procedures were performed identically, except for the injection of TLC-S, which was replaced with saline solution. Sham-operated animals served as a control. Twenty-four hours after induction of PA, animals were anesthetized, tracheostomized and connected to a spirometer specific for small animals. The following parameters were evaluated: Flow Rate (F), Tidal Volume (TV), Respiratory Frequency (RF) and Minute Volume (MV). Bronchoalveolar lavage (BAL) was performed to total cell count. Venous blood samples were collected to evaluate amylase and lipase. The study was approved by Animal Ethics Committees of Federal University Ceará under license 79/2013. **Results:** Serum levels of amylase(S=5670 ± 106.2; SH=5810 ± 811.2; T=8987 ± 163.3, lipase (S=573.1 ± 45.59;SH=581.1 ± 41.85;T=987.4 ± 049.2), There was a 2.04-fold increase in the number of BAL cells. F (S=22.46 ± 0.79; SH=21.24 ± 17.91; T=16.40 ± 10.63), TV(S=0.26 ± 0.01;SH=0.25 ± 0.02;T=0.21 ± 0.01) and MV (S= 43.94 ± 3.24; SH=40.81 ± 4.89; T=31.26 ± 2.83) were lower in animals with AP; RF remained unchanged. **Conclusion:** AP induced by retrograde infusion of TLC-S causing inflammatory and functional changes in the lung of male mice Swiss. **Financial Suport**: Capes and CNPq.

04.098

P and L-selectins inhibition prevents the development of ifosfamide-induced hemorrhagic cystitis in mice. Dornelas-Filho AF[1], Fernandes C[1], Teixeira MA[1], Wanderley CWS[1], Pinto FMM[1], Wong DVT[1], Silva RO[1], Sousa NRP[1], Almeida PRC[2], Ribeiro RA[1], Lima-Júnior RCP[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Pathology and Forensic Medicine

Introduction: Ifosfamide (IFS) is an alkylating agent with a broad spectrum of antineoplasic activity. The main adverse reaction is a dose-limiting Hemorrhagic Cystitis (HC) ocorring due to direct contact of its toxic metabolite acrolein with uroepithelium, causing edema, ulceration, neovascularization, hemorrhage and necrosis. The involvement of inflammatory mediators, such as nitric oxide (NO), IL-1β and TNF-a in IFS-HC has been reported by our group. However, the involvement of P and L-selectin in the pathogenesis of IFS-induced HC has not been elucidated yet. We aimed to investigate the possible protective effect of fucoidan, an inhibitor of P and L-selectin, against IFS-induced HC. **Methods:** Female Swiss mice (25g) were divided into groups (n=8) and treated with saline (5ml/kg) or IFS (400 mg/kg, i.p) or pre-treated with fucoidan (10mg, 30mg and 100 mg/kg, i.v) 30 min. previously IFS treatement (400 mg/kg, i.p). After 12 h, animals were euthanized, and the bladders were removed to evaluate the Bladder Wet Weight (BWW, mg/20g body weight), histopathologic analysis (edema and hemorrhage), myeloperoxidase activity (neutrophils/mg of tissue), and Vesical Vascular Permeability (VVP, pg of Evans blue\mg of tissue). Visceral nociceptive response was tested by mechanical stimulation of the abdomen using Von Frey test. Statistical analysis was performed using One Way Analysis of Variance (ANOVA) followed by Newman-Keuls test or Bonferroni. Macroscopic and microscopic scores were evaluated by Kruskal Wallis followed by Dunn's multiple comparison. Statistical significance was set at $P<0.05$. (CEPA 06/06) **Results:** The pre-treatment with fucoidan reduced the BWW in the doses of 10mg, 30mg and 100 mg/kg (54.47 + 13.44, $P<0.05$; 48.43 + 14.01, $P<0.001$; 45.5 + 11.34, $P<0.001$, respectively *vs* IFS 70.52 + 18.28) and also reduced VVP in the doses of 10mg, 30mg and 100mg (46.18 + 26.26, $P<0.01$; 41.60 + 16.39, $P<0.001$; 46.62 + 23.03, $P<0.01$, respectively *vs* IFS 110.9 + 46.62). The myeloperoxidase activity was reduced by fucoidan pre-treatment in the dose of 100 mg/kg (552.7 + 203.6 *vs* IFS 1269 + 706.2, $P<0.01$). In addition, edema and histopathological scores were decreased in 100 mg/kg pre-treated mice [1(1-2) vs IFS 3(2-3), $P<0.05$; 1(1-1) vs IFS 3(2-3); respectively]. The levels of IL-6 and IL-1β were reduced in the fucoidan pre-treated group (IL-6: 203 ± 82.98 *vs* IFS 432.1 ± 123, $P<0.05$; IL-1: 3.59 ± 2.61 vs IFS 11.42 ± 6.16, $P<0,001$). Furthermore, IFO-induced hypernociception was reduced by treatment with fucoidan (100 mg/kg) (IFS 1.8 ± 1.2 *vs* 4.83 ± 1.57, $P<0.01$). **Conclusion:** P and L-selectins inhibition attenuates experimental IFS- induced Hemorrhagic Cystitis (HC), suggesting the role of neutrophils in the pathogenesis of IFS-induced HC. **Financial Support:** CNPQ, Capes e FUNCAP.

04.099

Protective effect of N-acetylcysteine on irinotecan-induced experimental steatohepatitis.

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Introduction: Irinotecan (IRI)-based anticancer regimens have been used for the treatment of metastatic Colorectal Cancer. However, these regimens are associated with the occurrence of non-alcoholic steatohepatitis (NASH), which might limit the treatment. Recently, our group developed a novel IRI-induced mouse NASH model that suggested the involvement of interleukin-1 (IL-1), inducible nitric oxide synthase (iNOS), and Toll-like 4 receptor (TLR-4). Currently, there is no effective therapeutic option for IRI-related NASH. N-acetylcysteine (NAC), an antioxidant agent, is used in clinical practice for the treatment of liver damage induced by paracetamol. Then, we aimed to assess the protective effect of NAC on IRI-induced NASH. **Methods:** Male *Swiss* mice (25 g, n = 8) received saline (5 mL/kg, i.p), NAC (1,000 mg/kg, s.c), IRI(50 mg/kg, i.p), or NAC (10, 100, or 1000 mg/kg) + IRI 3x/week for 7 weeks. Weight variation and survival rate were assessed. At the 7th week, blood was collected for the determination of serum levels of ALT and AST (U/L), and the animals were killed for liver collection and weighing (mg/30 g of animal). Histopathological analysis was performed according to Kleiner's criteria for NASH (lobular inflammation [0–3], steatosis [0–3], and vacuolization [0–3]), as well as measurement of IL-1β production (pg/mL) and immunohistochemical analysis (IHC) of IL-1β and iNOS. For statistical analysis, ANOVA/student's Newman Keul test or the Kruskal Wallis/Dunn test was used. The level of significance was set at $p<0.05$. (CEPA 21/12). **Results:** IRI induced a significant ($p<0.05$) reduction in animal survival (44%), a marked increase in the serum concentrations of ALT (48.99 ± 13.4), AST (90.55 ± 19.7), local production of IL-1β (288.5 ± 35.86), liver wet weight (1,814 ± 159.3), and an increase in Kleiner's scores [5.5 (4–7)] *vs.* the saline group (survival: 100%; ALT: 17.31 ± 5.2; AST: 44.58 ± 5.4; IL-1β (104 ± 36.9); liver wet weight: 1,425 ± 39.5; Kleiner's scores: 0(0–0). Interestingly, NAC (10 mg/kg and 100 mg/kg) increased ($p<0.05$) the survival of animals injected with IRI (90% and 80%, respectively). In addition, NAC 10 mg/kg prevented the increase of ALT (26.95 ± 7) and AST (56.42 ± 4.8), liver wet weight (1,37 ± 68.2), inhibited tissue production of IL-1 (152 ± 23.92), and prevented the increase in Kleiner's scores (2[1–4]) *vs.* the IRI group. Shortening of the villi and the alteration of crypt size and architecture were also observed in IRI group (4[3-4]) compared with control group (0[0-1]). Furthermore, NAC (10 mg/kg and 1000 mg/kg) prevented these effects (1[0-1]; 0[0-1], respectively) *vs.* the IRI group. The IHC of the IRI group showed a significant increase in immunostaining for IL-1 (3[1–3]) and iNOS (3[3–3]) compared to the saline group (IL-1: 0[0–1]; iNOS: 1[1–2]), which was prevented ($p<0.05$) by NAC (10 mg/kg) (IL-1: 1[1–1]; iNOS:2[1–2]). **Conclusion:** Our study suggests that NAC prevents IRI-induced NASH, which seems to partially involve the maintenance of gut barrier integrity. **Support**: CNPq, Capes and FUNCAP.

04.100

Evaluation of safety and efficacy of the dry extract capsule of *Amburana cearensis* A C Smith in human neutrophils. Rocha TM, Lopes AA, Magalhães HIF, Silveira ER, Viana GSB, Leal LKAM

Introduction: *Amburana cearensis* AC Smith is a tree native of caatinga, referred to popularly as cumaru. The trunk bark and seeds are used frequently in the treatment of respiratory diseases such as bronchitis and asthma. Chemical and pharmacological studies conducted by our group made it possible to determine the antinociceptive, anti-inflammatory and bronchodilator potential of extracts and chemical constituents coumarin and amburoside of the stem bark of *A. cearensis*. The purpose of this study was to investigate the toxicity and the effect of phytotherapic, dry extract capsule of *A. cearensis* (CESAC), pro-inflammatory mechanisms in human neutrophils. **Methodology:** Polymorphonuclear cells predominantly neutrophils (80-90%) with feasibility of 90 ± 2.0% (excluding by tripan blue test) were obtained from human blood/subproduct ceded by the center of Hematology and hemotherapy in Ceará, according to the methodology described by Lucisano; Mantovani (1984) (CEP: Protocol N° 218/10). In assessing the toxicity/pattern of death (apoptosis and necrosis) were employed dyes acridine orange or ethidium bromide (LA/BE) (100 µg/mL). In the suspension of neutrophils ($2{,}5x10^6$cells/mL) were added the CESAC (10, 100 and 200 µg/mL), DMSO (control: 0.4%), HBSS (untreated cells) or Triton X-100 (cytotoxic drug-0.2%), and analyzed with the microscope Nikon with filters for fluorescence (515-560nm). To evaluate the effect of neutrophil degranulation (2, $5x10^6$cells/mL), these were incubated with the CESAC (5,10,25,50,100and200µg/mL), indomethacin (36µg/mL), DMSO or HBSS, and then the cells were activated by addition of PMA (0,1 µm) with consequent release of myeloperoxidase (MPO) and TNF-α, which were measured according to the methodology described by Úbeda *et al.* (2002) or guidelines described by the manufacturer (Bioscience, USA), respectively. **Results and discussion:** In the essay LA/BE, the CESAC at concentrations of 10,100 and 200µg/mL showed significant reduction in the number of viable cells (82,67 ± 0.9379,50 ± 0,67 and 70,83 ± 3,8%; respectively) when compared to cells in apoptotic process (11.42 ± 0.77 13.75 ± 0.56 and 20.75 ± 3.36%; respectively) and necrotic (5.91 ± 0.55; 6.83 ± 0.98 and 0.55 ± 8.41%; respectively). In the release of MPO, the CESAC (5,10,25,50,100 and 200 µg/mL) promoted inhibition of neutrophilic degranulation until 72% (CI_{50} = 13,36 µg/mL). In measuring the levels of TNF-α, the CESAC (5,10,50,100 and 200 µg/mL) promoted the reduction of the levels of this cytokine in up to 98% (CI_{50} = 6,85 µg/mL). **Conclusion:** In acridine orange test the CESAC showed a relative cytotoxicity, possibly related to the presence of coumarin in the plant. The CESAC showed an *in vitro* anti-inflammatory activity, related to the inhibition of neutrophil activation, in addition to the reduced levels of TNF-α. **Thanks financial support:** Capes, CNPq, FUNCAP.

04.101

Paradoxical effect of protease-activated receptor (PAR)-2 and 4 on macrophage phagocytosis *in vitro*. Barra A, Siqueira MVA, Matos NA, Freitas KM, Lopes MTP, Klein A
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Introduction: Proteinase-activated receptors (PARs) are G-protein-coupled receptors activated by serine proteinases, through their proteolytic cleavage at a specific site on the N-terminal amino acid sequence of the receptor. PARs are a family of four receptors namely PAR 1 to 4 and their activation has been shown to be implicated in many signs of inflammation. PARs are expressed on the surfaces of various cell types including leukocytes. No study has investigated the involvement of PARs in macrophage-mediated phagocytosis so far. Our goal was, then to investigate the role of PAR-2 and PAR-4 on the *in vitro* phagocytic activity of macrophages. **Methods:** C57Bl/6 (18-22g) mice were injected intraperitoneally (i.p.) with 6% thioglycolate and peritoneal cells were harvested after 72 h, by peritoneal lavage with phosphate buffered saline (PBS). Cells were centrifuged at 100 g for 4 min. Subsequently, the pellet was resuspended in culture medium for further use. The macrophages were incubated with LPS (10µg/ml) for 30 minutes. At the end of this period, increasing concentrations of the PAR-2 agonist SLIGRL-NH_2 (0.1-30 µM) or PAR-4 agonist GYPGK-NH_2 (0.1-30 µM) was added for 30 min in the presence or absence of their respective antagonists added 30 min beforehand (PAR-2: ENMD-1068, 30µM; PAR-4: AY-NH_2, 30µM), followed by incubation with zymosan 10 µg/ml for more 1 h. The index of phagocytosis was defined as the ratio between the total number of particles phagocytosed by the percentage of cells that phagocytosed. Statistical analysis was performed using one-way ANOVA followed by Tukey post-test. Experimental procedures were approved by the local animal ethics committee (protocol number 142/2014). **Results:** A concentration-dependent of the activation of PAR-2 receptor by SLIGRL-NH_2 led to an increase of the phagocytic activity of macrophages compared with controls (Ctrl, 4,85 ± 0,327; 0.1 µM, 5,52 ± 0,494; 0.5 µM, 6.06 ± 0,508; 1µM, 6,21 ± 0,476; 5µM, 6,53 ± 0,296; 15µM, 6,20 ± 1,26; 30µM, 8,20 ± 1,37*; *P<0.01). This increase was reversed by increasing concentrations of the antagonist ENMD-1068 in the presence of 30µM of SLIGRL- (Ctrl, 2.70 ± 0.167, 0 µM, 6.75 ± 0.150*; 0.1 µM, 3.75 ± 0, 25 *, 0.5 µM, 2.60 ± 0.20 *; 1µM, 2.62 ± 0.539 *; 3µM, 3.73 ± 1.13; 10µM, 3.93 ± 0.62; 30µM, 3.76 ± 0.603; 100µM, 2.88 ± 0.706; *P<0.05). In contrast, activation of PAR-4 by the agonist GYPGK-NH2 reduced the phagocytic activity which was concentration dependent (Ctrl, 4.75 ± 0.423, 0.1 µM, 4.45 ± 0.672, 0.5 µM, 4.48 ± 0.304; 1µM, 4 05 ± 1.08; 5µM, 3.75 ± 0.679, 15µM, 3.78 ± 0.922, 30µM, 2.58 ± 0.778 *, * P <0.05) **Discussion:** These results indicate a role for PAR receptors on the phagocytosis by *in vitro* macrophages. Once activation of these receptors showed an increase or a decrease in phagocytic activity of macrophages, our data suggest a complex modulatory role of PARs receptors under this condition. We suggest these receptors as attractive targets for modulation of diseases in which phagocytosis is an essential component. **Financial support:** CNPq / Fapemig.

04.102

Anti-inflammatory effects of Hypnea cervicornis aglutinin in rat experimental arthritis.

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Introduction: Rheumatoid arthritis (RA) is the most common chronic inflammatory arthropathy in humans, in which the joint pain is a frequent finding. Based on several *in vitro* studies reporting the anti-inflammatory effects of glycoconjugate-binding lectins, the activity of an agglutinin isolated from the red alga *Hypnea cervicornis* (HCA) was evaluated in the rat model of zymosan-induced arthritis. **Methods:** Male Wistar rats (200-220 g) (CEUA: 3207571/2014) received zymosan (Zy: 500 µg/25µL) or sterile saline into the right tibio-tarsal joint. A pressure was applied to the center of the paw with a wide area probe (4.15 mm^2) coupled to a digital analgesimeter, in order to cause joint flexion and paw withdrawal. To exclude the activation of local mecanonociceptors, a group of animals received lidocaine 2% (100 µL s.c.). To characterize the hypernociception, animals received indomethacin (5 mg/kg i.p., 30 min prior Zy) or morphine (4 mg/kg i.p., 5.5 h after Zy). HCA (1-3 mg/kg i.v.) was administered 30 min or 5.5 h after Zy. Animals were euthanatized 6 h after Zy or in the 14th day. Intra-articular fluid was collected for leukocyte counts and cytokines/growth factors determination. Data represent mean ± SEM of 8 animals per group. $P<0.05$ was considered significant (One-way ANOVA followed by Bonferroni's test). **Results:** Zymosan significantly reduced the withdrawal pressure threshold from the 4th hour until the 14th day. At the 6th hour, the Zy group displayed an increased number of leukocytes (46693 ± 8126 cells/mm^3) compared to the saline group (362 ± 53 cells/mm^3, $p<0.05$). In the 14th day, the number of leukocytes remained increased on zymosan group (650 ± 143 cells/mm^3 *vs* 15 ± 3 cells/mm^3, $p<0.05$). Increased levels of pro-inflammatory [IL-1β (45-fold), IL-6 (23-fold), TNF-α (4-fold)], immunomodulatory [IL-5 (2.5-fold)] and anti-inflammatory [IL-4 (4-fold); IL-13 (7-fold)] cytokines or growth factors [GROCK (15.5-fold), VEGF (8-fold), GM-CSF (7-fold), M-CSF(4-fold), MCP-1 (3-fold)] were detected in the fluid of Zy group. The paw withdrawal pattern was not altered by lidocaine. Morphine (62.02 ± 5.50 g) and indomethacin (59.59 ± 8.24 g) significantly reversed hypernociception evoked by zymosan (20.87 ± 2.54 g) at the 6th hour. The post-treatment with HCA (3 mg/kg) increased the pain threshold (37.36 ± 5.45 g) compared to zymosan (20.87 ± 2.54 g, $p<0,05$), and reduced the leukocyte migration (8869 ± 4552 cells, $p<0,05$). **Discussion:** Zymosan injected into the tibial-tarsal joint of rats evoked an analgesic-responsive hypernociception not related to stimulation of cutaneous mechanoreceptors, along with leukocyte influx and cytokines/growth factors release. HCA post-treatment reversed the acute inflammatory effects elicited by zymosan, revealing to be a promissing tool for arthritis study in animal models. **Financial** Agencies: Capes, CNPq.

04.103

Ferulic acid ethyl esther diminished knee incapacitation induced by carrageenan in rats

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Introduction: The inflammation is an adaptive physiologic response triggered by noxious stimuli or conditions as infection and tissue lesion. Success depends on a complex process from cell and humoral agents as neutrophils, leucocytes, mast cells and prostaglandins, interleukins and production of reactives oxygen and nitrogen species as nitric oxide. Drugs utilized to the treatment of inflammation as non-hormonal anti-inflammatory could be noxious to gastrointestinal tract and corticosteroids have many side effects specially when utilized by long time. Ferulic acid ethyl esther (FAEE) is an derivate from ferulic acid that have anti-inflammatory action. Previous studies indicate FAEE have more absorption than ferulic acid. The aim of this research was to evaluate the effect of FAEE on time of elevation paw and NO_3^- in carrageenan induced incapacitation in knee of rats. **Methods**: Wistar rats (200-220 g) and Swiss mice (20-25 g) were utilized for this experiment. Ethic Committee to animal research from UFPI (UFPI) early approved all proceedings CEEA/PI n° 08/2012. The incapacitation was induced by administration of carrageenan (100 ug). One hour before the animals (6 animals per group) were treated orally with vehicle (saline), indomethacin (10 mg/kg) and FAEE (25, 50, 100 mg/kg). The time of elevation paw (TEP) was accessed one hour before induction and every hour to fiftieth hour and twenty-four hours after induction during one minute each animal (Tonussi, Pain 48: 421 1992). In the end of the experiment, animals were euthanatized and blood was collected, centrifugated and the serum was utilized to measure levels of NO_3^- (nM) by Griess metrod (Green, Analytical Biochemistry 126: 131, 1982). In order to evaluate the central depressive effect, the open field test was performed to study spontaneous locomotion (number of invasions). Data of incapacitation were analyzed by Two way ANOVA and Bonferroni *post hoc* tests and One-way ANOVA followed by Tukey post hoc for levels of NO_3^- and open field with support of GraphPad Prism 6.0. The results are expressed by media ± standard error media ($p<0.05$). **Results and discussion:** Animals treated with FAEE (**25-** 1^{st}36.68 ± 1.8, 2^{nd}47.46 ± 2.63, 3^{th}48.5 ± 2.86, 4^{th}47.95 ± 2.14, 5^{th}46.06 ± 4.12; **50-** 1^{st}39.65 ± 2.32, 2^{nd}33.14 ± 5.97, 3^{nd}40.07 ± 2.97, 4^{th}40.35 ± 2.81, 5^{th}50.04 ± 3.19; **100-** 1^{st}43.51 ± 2.95, 2^{nd}33.67 ± 2.36, 3^{th}24.14 ± 2.29, 4^{th} 30.73 ± 2.01, 5^{th}36.32 ± 4.15) presented minor TEP than **control group** (1^{st}43.36 ± 3.29; 2^{nd}48.71 ± 2.68; 3^{th}51.95 ± 2.32; 4^{th} 56.32 ± 1.33; 5^{th}51.62 ± 2.52) in 2^{nd}, 3^{th}, 4^{th} and 5^{th} hours ($p<0.05$). Furthermore, these animals present less NO_3^- (**25** 8.040 ± 3.455; **50-** 6.302 ± 1.927; **100-** 4.822 ± 1.355) than control group (16.55 ± 1.295) but not in relation to indomethacin (2.627 ± 1.011). In open field test, animals treated with FAEE had no depressant effect (**50**:42.67 ± 5.72 and **100**:39.33 ± 5.83) in comparison with diazepam group (7.00 ± 1.069). The results suggest that FAEE (25, 50 ou 100 mg/kg) presents anti-inflammatory activity on carrageenan induced incapacitation. **Financial Support:** UFPI/Capes

04.104

Anti-inflammatory potential *in vitro* of isolated mixture of furan diterpenes of *Pterodon polygalaeflorus*. Leal NRF, Velozo LSM, Sousa TV, Vigliano MV, Coelho MGP UERJ

Introduction: Medicinal plants have been a useful source for the research of new biologically active compounds. Alcoholic extracts from fruits of Pterodon genus are commonly used in folk medicine as anti-rheumatic, anti-inflammatory and analgesic preparations[1,2]. Phytochemical studies of Pterodon genus have revealed the presence of alkaloids, isoflavones and diterpenes. Furan diterpenes were identified and isolated from Pterodon species.[3-7]. Studies have reported that the presence of furan diterpenes with skeleton vouacapan is involved with the anti-inflammatory, antinociceptive and antiproliferative properties of *Pterodon pubescens* crude extract[7-10]. In this study, the *in vitro* cytotoxic and anti-inflammatory effects of a furan diterpenes mixture from *Pterodon polygalaeflorus* extract (Ppg-02) were evaluated on RAW 264.7 macrophage cell line. **Methods:** The fruits of *Pterodon polygalaeflorus* were pulverized and subjected to static maceration with dichloromethane for 15 days to obtain extract. During evaporation of the solvent, it was observed the formation of a white precipitate in the extract. The precipitate was subjected to crystallization/recrystallization technique resulting in a white solid residue, which was identified as a mixture of furan diterpenes isomers (Ppg-02): methyl 6α-hydroxy-7β-acetoxy-17β-vouacapan-oate and methyl 6α-acetoxy-7β-hydroxy-17β-vouacapan-oate by gas chromatography coupled to mass spectrometer (GC-MS) analysis. Their structures were confirmed by comparison with the literature data. For *in vitro* analysis, RAW 264.7 cells were incubated for 24 h with LPS in the presence or absence of Ppg-02 (0.1 µg/mL to 50 µg/mL) and the nitric oxide production was measured indirectly by determining its metabolite, nitrite, in the supernatants by Griess reaction. RAW 264.7 cells were also incubated for 24 h with or without LPS, in the presence or not of Ppg-02 (0.1 µg/mL to 50 µg/mL). After 24 h, tetrazolium salt (MTT) was added to the plate and then incubated for 2 h. Afterwards, SDS 10% was added to the plate. The cell growth/cytotoxicity of macrophages was expressed as percentage of mitochondrial reducing activity (MRA) compared to control. **Results:** Ppg-02 decreased ($p<0.001$) 12.89%, 51.68% and 80.54% (10; 25; 50 µg/mL, respectively) of nitrite production by RAW 264.7 macrophages stimulated with LPS, when compared to the LPS-stimulated cells (control). The concentration required to obtain 50% of the effect (IC_{50}) on nitrite production was 23.4 µg/mL. Ppg-02 showed a significant cytotoxic effect on RAW 264.7 macrophages only at higher concentration. The percentage reduction of MRA ($p<0.001$) of macrophages in the absence of LPS was 46.6% at 50 µg/mL. Otherwise, LPS-stimulated cells showed MRA inhibiton ($p<0.05$) of 8.03%, 11.21%, 14.09%, 15.45% and 34.47% (0.1; 1; 10; 25; 50 µg/mL, respectively). **Discussion:** This study demonstrated the anti-inflammatory potential of substances with high purity (85%) by reducing the *in vitro* production of important inflammatory mediator (nitric oxide) at concentrations with low cytotoxicity. Thus, the previews results suggest a promising therapeutic approach for subsequent studies in inflammatory models. **Financial support:** Faperj, CNPq. **References:** 1. Pio Correa, M.; Dicionário das Plantas úteis do Brasil e das Exóticas Cultivadas, vol. I, 1975. 2. Lorenzi, H.; Árvores Brasileiras. Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil, vol. 1, 1998. 3. Mahjan, J. R.; *J. Chem. Soc. Perkin Trans* 1, 520, 1973. 4. Fascio, M.; *Phytochemistry* 15, 201, 1975. 5. Campos, A. M.; *Phytochemistry* 36, 403, 1994. 6. Arriaga, A. M.; *Fitoterapia* 71, 211, 2000. 7. Spindola, H. M.; *J Braz Chem Soc* 20, 569, 2009. 8. Silva, M. C. C.; J. Pharm. Pharmacol. 55, 135, 2004. 9. Vieira, C. R.; *Phytomedicine* 15, 528, 2008. 10. Spindola, H. M.; *BMC Pharmacol.* 10, 1, 2010.

04.105

Suppressor of cytokine signaling 2 (SOCS2) protein is involved in the mechanisms that control lung inflammation during infection with the pathogenic fungus *Paracoccidioides brasiliensis*. Santos PC[1], Ribeiro LS[2], Tavares CF[3], De Paula TP[1], Werneck SMC[1], Machado FS[2], Teixeira MM[2], Cisalpino PS[1], Souza DG[1] [1]UFMG – Microbiologia, [2]UFMG – Bioquímica e Imunologia, [3]TBSI – Biochemistry and Immunology

Introduction: *Paracoccidioides brasiliensis* is a thermodimorphic fungus and considered one of the agents of paracoccidioidomycosis (PCM), the most prevalent systemic mycosis in Latin America. PCM development depends on the virulence of infecting strain, the degree and type of immune response triggered, and intrinsic characteristics of the host. Despite the fact that a large number of individuals are exposed to the fungus, only a minority develop the disease. Therefore, the understanding of the protective characteristics of the immune response to *P. brasiliensis* infection is of interest as it may reveal targets for disease control. The suppressor of cytokine signaling (SOCS) proteins are key controllers of cytokine responses, which can down-regulate specific cytokine signals and consequently modify the immune response. In this study, we investigated the role of the suppressor of cytokine signaling 2 (SOCS2) protein in an experimental pulmonary infection by the pathogenic fungus *P. brasiliensis*. **Methods:** Male C57BL/6 wild-type mice (WT) and SOCS-2-deficient mice ($SOCS2^{-/-}$) were used in all experiments (CETEA-UFMG:163/2012). After anesthesia, mice were infected with 10^6 yeasts of Pb18 strain, by intratracheal injection, while uninfected mice received buffered saline by the same route. Mice were evaluated for survival and after three or fifteen days of infection mice were euthanized and their lungs removed for cytokine measurement by ELISA and evaluation of inflammatory infiltrates by myeloperoxidase, N-acetylglucosaminidase and eosinophil peroxidase assays. Moreover, the fungal load was determined in bronchoalveolar lavage fluid and lungs of infected mice. **Results and discussion:** The results showed that the absence of SOCS2 resulted in death of 100% of infected mice while no WT mice died after 30 days of infection. The high susceptibility of $SOCS2^{-/-}$ mice was associated with higher pulmonary fungal load, macrophage and eosinophil accumulation in the lungs and significant production of inflammatory cytokines such as TNF-γ, IL-6, IL-12 and IL-13, compared with WT mice. Furthermore, it was observed that the inflammatory phenotype found in $SOCS2^{-/-}$ mice resulted in high lung injury associated with fibrosis. Studies in the literature have shown that SOCS2 proteins are essential drivers of macrophage polarization and differentiation of Th2 and Th17 lymphocytes. Therefore, it is thought that a deficiency of this protein may alter the development of these cell types and consequently result in a susceptibility profile during infection by *P. brasiliensis*. **Financial Agencies:** CNPq, Capes, FAPEMIG.

04.106

Role of phosphoinositide 3-kinase gamma and platelet activating factor receptor signaling in an experimental model of fever. Ribeiro LS[1], Santos PC[2], Machado RR[3], Souza DG[2], Teixeira MM[1] [1]UFMG – Bioquímica e Imunologia, [2]UFMG – Microbiologia, [3]UFMG – Produtos Farmacêuticos

Introduction: Control of body temperature is thought to contribute to immune activity. In endothermal animals, this modulation is finely tuned by the hypothalamus, with local production of cytokines and lipid mediators. Fever is a response of the hypothalamic setpoint towards the detection of pyrogens either from infectious microrganisms or produced by the own body during an inflammatory reaction. Phosphoinositide 3-kinase gamma (PI3Kγ) is an enzyme downstream to G protein coupled receptors and its signaling leads to intracellular pathways like cell growth, survival, trafficking and gene activation. Platelet-activating factor (PAF) is a lipid mediator mostly produced by leukocytes. PAF acts on its receptor (PAFR) resulting in leukocyte activation, changes in vascular permeability and cytokine release. Since these structures are so important within the inflammatory process, the main goal of this work was to assess their importance for the development of fever in mice after pyrogen administration. **Methods**: 8 to 12 week, C57/B6 (wild-type), PI3Kγ- or PAFR-knockout mice were kept in an acclimatized room at 28 – 30 ºC, with chow and water *ad libitum* (CEUA-UFMG 355/2012). After a week in this environment, a temperature datalogger was surgically implanted inside their peritoneum. After recovery for 7 days, the animals were injected LPS (2,5 mg/kg, i.p.) and 6, 12 and 24 hours later, blood and hypothalamus were drawn for cytokine evaluation and qPCR. The datalogger was also recovered for analysis of temperature data recorded. **Results**: After LPS injection, wild-type mice presented a significant increase in the core temperature, peaking after 6 hours, while PI3Kγ$^{-/-}$ mice showed no signs of fever for the next 24 hours. PAFR$^{-/-}$ mice showed a delayed response, with maximum temperatures reached at 10 hours after stimuli. In the plasma, LPS injection also induced high levels of TNF-α in wild-type mice at the time of 6 hours p.i., but not in the PI3Kγ or PAFR knockout counterparts. In addition, IL-1β concentrations remained elevated in PAFR KO mice at the time of 12 hours, similar to that found in WT mice in 6 hours, endorsing the overdue peak of fever found in those animals. In the hypothalamus, mice lacking PI3Kγ or PAFR showed significantly lower levels of cicloxigenase-2 (COX-2) transcripts at the time of 6 hours, in comparison to the WT group. The amount of mRNA from inflammatory cytokines as IL-6 and TNF-α was also diminished in the knockout mice, compared to wild-type. **Discussion:** The PI3Kγ signaling pathway is essential for the induction of fever in mice. The response is associated with a smaller systemic concentration of TNF-α, leading to an reduced expression of COX-2 in the hypothalamus. In other hand, the PAFR knockout mice showed a retarded peak of fever, due to the maintenance of high peripheric levels of IL-1β, with reduced amounts of transcripts for TNF-α and IL-6 in the hypothalamus, associated with reduction in the expression of COX-2 in early time points. **Financial Support:** CNPq, Capes and Fapemig

04.107

Role of IL-1 and TNF receptors in ifosfamide-induced hemorrhagic cystitis in mice. Leite CA[1], Mota JM[2], Mello PH[3], Cunha FQ[3], Ribeiro RA[1] [1]UFC – Physiology and Pharmacology, [2]FMRP-USP – Clinics, [3]FMRP-USP – Pharmacology

Introduction: Hemorrhagic cystitis (HC) is a clinical burden related to ifosfamide (IFO)-based chemotherapy regimens. In spite of high-effective prophylaxis with mesna, some patients can develop subclinical HC. We demonstrated that interleukin-1 (IL-1β) and tumor necrosis factor (TNF-α) might play a role in the HC pathogenesis. In this context, IL-1 receptor (IL-1R) and TNF receptors 1 (TNFR1) and 2 (TNFR2) may be important to HC development. Our study aimed to evaluate the effect of the IL-1R, TNFR1 and TNFR2 in IFO- induced HC in mice. **Methods:** All proceedings were previously approved by local ethics committee (Protocol 11/14). Wild-type (WT) C57BL/6, IL-1R-/-, Caspase-1-/-, TNFR1-/- and TNFR1/R2-/- mice were given IFO (400 mg/kg, ip) and killed 12 h later. Bladders were harvested to wet weight measurement (BWW), macroscopic, histopathological evaluation and flow cytometry to neutrophils ($CD45^+$ $Gr1^{+)}$ and macrophages ($CD45^+$ $F4/80^{+)}$. Furthermore, in another set of experiments, animals were killed at 0, 3, 6 and 12 h to measure cytokines in bladder. We considered statistical significance at $p<0.05$. **Results:** WT mice showed increased BWW (45.95 ± 5.95 mg/20g of animal), hemorrhage (2 [1-2]), edema (3 [2-3]), neutrophil (19.09 ± 4.61 x 10^3) and macrophage (4.48 ± 0.58 x 10^3) infiltration when compared to controls (21.45 ± 1.15; 0[0-0]; 0[0-0]; 1.96 ± 0.50 x 10^3; 1.54 ± 0.42 x 10^3, respectively). IL-1β and TNF-α were increased at 3 h after IFO injection (250.80 ± 103.90; 399.60 ± 121.40 pg/mL) when compared to 0h (42.59 ± 8.03, 42.73 ± 4.10 pg/mL). IL-1R-/- mice did not developed HC, since the BWW (24.52 ± 2.95), hemorrhage (0[0-1]), edema (0[0-1]), neutrophil (4.61 ± 1.11 x 10^3) and macrophage (2.11 ± 0.46 x 10^3) infiltration were smaller than WT animals treated with IFO ($p<0.05$). In contrast, caspase-1-/- mice developed a full HC phenotype (BWW [63.33 ± 7.16], hemorrhage 1[1-2] and edema 1[1-2]). Although TNFR1 deletion did not prevent infiltration of neutrophils and macrophages. A significant reduction in BWW (38.79 ± 3.21) was also observed in TNFR1-/- mice when compared to WT. In the other hand, TNFR1/R2-/- mice treated with IFO presented an increase in BWW (80.89 ± 4.91), hemorrhage (3[3-3]) and edema (3[3-3]) when compared to WT treated with IFO. TNFR1/R2-/- treated with IFO did not present alteration in neutrophil and macrophage infiltration. However, TNFR1/R2-/- negative controls had already a higher macrophage accumulation (4.24 ± 1.59 x 10^3) when compared to WT treated with saline (0.71 ± 0.17 x 10^3). IL-1β and TNF-α were significant increased at 3 h after IFO injection (250.80 ± 103.90; 399.60 ± 121.40 pg/mL) when compared to 0h (42.59 ± 8.03, 42.73 ± 4.10 pg/mL) respectively. **Discussion:** We demonstrated the role of IL-1R in HC pathogenesis, in agreement with our previous data that showed the prevention of IFO-induced HC using a IL-1R antagonist. Also, we hypothezise that IFO-induced HC may be dependent of IL-1α, since caspase-1 genetic deletion was not able to prevent HC. Furthermore, HC appears to be partially dependent of TNFR and. TNFR2 might play a physiological role in preclude HC development. **Financial support:** CNPq, FUNCAP, Fapesp

04.108

Smoking-induced rheumatoid arthritis aggravation is dependent of aryl hydrocarbon receptor activation and is influenced by genetic polymorphism. Talbot J[1], Liew FY[2], Peres RS[1], Pinto LG[1], Oliveira RDR[1], Silva JR[1], Lima KWA[1], França RFO[1], Ryffel B[3], Cunha TM[1], Alves-Filho JC[1], Louzada-Junior P[1], Cunha FQ[1] [1]FMRP-USP – Pharmacology, [2]University of Glasgow, [3]CNRS

Introduction: Rheumatoid Arthritis (RA) is an autoimmune disease with unknown etiology that affects 1% of worldwide adult population. RA is characterized by joint pain, intense immune cells infiltration into the joints and bone and cartilage destruction. It have been described that genetic and environmental factors are associated to RA susceptibility. Cigarette smoking is the major environment risk factor related to increase RA development and severity. However, the mechanism of smoking-induced RA aggravation is unknown. The aim of this research was to identify this mechanism. **Methods**: Experimental arthritis was accessed by mBSA-induced arthritis (AIA) in WT, *Ahr* or *Il-17a* genetic-deficient mice (*Ahr*KO or *Il-17a*KO); and by collagen-induced arthritis (CIA) [FMRP-USP Animal Ethics Committee (038/2009)]. Mice were exposed to cigarette smoke (CS) in a smoking machine. After arthritis induction we evaluated: articular hyperalgesia, neutrophil infiltration into joints, articular histopathology and CD4 + IL-17 + (Th17) frequencies. We also collected blood samples from RA patients and healthy controls to isolate gDNA for genotyping using TaqMan Probes and to evaluate Th7 frequencies by flow cytometry. [HCFMRP-USP Human Ethics Committee (2981/2009)]. **Results and discussion:** We found that exposure to CS increase the incidence, hasten disease rise and aggravates AIA and CIA, also increasing Th17 frequencies. We observed that CS extract can increase Th17 *in vitro* differentiation. Indeed, CS effects were not observed in *Il-17a*KO, suggesting that smoking-induced arthritis aggravation is dependent of effects on Th17 function. Among the components of CS, hydrocarbons are of particular interest since they are described as ligands of aryl hydrocarbon receptor (AhR), which in turn has been described as an important receptor to Th17 development. We observed that CS-induced increase of Th17 *in vitro* differentiation can be blocked by AhR antagonist CH223191. Moreover, treatment of mice with CH223191 inhibited CS-induced arthritis aggravation and Th17 increase. The loss of CS effects was also observed in *Ahr* KO mice showing that CS-induced arthritis aggravation is AhR/Th17-dependent. Interestingly, the hydrocarbons benzo [b]fluoranthen and α-naptoflavone, present in high loads in CS, and the AhR agonist FICZ also increased Th17 *in vitro* differentiation and aggravated AIA. AIA aggravation induced by AhR agonist was lost in *Ahr* KO mice and was restored by transference of CD4 T cells from WT to *Ahr* KO. Further, we used a genetic association approach in humans with RA. We found an *AhR* genetic polymorphism (SNP; *rs2066853*) that increases AhR function and was related to higher Th17 frequencies in RA. Moreover, an interaction among the presence of this SNP and smoking increases the risk to RA (OR 2.66). Furthermore, smokers with this SNP are highly prone to show higher disease activity than non smokers and present higher Th17 frequencies. **Conclusion**: Smoking (hydrocarbons in smoke) increase Th17 differentiation and aggravates arthritis through AhR activation. Moreover, genetic polymorphisms at *AHR* can influence smoking effects on arthritis development. **Financial Support:** Fapesp, CNPq, FAEPA, Capes

04.109

Fructose 1,6-bisphosphate, an intermediate of glycolysis, promotes anti-inflammatory effects in experimental arthritis via adenosine 2a receptor. Veras FP, Peres RS, Pinto LG, Saraiva ALL, Cunha FQ, Alves-Filho JC FMRP-USP – Pharmacology

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease, which leads to deformity and destruction of joints. Several drugs are used to treat this disease, which may exhibit adverse and toxic effects. Therefore, the study of new drugs with therapeutic effectiveness and low toxicity is necessary. Anti-inflammatory effects of fructose 1,6-bisphosphate (FBP), an intermediate carbohydrate in the glycolytic pathway, have been described, *in vitro* studies. However, these effects on experimental model of diseases, *e.g.* arthritis, remain unclear. The aim of our study was to evaluate the effect of FBP on zymosan-induced arthritis (ZIA) and investigate the possible mechanism involved in this phenomenon. **Methods:** C57BL/6 mice (n=5 per group) were treated with FBP (10, 30 and 100 mg/kg) 24 h and 30 min before the zymosan injection (30 µg/i.a). We evaluated leucocyte migration in the joint cavity by articular lavage, as well as hypernociception threshold by electronic Von Frey, joint swelling and quantification of cytokines in the joint by ELISA. Moreover, we quantified adenosine in mice serum by HPLC. All animal care and experimental procedures were in accordance with the Ethics Committee (53/2013). **Results and discussion:** FBP decreases dose-dependently neutrophil infiltration, hypernociception and swelling. Furthermore, there was a decrease of pro-inflammatory cytokines (IL-1β, TNF-α and IL-6) in the inflammatory site. Otherwise, anti-inflammatory cytokine IL-10 levels were higher in treated mice. FBP increased levels of adenosine (ADO) and the blockade of ADO receptor (A2AR), with A2aR antagonist, abolished the anti-inflammatory effects of F1,6BP. Additionally, concomitant treatment of FBP plus inhibitors of ectonucleotidases CD39 and CD73, that produce extracellular ADO from degradation of ATP, also reverted the antiinflammatory effects of F1,6BP. These findings show that FBP exhibits anti-inflammatory effect in experimental arthritis by an adenosine-dependent mechanism via activation of A2a receptor. **Financial Support:** Capes, Fapesp

04.110 *Hypnea musciformis* polysaccharides in TNBS-induced colitis. Dias GJJ, Brito TV, Barbosa ALR UFPI – Experimental Physiopharmacology

Introduction: Algae can be found in various kinds of locations, occurring in fresh and saltwater environments, on tree trunks, rocks, deserts and glaciers (Raven et al., 2007). A variety of bioactive complexes can be found in algae, these principles are widely used in the pharmaceutical investigation. The *Hypnea musciformis* is a type of seaweed easily distinguished from the other *Hypnea* spp because of the presence of flattened, wide hooks on the ends of the branches. The polysaccharides (PLS) are the main means of study, because they have unique characteristics in their structure and biological interactions. Aware of this therapeutic potential, this study aim to evaluate the anti-inflammatory potential of PLS extracted from *H. hypnea* in TNBS-induced colitis. The concentration of glutathione (GSH) and malondialdehyde (MDA), measures of oxidative stress, nitric oxide (NO) production from the measurement of nitrite and nitrate, and the activity of IL-1β and TNF-α acting as a result of prostaglandins production by Cox-2 activation were also evaluated. **Methodology**: Male Wistar rats were used. Colitis was induced with 20 mg of TNBS solution in the rat colon, using 800µl per animal. One hour before the administration animals were divided in 6 groups: control group (treated with saline solution), TNBS, dexamethasone, PLS at 10, 30 and 60 mg/kg. In the second and third day after colitis induction, the animals were treated with dexamethasone or PLS. The rats were sacrificed in the third day 1 hour after the treatment and the abdomens opened for the removal of the distal colon, washed with physiologic solution pressed into a wax block and then the samples were submitted to biochemical analyses. The Project was approved by the Ethic Committee of The Federal University of Piaui (UFPI) (Protocol: 036/12). **Results**: Treatment with PLS reduced the MDA level from 162.5 ± 25.63 nmol/g (TNBS) to 76.27 ± 16.30 nmol/g (TNBS + PLS 60mg/kg) reaching similar values then control group (65.07 ± 17.93 nmol/g). The level of GSH was significantly reduced in the colon (91.56 ± 14.96 mg/g) in TNBS-treated animals in comparison to the control group (240.2 ± 18.55 mg/g). PLS reestablished GSH normal levels (225.4 ± 32.47mg/g). There was an increase in the level of NO_3/NO_2 in TNBS-treated animals (colon tissue = 0.22 ± 0.03 mM) in comparison to control group (0.08 ± 0.01uM). The treatment with PLS reduced the levels of nitrate and nitrite (0.10 ± 0.00 uM). Finally, the levels of IL-1β and TNF-α increased significantly in the TNBS group (478.7 ± 139.8 pg/mL and 99.29 ± 18.42 pg/mL, respectively) compared to control group (125.8 ± 12.44pg/mL and TNF-α: 41.23 ± 85.95 pg/mL, respectively). The treatment with PLS significantly reduced the levels of these cytokines (IL-1β:108,8 ± 31.03 pg/ml and TNF-α 277.9 ± 27.20 pg/mL). **Discussion**: The results of this study showed the protective effect exerted by *H. muscifomis* PLS in TNBS-induced colitis in rats. PLS protective effect was related to its antioxidant properties, reducing MDA formation and increasing level of endogenous GSH. The production of NO, another important proinflammatory mediator, was also regulated through the administration of PLS.

04.112

Eosinophil deficiency improves psoriatic like skin lesions in mice. Dourado LPA[1], Rocha RPF[1], Silva RC[2], Oliveira VLS[2], Dias ACF[2], Souza DG[1], Teixeira MM[2], Amaral FA[2] [1]UFMG – Departamento de Microbiologia, [2]UFMG – Departamento de Bioquímica e Imunologia

Introduction: Psoriasis is a chronic autoimmune skin disease of unknown cause that involves dysregulated interplay between immune cells and keratinocytes. Topical application of imiquimod (IMQ), a Toll like receptor 7 and 8 ligand can induce psoriasis by stimulating production of inflammatory cytokines leading to leucocyte migration. In this context, we investigated the contribution of eosinophils to psoriasis-like skin inflammation in mice induced by IMQ. **Methods:** The use of animals in this study was approved by the Ethics Committee on Animal Experimentation of UFMG (CEUA-7/2013). Wild type (WT) and eosinophil deficient (ΔdblGATA-1) BALB/c mice were shaved on the back skin and received daily, for 5 consecutive days (days 1- 5), topical applications of imiquimod (3.125 mg) from a commercially available cream (5%) (Ixium; FQM).Control mice were treated with vaseline cream. In order to evaluate the induction of the experimental psoriatic lesion, the scaling of the back skin was scored daily throughout the experiment. On day 6, mice were euthanized and spleen and back skin fragments were collected. The spleen mass was evaluated. The levels of eosinophilic peroxidase (EPO) and myeloperoxidase (MPO) were measured in skin as an indirect quantification of neutrophil and eosinophil infiltration respectively. In addition, IL-36 gene expression was evaluated in the skin by qPCR. **Results and discussion:** Daily application of IMQ on WT mice skin induced a significant spleen enlargement with an increase in weight of approximately 2 fold. The treatment also induced a lesion with phenotypic and histological features of psoriasis such as the marked scaling termed hyperkeratosis. Also, increased levels of EPO and MPO in the skin of IMQ treated mice were observed. The deficiency of eosinophil improved the psoriatic lesion as demonstrated by a decrease in scaling and neutrophil infiltrate in the skin. In addition, splenic enlargement was less expressive than that observed in WT animals. However, the expression of the pro-inflammatory cytokine IL-36 was significantly increased in the skin of eosinophil deficient mice. More studies are needed to clarify the mechanisms underlying the participation of eosinophil in experimental psoriasis inflammation. **Financial support:** CNPq

04.113

TNF receptors in murine melanoma progression – possible role on T regulatory cells (TREGS) and tumor associated macrophages (TAMS). Melo PH, Mota JMSC, Leite CAVG, Prado DS, Cunha FQ, Alves JCF FMRP-USP – Pharmacology, FMRP-USP – Clinics

Introduction: TNF is a classical mediator of inflammatory response, but also display immunoregulatory functions through two distinct receptors that are, in part, responsible for its dual effects. Activation of TNFR2 increases proliferation and function of Tregs, the component of peripheral tolerance and immune suppression, while activation of TNFR1 promotes tumor cell death and inflammation (Chen *et al.*, 2012). In this context, Tregs are found infiltrating tumors in a vast array of tumor types, which is related to worse cancer progression (Shindo and Yoshida, 2010). **Aim:** To evaluate the role of TNF and its receptors in the progression of murine B16-F10 melanoma. **Methods and Results:** All experiments were approved by local ethics committee (Protocol number: 152/2011).WT, TNFR1$^{-/-}$ and TNFR1/2$^{-/-}$ mice were subcutaneously inoculated with 3 x 10^4 cells of B16-F10 melanoma and cancer progression was evaluated through tumor volume and survival. We found that TNFR1$^{-/-}$ mice had increased tumor volume and diminished overall survival compared to WT and TNFR1/2$^{-/-}$ mice. TNFR1/2$^{-/-}$ mice showed an intermediate tumor volume and survival when compared to the others groups. In a different set of experiments, animals were killed 14 days after B16 cells inoculation for tumor and spleen harvesting. Tregs (CD4$^+$ FoxP3$^+$ in CD45$^+$ cells) and TAMs (F4/80$^+$ CD206$^+$ in CD45$^+$ cells) were assessed by flow cytometry. TNFR1$^{-/-}$ mice showed increased TAM accumulation (27% ± 2. 4) compared to WT mice (15.2% ± 2.1) and TNFR1/2$^{-/-}$ mice (18.3% ± 3.2). Tumoral Tregs frequency was reduced in TNFR1/2$^{-/-}$ mice (4.7% ± 1.1), while TNFR1$^{-/-}$ mice presented a minor increase (11 ± 2.2) compared to WT mice (8% ± 2.1). By the other side, TNFR1$^{-/-}$ mice presented an increased splenic Tregs (14.6% ± 1.2), whereas TNFR1/2$^{-/-}$ mice had a slight decrease (8.7% ± 0.7) compared to WT mice (10.9% ± 1.1). **Conclusion:** Our results suggest that TNFR1 may contribute to immune response against melanoma, whereas TNFR2 could attenuates that effect, leading to an increase in Tregs and TAMs accumulation. We hypothesise that TNFR2 pro-tumoral effects can be, in part, mediated by Tregs and TAMs accumulation. **Financial Support**: CNPq, FAEPA and Fapesp

04.114

Anti-inflammatory and antioxidant activity of dry extract capsules of *Amburana cearensis* A C Smith. Albuquerque AA[1], Lopes AA[1], Leal LKAM[1], Araruna SM[1], Silveira ER[2], Viana GSB[3] [1]UFC – Farmácia, [2]UFC – Química Orgânica e Inorgânica, [3]UFC – Fisiologia e Farmacologia

Introduction: The bark of stem of *Amburana cearensis* A C Smith, popularly known as cumaru, is used to treat respiratory diseases such as bronchitis and asthma. Previous pharmacological studies have shown anti-inflammatory, antioxidant and antinociceptive activity of extract and isolated molecules of *A. cearensis*: including coumarin and amburoside A. Thus, the objective of this study was to investigate the potential anti-inflammatory and antioxidant of capsules of dry extract *A. cearensis* (CESAC). **Methodology:** This study was approved by the Ethics and Animal Research committee of UFC, Protocol N ° 49/09. Male albino rats (*Rattus norvegicus*) Wistar (120 to 180g) or male albino mice (*Mus musculus*) Swiss (25 to 30g) were used. In the trial of edema of paw induced by carrageenan (Cg), Swiss mice were treated orally with CESAC in doses of 100,200 and 400 mg/kg, indomethacin (5 mg/kg, i.p.) or Tween 80 (control – 4%, v.o.) 60 min before the injection of Cg 1% in the paw. The volume of paw was measured at 1, 2, 3 and 4 hours after injection of Cg. In the trial of broncho-alveolar lavage (BAL), albino mice were sensitized and challenged with ovalbumin (OVA) and pretreated with CESAC (100,200 and 400 mg/kg, v.o.), dexamethasone (5 mg/kg) or Tween 80 (control 4 % in distilled water, v.o.). Subsequently, the collection of BAL was executed to leukocyte counts and determination of the levels of reduced glutathione and the levels of nitrite/nitrate. For *in vitro* antioxidant activity of CESAC (10, 50 and 100 µg/mL), it was performed the test of scavenging activity of the DPPH radical at which the absorbance was determined at 517 nm. **Results and discussion:** In the trial of paw edema, the CESAC promoted reduction of volume of paw in concentration of 200 and 400 until the 4th hour (88.42 ± 8.9 µL and 84,66 ± 9,2 µL, respectively) compared to the control group (4th hour: 139.74 ± 7.20 µL), and indomethacin, standard drug, also inhibited the volume of edema (4th hour: 80.21 ± 5.00 µL). In BAL, CESAC (100, 200 and 400 mg/kg, v.o.) significantly reduced the number of total leukocytes (0.2389 ± 0.018. 0.1094 ± 0.019 and 0.1073 ± 0.018. respectively) compared to the control group (0.5395 ± 0.043); also there was a decreased the levels of nitrite/nitrate (12.88 ± 1.25. 11.68 ± 11.71 and 11.56 ± 1.12. respectively) when compared to the control group (24.30 ± 2.8) and an increase in glutathione leading to normal levels (Normal/without OVA: 0.03095 ± 0.002; CESAC 400 mg/kg: 0.03055 ± 0.00098). In the DPPH test, only CESAC in concentration of 100 µg/mL showed significant inhibition (22%) of DPPH. The CESAC demonstrated anti-inflammatory activity in experimental models of paw edema and of bronchoprovocation induced by antigen, that seems to be related to the blockade on leukocyte accumulation in inflammatory focus and on modulation of oxidative stress. **Financial support and acknowledgment:** Capes, FUNCAP and CNPq

04.115

Diet induced obese mice developed lung insulin resistance via protein nitration and inhibition of key proteins in the insulin signaling. Calixto MC, André DM, Sollon C, Anhê GF, Antunes E Unicamp

Obesity and asthma are prevalent and increasing diseases, and both have significant impact on global public health. The increase in prevalence of asthma and obesity has led researchers to suggest that obesity may be an important factor in the development of asthma, or even worse a pre-existing asthma. High fat diet induced obesity enhances the pulmonary eosinophilic inflammation in ovalbumin (OVA)-challenged mice (Calixto *et al.*, *Br J Pharmacol*, 159, 617, 2010). Recently data have emerged suggesting that insulin resistance associated with obesity plays an important role in the development of asthma, explaining, at least in part, the association between asthma and obesity (Agrawal, *Am J Respir Cell Mol* Biol, 44, 270, 2011; Calixto *et al.*, Plos One, 8, 2013)

Considering that NO is indicated as the key mediator in obesity and systemic insulin resistance (Carvalho-Filho *et al*, 2006;. Pilon, *et al*, 2010) and levels of this mediator are elevated in bronchoalveolar lavage of obese mice, it becomes relevant to study whether excessive NO production is directly related to development of insulin resistance in obese mice lungs, which may occur through nitration of essential proteins for glucose uptake.

In the present study we evaluated the activation of key proteins in the insulin signaling pathway, such as insulin receptor (IR), insulin receptor substrate-1 (IRS-1) and AKT in obese mice lungs. For this, male C57Bl/6/JUnib mice received a high-fat diet or standard-chow diet for 12 weeks. Obese and control mice were anesthetized and intravenously stimulated with insulin for 5 min. Then mice were sacrificed and lungs were removed for analysis of protein expression through Western blott and immunopreciptation. The experimental protocols were approved by the Ethics Committee of University of Campinas (N$^{o.}$ 1496-1).

After *in vivo* stimulation with insulin we don't observe IR and AKT phosphorilation in obese mice lung when compared to basal phosphotilation, unlike lean animals which showed an increase of 46% and 53% for IR and AKT respectively. Besides it, the analysis of IR and IRS-1 phosphotyrosine expression were decreased in obese lung compared with lean ($p<0,005$). This data reinforce a failure in insulin signal, a fact already predicted by low rate of glucose uptake after intraperitoneal stimulation with insulin presented by obese mice (3.8 min^{-1}/% and 1,9 min^{-1}/% for lean and obese mice respectively). Moreover, obese mice lung showed an increase in nitration of tyrosine residues both on IR and AKT ($p<0.05$). Obese mice also presented increased expression of iNOS (39%) and a decrease ($P<0.05$) in superoxide dismutase (SOD) activity in lung tissue compared with lean group. Our data reinforces the hypothesis that insulin resistance mediates the association of obesity and asthma and suggests that obese mice presents a local insulin resistance caused by the increase in NO reactivity with anion superoxide and subsequent peroxynitrite formation, resulting in elevated lung nitrative stress. The nitrative stress leads to covalent modifications and inhibition of several key proteins in the insulin signaling.

04.116

Irinotecan-induced alterations in the myenteric plexus. Gomes AS[1], Costa DVS[1], Barreto Junior JEF[2], De Sá ISLB[2], Andrade MN[2], Aguiar BF[2], Portela VS[2], Alcântara JR[3], Martins CS[1], Moura Neto LI[4], Brito GAC[1] [1]UFC – Morfologia, [2]UFC – Faculdade de Medicina, [3]UECE – Nutrição, [4]UFC – Enfermagem

Introduction: Irinotecan(CPT-11) is a semi-synthetic camptothecin derivative and has been used in the treatment of colorectal cancer. The intestinal mucositis is a major side effect of cancer therapy and may contribute to structural and/or neuronal alterations of the gastrointestinal smooth muscle, especially in the myenteric plexus. The aims of this study were to evaluate the effect of irinotecan-induced toxicity on the measurement of MPO(myeloperoxidase) and morphology of neurons in the myenteric plexus: immunoreactive nitric oxide synthase (NOS, a marker for inhibitory neurons) and ChAT (a marker for excitatory neurons). **Methods:** The intestinal mucositis was induced by intraperitoneal administration of CPT-11 (200 mg/kg) in rats. After three days, the animals were sacrificed and intestinal segments were removed for measurement of MPO, fixed in 4% PFA and dissected to obtain the myenteric plexus. Immunofluorescence using NOS and ChAT polyclonal antibody as the primary antibodies and qualitative analysis with a fluorescence microscope were performed. Experimental protocols were performed according to the guidelines approved by the research Ethics Committee of the department of physiology and pharmacology Federal University of Ceará(nº 008/11). Results: The animals treated with CPT-11 showed an increase of neutrophil infiltration into the duodenum (15.88 ± 0.6 MPO U / mg), jejunum (13.43 ± 0.6 MPO / mg) and ileum (12.20 ± 0.8 U MPO / mg) compared with the control group (2.20 ± 0.3, 1.67 ± 0.23, 2.04 ± 0.76 U MPO / mg, respectively). Confocal microscopy of the myenteric plexus of the small intestine of animals with intestinal mucositis by CPT-11 showed an increase of ChAT-immunoreactive neurons. Whereas population of inhibitory neurons (NOS) exhibited decreased when compared to the control group. **Discussion:** Our data demonstrated the presence of ChAT and NOS immunoreactivity in neurons of the myenteric plexus of the segments studied in rats treated with CPT-11. These data are important to understand how the enteric nervous system of the intestine respond to anticancer treatments.

04.117

Ethanol extracts from chemotypes of *Myracrodruon. urundeuva* reduce proinflammatory response of activated human neutrophils. Araujo EVO[1], Milet RRC[2], Pierdoná TM[3], Rocha TM[4], Silveira ER[5], Leal LKAM[4] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Estudos Farmacêuticos e Cosméticos, [3]UFC – Fisiologia e Farmacologia, [4]UFC – Farmácia, [5]UFC – Química Orgânica e Inorgânica

Introduction: In Northeast Brazil the Aroeira-do-sertão (*Myracrodruon urundeuva* Fr. All., Anacardeaceae) is extensively used by traditional medicine for the treatment of inflammatory diseases and gastric ulcer. Previous chemical and pharmacological studies established the anti-inflammatory and gastroprotective potential of extracts and molecules (flavonoids) from wild or cultivated plants. In this context, considering that neutrophils are one of the most important cells involved in inflammation response, the objective of the present study was to evaluate the anti-inflammatory potentials of ethanol extracts of leaves from *M. urundeuva* on human neutrophils. **Experimental part:** The ethanol extract of leaves from *M. urundeuva* – chemotypes myrcene (M), α-pinene (P), Δ3–Carene (C) and limonene (L) were produced by maceration or decoction (EEMUM or EEMUD). Polymorphonuclear cells ($2.5x10^6$cells/mL), predominantly neutrophils (80-90%) with viability of 89 ± 2.0% established by the exclusion with Trypan blue were isolated from human blood (Boyum, 1968) (Approval COMEP: NS29). To evaluate the effect of test drugs on neutrophil degranulation, firstly the cells were incubated with extracts (100 µg/mL), HBSS (negative control), indometacin (INDO, 36 µg/mL, standard drug) or DMSO 1% (vehicle/control) during 15 min before the addition of PMA (0,1µM). The measurement of myeloperoxidase (MPO) release by stimulated cell was determined by spectrophotometry (620 nm) (De Young *et al.*, 1989). The cytotoxicity of extracts (10 – 100 µg/mL) or Triton X-100 0.2% (cytotoxic standard) determined by lactate dehydrogenase (LDH) activity (LDH liquiform of Labtest Diagnosis, Brazil). **Results/Discussion:** Like indomethacin, a non-selective inhibitor of cyclooxygenase, the EEMUD – chemotypes myrcene, α-pinene, Δ3 – Carene and limonene (100 µg/mL) inhibited the neutrophil degranulation process of human neutrophil induced by PMA reducing significantly the release of MPO by cells (46.5 ± 5.3, 80.0 ± 3.0, 64.1 ± 6.9 and 47.0 ± 7.7, respectively). Similar results were observed for the EEMUD – chemotypes myrcene, α-pinene, Δ3 – Carene and limonene yielding an inhibition in about 60%. Among the extracts evaluated only the EEMUM- chemotype myrcene (65.6 ± 8.12 U/L) interfered significantly with the LDH activity released by human neutrophils when compared to the control group (22.4 ± 2.8 U/L). In summary, the present study showed that both extracts (EEMUM or EEMUD) from *M. urundeuva* inhibited human neutrophil degranulation. However, the toxicity showed by EEMUM-chemotype myrcene was possibly involved in its effect. **Financial support:** CNPq

04.118

Pretreatment of lipopolysaccharide-injected rats with pravastatin increases the number of circulating platelets by mechanisms envolving augment of plasma thrombopoietin levels. Naime ACA[1], Lopes-Pires ME[1], Latuf P[2], Vassalo J[2], Lima CSP[3], Landucci ECT[1], Antunes E[1], Marcondes S[1] [1]Unicamp – Farmacologia, [2]Unicamp – Anatomia Patológica, [3]Unicamp – Clínica Médica

Introduction: Sepsis is still a cause of high mortality in hospitals all over the world. It is a very complex clinical condition and up to now there is no effective treatment. In the last decades, works have described the important role of platelets in sepsis, since the severity of the condition is correlated to the number of circulating platelets. Statins, besides their action on lowering the cholesterol levels, have been successfully used in the treatment of inflammatory diseases. Therefore, in the present study we decided to investigate the effect of pravastatin in the number of circulating platelets in model of experimental sepsis induced by lipopolysaccharide (LPS). **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – Unicamp, protocol number 2097-1). Male Wistar rats were treated with saline or pravastatin 20 mg/kg (once a day, by oral gavage, for 7 days). In the sixth day, rats of both groups received a single injection of saline or LPS (from E. coli, 1 mg/kg) and after 48h arterial blood was collected. Platelet counts were carried in peripheral blood using Newbauer chamber. The number of megakaryocytes was determined by histology of the bone marrow. Plasma thrombopoietin concentrations were measured by ELISA. **Results:** The pretreatment of saline-injected rats with pravastatin reduced significantly the number of circulating platelets (reduction of 38%). In this group, pravastatin increased 40% the number of megakaryocytes in the bone marrow and reduced by 42% the plasma thrombopoietin concentration. Injection of rats with LPS reduced 6.8-fold the platelet counts compared to the saline-injected rats, which was accompanied by increasing of megakaryocytes number (increase of 40%) and decreasing of thrombopoietin levels (reduction of 47%). Pretreatment of LPS-injected rats with pravastatin prevented significantly the fall in the number of circulating platelets (6.3 ± 0.2, 0.9 ± 0.1 and 2.6 ± 0.3 $x10^8$ platelets/ml in saline-injected rats, in LPS-injected rats and in rats pretreated with pravastatin and injected with LPS, respectively). Pravastatin reduced the number of megakaryocytes in LPS-injected rats to counts similar to saline-injected rats. On the other hand, pretreatment of rats with pravastatin increased significantly plasma thrombopoietin concentration compared to the rats injected only with saline or LPS. **Conclusion:** Pravastatin increases the number of circulating platelets in LPS-injected rats by mechanisms involving enhancing of plasma thrombopoietin concentration. **Supported by:** CNPq

04.119

The effects of alpha-bisabol-loaded nanocapsules in model of acute pulmonary inflammation LPS-induced in mice. D'Almeida APL[1], Ciambarella BT[1], Souza ET[1], Marques S[1], Terroso T[2], Pohlmann AR[2], Guterres SS[2], E Silva PMR[1], Cordeiro RSB[1], Martins MA[1], Bernardi A[1] [1]IOC-Fiocruz, [2]UFRGS – Farmácia

Introduction: Alpha-bisabolol is a sesquiterpene alcohol obtained by essential oil from plants with an anti-inflammatory and antioxidant activity. Many researches have consolidated the concept of nanotechnology in drug delivery with many advantages. This work aims to evaluate the anti-inflammatory effects of alpha-bisabolol-loaded nanocapsules (α-bis NC) on pulmonary inflammatory model LPS-induced in mice. **Methods:** Mice were pretreated with free alpha-bisabol (α-bis) (30, 50, 100 mg/kg, p.o.) or α-bis NC (30, 50, 100 mg/kg, p.o.) or drug-unloaded nanocapsules (NC) (100 mg/kg p.o) 4 h before saline (25 µl/animal, i.n.) or LPS provocation (25 µg/25 µl saline, i.n.), and several pivotal parameters of acute lung injury were monitored 18 h post-provocation. Airway hyper-reactivity (AHR), concerning both lung resistance and elastance, was assessed following animal exposure to increased concentrations of aerosolized methacholine (3-27 mg/ml), using invasive whole-body plethysmography. Total and differential leukocyte numbers were quantified in samples of bronchoalveolar lavage fluid (BALF), with the help of Neubauer chamber and cytocentrifuged slides stained with May-Grünwald-Giemsa, respectively. Pro-inflammatory cytokines and chemokines were quantified in lung tissue samples using ELISA. **Results:** We found that α-bis NC (30, 50 and 100 mg/kg) significantly reduced LPS-induced AHR concerning both lung elastance (54%, 61% and 50%, respectively) and resistance (50%, 44% and 44%, respectively) when compared with NC treated mice. In the BALF the total number of leukocytes (46,5 ± 21,36 to 514,7 ± 222,3; mean ± SD; $x10^3$ cells/ml; n=5-7; $p<0,05$) and neutrophils (0,98 ± 0,68 to 514,8 ± 128,6; $p<0,001$) were increased in NC treated and LPS-induced group when compared to saline group. Again, α-bis NC (30, 50 and 100 mg/kg) significantly reduced LPS-induced elevation in total leucocyte and neutrophil numbers (0,71 ± 0,26, 0,11 ± 0,13 and 0,04 ± 0,07, respectively; $p<0,001$) when compared with the NC treated group (514,8 ± 128,6). Remarkably, α-bis (30, 50 and 100 mg/kg) presented a blockade of neutrophil recruitment, which was significantly higher compared with that noted following the respective free-treated doses (454,8 ± 273 to 0,71 ± 0,26, 488,1 ± 92,24 to 0,11 ± 0,13 and 458,3 ± 331 to 0,04 ± 0,07, respectively; $p<0,001$). Moreover, α-bis NC (50 mg/kg) reduced the levels of TNF-α (34,93 ± 7,9 to 18,4 ± 8,3; pg/mg protein; $p<0,05$), KC (10,7 ± 25,19 to 4,36 ± 1,26), MIP-1 (34,8 ± 14,5 to 1,98 ± 0,38) and MIP-2 (68,7 ± 19,95 to 14,45 ± 2,53). **Conclusion:** Our results suggest that α-bis NC present a potential anti-inflammatory effect on LPS-induced pulmonary inflammation in mice. Studies trying to clarify the action mechanism of alpha-bisabolol in the pulmonary inflammatory process are in progress. **Financial support:** CNPq, Faperj. Ethics Committee of Animal Use: CEUA – LW23/10.

04.120

Toll-like receptor-7 agonist resiquimod decreases established allergen-induced asthma changes in mice: impact on eosinophil survival. Ghilosso-Bortolini R, Ciambarella BT, Olsen PC, Azevedo C, Cotias AC, Dias DF, Arantes ACS, Silva PMR, Martins MA IOC-Fiocruz

Introduction: Allergic asthma is a chronic inflammation of the bronchial airways, marked by eosinophilic infiltration, airway hyper-reactivity (AHR) and lung remodeling. Inhaled glucocorticoids (GCs) are by far the most effective therapy for controlling asthma, but adverse effects and GC resistance clearly limit their effectiveness. Prior studies reported that modulation of the immune response through Toll-like receptor (TLR)-7 activation can prevent experimental asthma, but the mechanism of action remains unclear. In the current study we investigated the potential of TLR-7 agonist Resiquimod, as compared to Dexamethasone, to alter AHR in mice with established allergic eosinophilic inflammation. We also compared the impact of Resiquimod and Dexamethasone on eosinophil survival concerning cells recovered from the bronchoalveolar effluent. **Methods:** Male A/J mice were actively sensitized with a mixture of ovalbumin (OVA) and $Al(OH)_3$ on the days 0 and 7, followed by a sequence of intranasal instillations of OVA (50 µg/25 µL, i.n.), once a week (wk) during 4 wks. Resiquimod (200 µg/25 µL, i.n.) and Dexamethasone (1 mg/kg, orally) were separately administered, the former 48 h and the latter 1 h before the third and fourth allergen provocation. Analyses were carried out 24 h after the last OVA provocation. Changes in lung resistance and elastance following aerosolized methacholine (3, 9, and 27 mg/ml), reflecting the AHR status, were measured by invasive whole-body plethysmography. Cells recovered by bronchoalveolar lavage (BAL) were stained with PE Anti-Mouse Siglec-F (BD Pharmigen) and with FITC Annexin V (Apoptosis Detection Kit-BD Pharmingen) and analyzed for survival state using FACSCalibur flow cytometer and CellQuest software (BD Biosciences). All animal procedures were done under official license (CEUA-Fiocruz 034/09). **Results:** As expected, sensitized mice subjected to OVA challenge reacted with AHR, 24 h post-challenge, attested by higher values of lung resistance (AUC_R=153.8 ± 20.5) (mean ± SEM)(n=7)($p<0.05$) and elastance (AUC_E=5841 ± 385; n=7)($p<0.05$) triggered by methacholine, as compared to the values from sham-challenged ones (AUC_R=91.1 ± 11.9; n=8; AUR_E=3068 ± 525.4; n=9). Notably, the state of increased resistance and elastance were significantly inhibited ($p<0.05$) in mice treated with either Resiquimod (77.0% and 76.5%, respectively) or Dexamethasone (99.2% and 68.3%, respectively). Interestingly, in this model, Resiquimod, compared to Dexamethasone, was significantly more effective in triggering eosinophil apoptosis. The number of apoptotic eosinophils recovered from the BAL fluid increased from 1262 ± 235 (n=6) in samples from OVA-challenged mice to 2874 ± 1036 (n=6) following OVA + Dexamethasone and 15313 ± 5732 (n=4) ($p<0.05$) after OVA + Resiquimod. **Discussion:** These results show that local treatment with Resiquimod is as effective as orally administered Dexamethasone in decreasing established allergen-induced AHR in mice. They also suggest that this TLR7 agonist induces eosinophil apoptosis, which may contribute to its protective effect in asthma. **Financial support:** Fiocruz, CNPq, Faperj, TIMER consortium.

04.121

Early pro- and anti-inflammatory responses to LPS-induced epididymitis in rats. Silva EJR[1], Mirim AFMN[1], Avellar MCW[1] [1]Unifesp-EPM – Farmacologia

Introduction: Bacterial infection ascending from the urethra into the epididymis usually causes epididymitis, an inflammatory condition that may lead to infertility in men. Gram-negative bacteria *Escherichia coli* are one of the most prevalent etiological factors of epididymitis. Our aim was to investigate the local acute inflammatory responses of the rat epididymis using an experimental model of epididymitis induced by the injection of lipopolysaccharide (LSP) from *E. coli* into the lumen of the vas deferens, thus mimicking the clinical condition. **Methods:** The study was approved by the Research Ethics Committee from Unifesp/EPM (process 0310/12). Wistar rats (90 days) were anesthetized with ketamine/xylazin (100/10 mg/kg, i.p.). A scrotal incision was made to expose the epididymal portion of the vas deferens. Twenty-five microliters of sterile saline (0.9% NaCl, control) or different doses (5-25 µg/epididymis) of ultrapure LPS from *E. coli* (O55:B5, S-type) dissolved in sterile saline were injected into the lumen of the vas deferens near to the cauda epididymis using a 30-G needle and a 0.1-ml Hamilton syringe. Rats were sacrificed 0.5, 1, 2, 4, 6, 8, 16, 24 and 48 h after injections. Cauda epididymides were harvested and subjected to RNA extraction and reverse transcription (RT) quantitative polymerase chain reaction (qPCR) for the evaluation of the expression of pro- and anti-inflammatory genes. **Results:** Macroscopic examination of control epididymis showed no signs of inflammation. Epididymides from LPS-treated rats, however, showed slightly swollen cauda with increased vascularization between 6 h and 24 h after injection, indicating the presence of an ongoing local inflammatory reaction. qPCR analysis demonstrated that intravasal LPS injection induced a dose- and time-dependent up-regulation in the expression of pro-inflammatory genes *Il1b* and *Tnf*, as well as in the anti-inflammatory gene *Nfkbia* in the cauda epididymis in comparison to control rats, reaching a maximum peak 6 h after inoculation of 25 µg LPS (87-, 29- and 6-fold increase for *Il1b*, *Tnf*, and *Nfkbia* transcripts, respectively; $p<0.05$; ANOVA followed by Tukey test, n=4 rats/group). Further analyses performed with samples from rats treated with 25 µg LPS indicated an increase in the expression of the pro-inflammatory genes *Cd14* (8-fold increase), *Il6* (355-fold increase), *Infg* (3-fold increase), and *Nos2* (213-fold increase) 6 h after LPS injection, whereas the expression of the anti-inflammatory cytokine *Il10* was increased 6 h and 24 h after the endotoxin injection (3- and 2-fold increase, respectively). We observed no changes in the transcript levels of the pro-inflammatory genes *Tlr4*, *Cox1* and *Rantes*, as well as the anti-inflammatory genes *Il4* and *Tgfb1*. **Discussion:** Our findings indicate that the epididymis is able to mount a rapid inflammatory response to a luminal LPS exposure. The persistent up-regulation of *Il10* transcript suggests that this anti-inflammatory cytokine could be involved in the modulation of the inflammatory response in this organ. Altogether, our results shed light into the understanding of the players involved in the earlier stages of epididymitis, which may affect its clinical outcomes. **Financial Support:** Science without Borders Program (CSF/CNPq), CNPq, Fapesp, and Capes.

04.122

The atypical chemokine receptor d6 plays a protective role during experimental sepsis.

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Introduction: Sepsis is a systemic inflammatory response resulted from the inability of the immune system to control infections. During an infection, neutrophils are the first cell line to reach the primary focus, and chemokines have a fundamental role in recruiting these cells. However, in sepsis, these chemokines contribute to the neutrophil infiltration to remote organs and to multiple organ failure. Under normal physiological conditions neutrophils do not express the CC chemokine receptor subfamily and, as consequence, do not respond to CC chemokines. On the other hand, our group has shown that during sepsis neutrophils become responsive to these chemokines and express CC chemokine receptors, as CCR2 (1) and CCR5. Recently, a new chemokine receptor named D6 (2) has been studied and it has been described as an atypical receptor due to its involvement in the removal and degradation of CC inflammatory chemokines. However, to date, there are no studies showing the involvement of D6 in sepsis. **Methods:** All experiments were performed according to our institution's ethical guidelines (169/2011). Sepsis was induced in C57BL/6 and D6 deficient mice ($D6^{-/-}$) by cecal ligation and puncture (CLP). Neutrophil migration to the peritoneal cavity, bacteremia, markers of organ damage, neutrophil infiltration and chemokine levels on remote organs were determined 24 hours after CLP. The survival rate of animals was assessed twice a day, until the 10º day after sepsis induction. The means of the parameters evaluated in WT and $D6^{-/-}$ mice submitted to CLP were analyzed by ANOVA, followed by Bonferroni test, or by t test and the survival rate by the Mantel-Cox log rank test. **Results and discussion:** It was observed that $D6^{-/-}$ mice under CLP-surgery exhibited a significant reduction in the survival rate, as compared to WT animals (WT: 88% and $D6^{-/-}$: 33%). However, neutrophil migration to the peritoneal cavity, bacterial load in the peritoneal exudate and in the blood were similar between WT and $D6^{-/-}$ mice, 24 hours after CLP. On the other hand, we showed increased neutrophil infiltration, assessed by myeloperoxidase (MPO), and chemokine levels in the lung (MPO- WT: 15120 ± 502.3 and $D6^{-/-}$: 19350 ± 807.4; CCL3- WT: 791.2 ± 289.2 and $D6^{-/-}$: 2864 ± 487.3), heart (MPO- WT: 119.8 ± 25.8 and $D6^{-/-}$: 208.2 ± 19.7; CCL2- WT: 656.8 ± 60.8 and $D6^{-/-}$: 837.5 ± 47.2; CCL3- WT: 34.82 ± 20.8 and $D6^{-/-}$: 731.4 ± 284.5) and kidney (CCL5- WT: 785.2 ± 142.7 and $D6^{-/-}$: 1717 ± 204.5) of $D6^{-/-}$ mice. These mice also showed higher levels of biochemical markers of lesion in the heart (CK-MB; WT: 376.5 ± 34.1 and $D6^{-/-}$: 554.8 ± 75.2), kidney (BUN; WT: 59.7 ± 13.4 and $D6^{-/-}$: 116.3 ± 23.4) and liver (TGO; WT: 117.5 ± 2.9 and $D6^{-/-}$: 230.4 ± 33.7) then WT animals. In conclusion, our data indicate that D6 has a protective role during sepsis, mediating the reduction of chemokine levels in remote organs and, consequently, the reduction of organ damage. **References: 1**) Souto FO. *Am J Respir Crit Care Med*, 183, 234, 2011. **2**) Jamieson T. *Nat Immunol*, 6, 403, 2005. **Financial support:** CNPq, Fapesp, Capes, FAEPA

04.123

Treadmill exercise induces neutrophil recruitment into muscle tissue in a reactive oxygen species-dependent manner. An intravital microscopy study. Silva AN[1], Bernardes PTT[1], Rezende BM[1], Gomes EC[1], Pinho V[1], Marques PE[1], Lima PMA[2], Coimbra CC[2], Menezes GB[1], Teixeira MM[3] [1]UFMG – Morfologia, [2]UFMG – Fisiologia e Biofísica, [3]UFMG – Bioquímica e Imunologia

Introduction: Intense exercise is a physiological stress capable of inducing the interaction of neutrophils with muscle endothelial cells and their transmigration into tissue. Mechanisms driving this physiological inflammatory response are not known. Here, we investigate whether production of reactive oxygen species is relevant for neutrophil interaction with endothelial cells and recruitment into the quadriceps muscle in mice subjected to the treadmill fatiguing exercise protocol. **Methods:** This study received prior approval from the local animal ethics committee (Animal Ethics Review Board – Comitê de Ética em Experimentação Animal-CETEA/UFMG-UFMG, Certificate number 17412/12). Mice exercised until fatigue by running for 56.3 ± 6.8 min on an electric treadmill. Skeletal muscle was evaluated by intravital microscopy at different time points after exercise, and then removed to assess local oxidative stress and histopathological analysis. **Results:** We observed an increase in plasma lactate and creatine kinase (CK) concentrations after exercise. The numbers of monocytes, neutrophils, and lymphocytes in blood increased 12 and 24 hours after the exercise. Through intravital microscopy, numbers of rolling and adherent leukocytes increased 3, 6, 12, and 24 hours post-exercise. Using LysM-eGFP mice and confocal intravital microscopy technology, we show that the number of transmigrating neutrophils increased 12 hours post-exercise. Mutant $gp91^{phox-/-}$ (non-functional NADPH oxidase) mice and mice treated with apocynin showed diminished neutrophil recruitment. SOD treatment promoted further adhesion and transmigration of leukocytes 12 hours after the exercise. **Conclusion:** These findings confirm our hypothesis that treadmill exercise increases the recruitment of leukocytes to the postcapillary venules, and NADPH oxidase-induced ROS plays an important role in this process.