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IMMUNOPHARMACOLOGY

INFLAMMATORY CYTOKINES AND CHEMOKINES REDUCTION IN SPINAL CORD IMPROVED EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AFTER O-TETRADECANOYL-GENISTEIN TREATMENT

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder that affects the central nervous system. Experimental autoimmune encephalomyelitis (EAE) is a model for the study of MS. Genistein has been shown to down-modulate pro-inflammatory cytokines like IFN- γ , IL-12 and TNF- α , and reverse clinical signs of EAE. The esterification *in vivo* may be responsible for the increase of biological activity of genistein. The aim was evaluate the effect of 7-O-tetradecanoyl-genistein (TDG) treatment, analog of genistein, in EAE model.

Methods and Results: The mice were subcutaneously (s.c.) immunized on both sides of the tail base with 100 μ g of myelin oligodendrocyte glycoprotein peptide (MOG) 35–55. One of these immunized groups was treated with TDG (TDG group) at a dose of 200mg/kg s.c. daily for a total of seven doses. This treatment was initiated in the 14th day post-immunization (dpi). A second immunized group received no treatment (NT-EAE group). Genistein was used as a reference compound on the third immunized group (genistein group) at a dose of 200mg/kg s.c., because of its proven effects on EAE. Animals were monitored daily and neurological impairment was quantified. Mice were sacrificed in the 21st dpi and spinal cords were removed, macerated and the supernatants collected to determine the concentration of cytokine and chemokine. TDG was effective in treating mice with EAE and the effect was notable on 18th dpi with a clinical score (2.4 ± 0.245) lower than the NT-EAE group (4.0 ± 0.447). The reduction of the clinical signs in TDG animals could be correlated with lower levels of inflammatory cytokines in relation to the NT-EAE group: IL-6 (538.7 ± 54.56 vs 672.0 ± 23.38), IL-17 (825.0 ± 67.56 vs 994.7 ± 85.88) and IFN-g (375.3 ± 82.64 vs 3986.0 ± 138.1) and increased levels of TGF- β (4459.0 ± 163.9 vs 1839.0 ± 69.0) and IL-10 (3012.0 ± 79.01 vs 1362 ± 24.64) in the spinal cord. The chemokines CCL20 and CCL5 were reduced in the TDG group when compared to NT-EAE group which can be related with the reduction of the inflammatory infiltrate in the spinal cord.



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Conclusion: This study suggests that TDG acts ameliorating signals in EAE through reduction of inflammatory cytokines and chemokines in the spinal cord. Further investigations are necessary to indicate the therapeutic potential of this analogue for the treatment of MS.

Financial support: CAPES, CNPq and FAPEMIG

EVALUATION OF ANTI-INFLAMMATORY PROPERTIES OF AQUEOUS FRACTION AND ISOLATED FLAVONOIDS FROM CROTON ANTISYPHILITICUS MART. IN IN VIVO EXPERIMENTS.

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Introduction: *Croton antisyphiliticus* is a plant found in the Brazilian savanna and it is used in the folk medicine to inflammatory disorders. This study evaluate the anti-inflammatory effect of the aqueous fraction (Aq) and its isolated flavonoids: Quercetin (QC) and Vitexin (VOG), obtained from *C. antisyphiliticus*, upon leukocytes migration, exudation, adenosine deaminase (ADA) and myeloperoxidase (MPO) activities and nitric oxide levels (NOx), using the murine model of pleurisy induced by carrageenan (Cg). **Methods:** Aerial parts of the herb were dried, macerated and extracted with ethanol to obtain the crude extract, which was partitioned with different solvents to obtain the Aq, which was chromatographed on silica gel column eluted with ethyl acetate/ethanol to obtain two flavonoids: QC and VOG. Swiss mice (18-20 g) were used in the pleurisy model which was performed as described in *Brit.J.Pharmacol.183:811-19,1996*. The animals were previously challenged with Evans blue dye (25 mg/kg, i.v.) to evaluate the exudation. Different groups of animals were pretreated with the Aq (5-50 mg/kg), QC (5-25 mg/kg) or VOG (5-25 mg/kg) administered by intraperitoneal route, 0.5h before Cg injection administered by intrapleural route. The inflammatory parameters were analyzed after 4h. To analyze ADA and MPO activities and NOx levels, different groups of animals were treated (0.5h before Cg) with Aq (25 mg/kg), QC (10 mg/kg) or VOG (10 mg/kg) and the inflammation was also analyzed after 4h. This study used 6 animals per group and it was approved by the Institutional Ethics Committee (protocol number: PP00617). **Results:** The Aq (25-50 mg/kg), QC (10-25 mg/kg) and VOG (10-25 mg/kg) inhibited leukocytes migration (Aq: 55.7±3.8% to 58.6±3.8%; QC: 41.7±5.7% to 47.4±7.2%; VOG: 29.8±5.4% to 35.8±7.2%), neutrophils (Aq: 57.9±3.1% to 63.4±3.0%; QC: 44.0±4.8% to 48.4±7.3%; VOG: 29.3±5.0% to 34.5±7.9%) and exudation (Aq: 44.2±6.4% to 54.7±4.5%; QC: 14.3±2.0% to 29.0±6.5%; VOG: 22.0±8.4% to 22.9±8.5%)(P<0.05). Aq (25 mg/kg), QC (10 mg/kg) and VOG (10 mg/kg) inhibited ADA by 40.1±11.5%; 57.9±6.5% and 67.2±10.0%, MPO by 45.8±6.5%; 66.6±2.6% and 66.8±3.0% and NOx by 52.5±4.5%; 33.7±10.5% and 50.8±7.7%, respectively (P<0.01). **Conclusion:** *C. antisyphiliticus* showed an



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important anti-inflammatory activity by inhibiting leukocytes migration and exudation. These inhibitory effects may be associated with the decreasing of ADA and MPO activities and NOx levels.

Financial support: CAPES

TOXICITY AND IN VIVO ANTI-INFLAMMATORY ACTIVITY OF NOVEL THALIDOMIDE ANALOGUES CONTAINING DIAMINES AND OPEN PHTHALIMIDE STRUCTURE

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Introduction: Thalidomide exhibits both anti-inflammatory and immunomodulatory effects, improving clinical symptoms in a variety of diseases including erythema nodosum leprosum. In previous study from our laboratory it has been shown that thalidomide analogues containing diamines and open phthalimide structure were synthesized and showed high inhibitory in vitro activity on key molecules such as TNF- α , IL-12, IFN- γ , IL-6, CXCL9, CXCL10 and CD80. In contrast, some compounds induced an increase in IL-10 production. In this study, the anti-inflammatory activity of two thalidomide analogues (GI-16 and SC-15) were evaluated in vivo using the carrageenan-induced mouse paw inflammation and the LPS-induced lung inflammation in C57Bl/6 mice. In addition, the acute and sub-chronic toxicity of the compounds on rats were evaluated. **Methods and Results:** Treatment with both GI-16 and SC-15 (50mg/kg) reduced significantly (53-77%) the paw oedema over 24 h evoked by subplantar injection of 2% carrageenan (30ul). GI-16 and SC-15 (50mg/kg) greatly inhibited LPS-induced TNF- α (around 34%) and IL-6 (89% and 73%, respectively) production in bronchoalveolar lavage. In contrast, thalidomide and SC-15 enhanced IL-10 ($p < 0,05$). As expected, thalidomide (50mg/kg) and the reference drug dexamethasone (10 mg/kg) caused significant inhibition of both carrageenan-induced oedema and of TNF and IL-6 production 24 h after intratracheal administration of LPS (50ul; 200ug/ml). Female and male Wistar rats administered with GI-16 and SC-15 (20mg/kg) did not develop any clinical signs of acute toxicity (single dose) or sub-chronic



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toxicity (every other day for 28 days) either immediately or during the post-treatment period. No mortality occurred in both control and treated animals either immediately or during the 28-days period. Body weight gain over time was similar in all groups. There was no significant alteration in enzyme activity of AST, ALT and AP in both GI-16 and SC-15 treated rats. A similar trend of results was observed with regard to serum levels of glucose, urea, creatinine, total cholesterol and triglyceride during the 28-days experimental period. GI-16 and SC-15 caused no significant alterations in HB, RBC and WBC count. Sections of liver, kidneys and heart tissues of the studied rats (n=5/group) showed no pathological alterations under light microscopy.

Conclusions: These results strongly reinforce the anti-inflammatory effects of GI-16 and SC-15, which make them very attractive drug candidates to treat a broad range of inflammatory diseases.

Financial support: CNPq, FAPEMIG and CAPES.

THE ROLE OF THE CYTOKINE MIF IN THE INFLAMMATORY RESPONSE INDUCED BY URIC ACID CRYSTALS INJECTION IN MICE

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Introduction: Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that is expressed by a wide variety of cells and plays an important role in the pathogenesis of numerous inflammatory and autoimmune diseases. Gout is an inflammatory arthropathy caused by the precipitation of monosodium urate (MSU) crystals in the joint, which is associated with the production of important inflammatory mediators, such as the cytokines IL-1 β , TNF- α and CXCL1, as well as neutrophil recruitment. This study aimed to evaluate the participation of MIF in the inflammatory response induced by MSU using a model of gout in mice. **Methods and Results:** All experiments were performed in male C57/BL6 mice (8-12 weeks old; 6-10 animals per group). Injection of recombinant mouse MIF intra-articularly (100 ng; 6 h) induced an intense neutrophil accumulation into the joint cavity (V: 0.10 ± 0.09 ; MIF: 6.81 ± 3.4 - cells $\times 10^4$). This was associated with an elevated production of the neutrophil-related chemokine CXCL1 (V: 12.33 ± 8.37 ; MIF: 134.29 ± 28.59 - pg/cavity) as well as high levels of IL-1 β (V: 83.48 ± 21.08 ; MIF: 150.83 ± 19.26 - pg/cavity) (ELISA). MSU crystals injection induced MIF expression on synovial tissue at earlier time points (1, 3 and 6 h - WB). To verify the importance of MIF in the gouty inflammation, we used the MIF inhibitor (ISO-1; 50 μ g; 40 min before MSU crystals injection). 15 h after MSU injection there was a reduced neutrophil infiltration (V: 3.85 ± 1.64 ; MSU: 84.16 ± 8.50 ; ISO-1: 30.33 ± 4.98 - cells $\times 10^4$) and IL-1 β (V: 46.53 ± 14.02 ; MSU: 243.10 ± 62.75 ; ISO-1: 110.06 ± 42.26 - pg/cavity) production compared to vehicle-treated mice. However, ISO-1 treatment was not effective in reducing CXCL1 production (V: 13.48 ± 2.31 ; MSU: 49.49 ± 11.39 ; ISO-1: 47.49 ± 4.54 - pg/cavity). The reduced inflammatory



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response by ISO-1 treatment was effective in decrease mechanical hypernociception (MSU: 4.61 ± 0.32 ; ISO-1: 2.55 ± 0.41 - g). **Conclusion** These results suggest that MIF plays an important role in the pathogenesis of gout, which have a major contribution to neutrophil recruitment to joint cavity after MSU crystals injection.

Financial support: CNPq, FAPEMIG, CAPES.

VASCULAR ADHESION PROTEIN-1 (VAP-1) INHIBITOR IMPAIRS NEUTROPHIL BUT NOT EOSINOPHIL RECRUITMENT IN MICE MODELS OF PULMONARY INFLAMMATION

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Aim: Vascular Adhesion Protein-1(VAP-1) is a cell surface and circulating enzyme implicated in leukocyte adhesion to endothelium. VAP-1 has been shown to play a significant role in leukocyte rolling and transmigration, in complementary pathway with Selectins and Integrins. This study aimed to evaluate the role of VAP-1 inhibitor in mice model of pulmonary inflammation induced by *Klebsiella pneumoniae* infection and in sub-lethal CLP-model, on which PMNs plays a central role, as also evaluate the role of VAP-1 inhibitor in Ovalbumin (OVA) induced allergy on which eosinophils are the major mediators.

Methods and Results: To evaluate the role of VAP-1 in pneumonia caused by *K. pneumoniae* infection, mice were intra-tracheally instilled with 10^7 CFU and treated orally with 2 mg/Kg of VAP-1 inhibitor daily. The infected mice presented about 15% of lethality at day 3 and 50% at day 5, while the VAP-1 inhibitor treated mice had no lethality and its bacterial load in the lung was equal to the untreated animals. Infection induced elevated leukocyte influx into the airways, markedly by increased number of PMNs, macrophages and lymphocytes. However, reduced leukocyte number was found in the VAP-1 inhibitor treated mice. Moreover, the treatment with VAP-1 inhibitor reduced TNF- α lung levels, but not IL-1 β , IL-6 and CXCL1/KC induced by infection. For the sub-lethal CLP-model mice were exposed to cecal ligation and perforation-induced sepsis and treated orally with 6mg/Kg or 0.6 mg/Kg of VAP-1 inhibitor daily, during 7 days. Treated mice showed less 40% of lethality in comparison with vehicle. Treatment led to a reduction of total leukocytes number in lung, markedly by a decrease in the neutrophil influx. The challenge with OVA increased leukocyte influx into airways, mostly eosinophils, and elevated Eosinophil Peroxidase lung levels. Nevertheless, the VAP-1 inhibitor-treated mice failed to reduce lung eosinophil accumulation.

Conclusion: The protection from lethality by VAP-1 inhibition must be due to observed reduction of PMN influx, in *K. pneumoniae* infection and CLP sepsis model, and also decrease of TNF- α levels in the lung, even without reducing the bacterial load, in *K. pneumoniae* infection. The VAP-1 blockade was capable of reducing the neutrophils influx into airways, but not eosinophils. Therefore, VAP-1 can be an important target in the control of neutrophil-mediated lung inflammatory diseases.



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ASSOCIATION OF EXTRACTS FROM *Caulerpa mexicana* WITH PROBIOTICS REDUCED CLINICAL SIGNS OF ULCERATIVE COLITIS IN MICE AND Th1 AND Th17 CYTOKINES LEVELS

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Introduction: The regulation of the inflammatory response is essential to avoid or minimize excessive inflammatory process and several studies have investigated new drugs that may contribute to this, especially drugs provided by natural sources. The aim of this study was to evaluate the effect of association of extracts of the green algae *Caulerpa mexicana* with the probiotic *Enterococcus faecium* 32 in experimental colitis model.

Methods and Results: The ulcerative colitis, an inflammatory bowel disease, was experimentally induced in C57Bl/6 male mice by dextran sulfate sodium (DSS) administered in the drinking water during seven days. The disease activity index (DAI) was evaluated daily to each animal as a clinical assessment and was based on weight loss, stool consistency and presence of blood in the stools. After mice euthanasia, the colons were removed, and the colon culture was proceeded for Th1, Th2 and Th17 cytokines dosage in the supernatants. Simultaneous treatment of mice with methanolic extracts of *C. mexicana* was able to reduce the disease activity index ($p < 0.05$) when compared to positive controls. Furthermore, the treatment of mice with *C. mexicana* extracts associated with the probiotic also decreased the secretion of IFN-g ($p < 0.05$), IL-12 ($p < 0.01$), IL-17 ($p < 0.05$) and IL-6 ($p < 0.01$) in supernatants of colon culture. We did not observe any difference in the IL-4 levels.

Conclusion: We concluded that administration of methanolic extracts of *C. mexicana* associated with the probiotic *Enterococcus faecium* 32 resulted in a reduction of inflammatory parameters of ulcerative colitis induced by DSS, with possible negative regulation on Th1 and Th17 cytokines production.

Financial support: CNPq, BNB, CAPES

CYTOTOXICITY EVALUATION OF SOYBEAN BIOTRANSFORMED BY FUNGUS IN CULTURE OF HUMAN ENDOTHELIAL CELLS

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Introduction: Cardiovascular diseases (CVD) are the main cause of death in postmenopausal women in developing countries. This is due to loss of cardiovascular protection attributed to estrogen deficiency that occurs physiologically during this period. Two large studies showed that estroprogestative Hormone Replacement Therapy (HRT) proposed for this phase has as side effects breast cancer and endometrial. Searching for alternative therapies to HRT, the literature suggests phytoestrogens, especially isoflavones present in soy as a drug to ameliorate menopausal symptoms, because it acts as agonists for estrogen receptors, promoting beneficial effects on endothelium. In this work we focused a preparation of soybean biotransformed by the fungus *Aspergillus awamori* to investigate its effects on the production of vasoactive factors in Human Umbilical Vein Endothelial Cell (HUVEC). **Methods and Results:** 1) HUVEC extraction was performed according Climacteric. 15:186 -194.2012.; 2) The HUVEC were identified by the presence of the marker CD31 (PECAM-1, Platelet-Endothelial Cell Adhesion Molecule-1) by flow cytometry 3)The soybean extract not biotransformed (SE) and the extract biotransformed, alcoholic and aqueous fractions (SEBFalc and SEBFAQ) were produced in accordance to J. Appl. Microbiol. 106:459-466,2009. ; 4) The cytotoxicity assays were performed using the MTT method as described by J. Immunol. Methods. 16:55-56, 1983. The soy metabolites, Daidzein (D) and Genistein (G) were used as standards. The cell viability assay by MTT showed nearly 100% of viable cells to 0,025mg/mL of SEBFalc (93,1% ± 15,0) and SE (111,5%±21,2) and 0,003mg/mL for the SEBFAQ (101,92%±18,7). The concentrations of the standards D and G present in soy preparations were determined by HPLC: 0.0151µg/mL and 0.0125µg/mL, respectively. **Conclusion:** These concentrations are defined and will be used in the future to determine the effect of these soy preparations in the production of vasoactive factors endothelium-derived.

Financial support: FAPESP.

PAF-R ENGAGEMENT MODULATES CLASSICAL AND ALTERNATIVE ACTIVATION OF MOUSE MACROPHAGE

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Introduction: The platelet activating factor receptor (PAF-R) is a G-protein coupled receptor present in the plasma and nuclear membrane of macrophages and other cells. Depending on the experimental model, the engagement of PAF-R in macrophages has been associated to either suppressor or activation functions. Here, we investigated whether PAF-R is involved in macrophages differentiation towards the classical (M1) and alternative (M2) activation phenotype.

Methods: Murine (C57BL/6 and BALB/c) bone marrow-derived macrophages (BMDM) were obtained by 6-days culture of bone marrow cells with 20% L929 culture supernatant. Of the adherent cells, 96% were positive for the specific macrophage markers F4/80 and CD11b. Polarization stimuli employed for M1 were LPS (50 ng/mL)/IFN-g (5 ng/mL) and for M2 was IL-4 (20 ng/mL). After stimulation, PAF-R, PAF acetylhydrolase (PAF-AH), arginase-1 (Arg-1), mannose receptor (MR), IL-12 and IL-10 were evaluated by real time RT-PCR; Arg-1 and iNOS by western blotting; MCP-1, IL-6 and TNF- α in supernatants by ELISA. **Results:** M1 cells from both mouse strains showed high IL-12, IL-10, iNOS, MCP-1, TNF- α and IL-6, but not Arg-1 nor MR mRNA expression. M2 cells expressed high MR and Arg-1, low IL-10 and did not express IL-12 mRNA. PAF-R mRNA expression was higher in M1 compared to M2. BALB/c mice expressed higher levels of PAF-R mRNA than C57BL/6, but lower levels of PAF-AH. In macrophages from C57BL/6, the PAF-R antagonist (WEB2170, 50 mM) added 30 min before polarization stimuli, reduced MCP-1, TNF- α , iNOS, MR and strongly inhibited the M2 marker Arg-1 mRNA expression. In macrophages from BALB/c, blocking the PAF-R reduced only the expression of Arg-1, but had no effect on the expression of M1 markers. PAF-R antagonist reduced more intensively the expression of IL-10 compared to IL-12 mRNA and this was observed in both mouse strains. Thus, PAF-R seems to modulate more selectively the M2 phenotype.



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Conclusion: Taken together, these results show that, during macrophage activation, PAF-R engagement by endogenous ligands influences macrophage polarization, preferentially towards M2 profile.

Financial support: FAPESP, CNPq and CAPES (Proex).

PROTECTIVE EFFECT OF *Cissampelos sympodialis* ON LIPOPOLYSACCHARIDE-INDUCED ACUTE LUNG INJURY IN MICE

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Introduction: *Cissampelos sympodialis* Eichl (Menispermaceae) is used in Brazilian folk medicine to treat lung diseases. Studies using the alcoholic fraction of leaves (AFL) showed to inhibit inflammatory cell migration into the airways and the release of inflammatory mediators such as histamine. Acute lung injury (ALI) is a clinical problem, which is characterized by disruption of lung epithelial and endothelial cells, alveolar permeability, inflammatory cell influx followed by severe hypoxemia, thus leading to morbidity and/or mortality. The goal of this study was to evaluate the AFL in the experimental model of ALI.

Methods and Results: Swiss female mice (n=6) were orally pretreated with 50, 100 mg/kg or 200 mg/kg AFL or dexamethasone (5 mg/kg, intravenous) 1h before challenging with 50 μ l of LPS (2.5 mg/mL, intranasal) or PBS. After 24h the bronchoalveolar lavage was obtained to characterize leukocyte migration and total protein concentration. Furthermore the lungs were collected and processed to qualitative histological analysis with H&E stain. The experimental protocols were approved by the Ethics Committee on Animal Research / UFPB with protocol number 0309/11. Animals pretreated with 50 or 100 mg/kg of AFL showed significant ($p < 0.05$) reduction of total inflammatory cells (55.2% and 34.1%, respectively), polymorphonuclear cells (63.8 and 40.7% respectively) and protein leakage concentration (30.2% and 21.7% respectively). The pretreatment with these doses also attenuated the development of pulmonary edema, vasodilatation and maintenance of the epithelium integrity. However the dose of 200 mg/kg did not reduce the cell migration, protein leakage or histological inflammatory pattern. As expected, dexamethasone effectively decreased all parameters evaluated.

Conclusion: The results indicated that AFL is effective in modulating the ALI parameters by inhibiting cell migration to the lungs probably by decreasing lung vascular permeability. The best dose to treat ALI was the lower one, suggesting the presence of compounds with antagonist effects.

Financial support: CNPq/CAPES/INCT Câncer

ANTI-INFLAMMATORY EFFECT OF GAMA-TERPINEN IN EXPERIMENTAL MODEL OF ACUTE LUNG INJURY

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Introduction: Genus *Eucalyptus* is rich in essential oils such as gama-terpinen which is a monoterpene and it has being described with anti-inflammatory activities. Acute lung injury (ALI) is a disease that affects a wide population. The pathology is characterized by lung cell influx, edema, expression of pro-inflammatory mediators, lung epithelium and endothelium injury. Corticosteroids have being used for the treatment of ALI, but these drugs do not have effective effect. Therefore research to demonstrate molecules with beneficial effects on ALI is essential. The objective of this study was to evaluate the effect of gama-terpinen in experimental model of ALI.

Methods and Results: Swiss female mice (n=6) were challenged with 50 μ L of intranasal PBS or LPS (2.5 mg/mL) and one hour after the animals were orally treated with 12.5 mg/kg, 25 mg/kg or 50 mg/kg of gama-terpinen, dexamethasone (5 mg/kg, intravenous) or vehicle soy oil. Bronchoalveolar lavage (BAL) was obtained to characterize leukocyte migration and protein concentration by colorimetric assay. Furthermore the lungs were collected and processed to qualitative histological analysis with hematoxylin and eosin (H&E) stain. Experimental protocols were approved by Ethic Committee on Animal Research / UFPB. Animals treated with 25 or 50 mg/kg of gama-terpinen showed significant ($p < 0.05$) reduction of total inflammatory cells (49.4% and 62.6%, respectively) and polymorphonuclear cells (68.6% and 65.5%, respectively). However the dose of 12.5 mg/kg was not effective in decreasing total cell migration to the lung, only neutrophil had reduction. The protein leakage was not reduced by treatments. The treatment with the three doses also attenuated the development of pulmonary edema, vasodilatation and maintained the integrity of the epithelium. As expected, dexamethasone effectively decreased all parameters evaluated.

Conclusion: According to the study, gama-terpinen is able to decrease the inflammatory cell migration to the lung in the experimental model of ALI independently of the vascular permeability.

Financial Support: CNPq/CAPES/INCT Cancer.

***OCIMUM GRATISSIMUM* L. ATTENUATES INFLAMMATORY PARAMETERS INVOLVED IN THE LIPOPOLYSACCHARIDE-INDUCED TLR4 ACTIVATION, *IN VITRO*.**

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Introduction: *Ocimum gratissimum* is commonly used in folk medicine to treat inflammatory disease such as asthma and others immune-mediated diseases. Lipopolysaccharide (LPS) is a component from gram-negative bacteria responsible to induce inflammation by activating Toll-like receptor 4 (TLR4) signal pathway, producing mediators such as pro-inflammatory cytokines (IFN- γ and TNF- α) and Nitric Oxide (NO).

The aim of the present study was to evaluate the anti-inflammatory effect of *Ocimum gratissimum* in stimulated culture with LPS.

Methods and Results: Spleen cells of BALB/c mice were stimulated with 20 $\mu\text{g}/\text{mL}$ of LPS with or without *Ocimum gratissimum* methanolic extract (Og) at a concentration of 50 or 5 $\mu\text{g}/\text{mL}$. After 72h of culture, the supernatants were collected and IFN- γ levels were determined by ELISA. Cell proliferation was estimated based on MTT-tetrazolium method. In order to evaluate nitric oxid production, BALB/c mice received 20 $\mu\text{g}/\text{animal}$ of LPS, i.p.. After 72h of injection, peritoneal lavage was performed to obtain the macrophages. Nitric oxid production in macrophage culture was induced by 5 $\mu\text{g}/\text{ml}$ of LPS stimulation *in vitro*. Og was added at 1-100 $\mu\text{g}/\text{mL}$. After 24h incubation, NO production was determined by Griess' reaction. Our results have shown that LPS increased spleen cells proliferation ($p < 0.001$) as well as IFN- γ in culture ($p < 0.05$) and NO production in macrophage culture ($p < 0.001$). Og decreased



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LPS-induced IFN- γ production at both concentrations 50 μ g/mL ($p < 0.01$) and 5 μ g/mL ($p < 0.05$), but did not affect cell viability. In addition, Og in both concentrations tested (100 and 50 μ g/mL) reduced the LPS-induced NO production ($p < 0.001$ and $p < 0.05$, respectively).

Conclusion: Og attenuates inflammation using a *in vitro* model of LPS-induced TLR4 activation. Further studies are needed to further elucidate the mechanism whereby Og exerts its anti-inflammatory activity.

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EFFECTS OF ISONIAZID ON A HUMAN IN VITRO GRANULOMA MODEL

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Introduction and objectives: Tuberculosis (TB) is a major public health threat worldwide caused by *Mycobacterium tuberculosis* (Mtb) and the granuloma is the hallmark structure of this disease. One of the main anti-TB drugs used is isoniazid (INH), which inhibits mycobacterial mycolic acid synthesis. In addition, *in vitro* studies have suggested that INH induces apoptosis in different cell lineages and this might be an indirect effect of this drug. In this context, we aim to investigate the effects of INH on cellular dynamics of a human *in vitro* granuloma model.

Methods and results: Peripheral blood was obtained from healthy donors by informed consent and PBMCs were isolated by Ficoll-Hypaque gradient. Cells were seeded at a density of 6×10^5 cells in 200 μ l supplemented RPMI medium into the agarose-coated 96-well plates and exposed to mycobacteria to generate granuloma-like structures. After 3 days, cells were treated with 0.1 or 100 μ M of INH for 24 hours. Cell death and proliferation were evaluated by flow cytometry using annexin V/propidium iodide and carboxyfluorescein diacetate, succinimidyl ester, respectively. Compared to untreated cells, exposure to low concentration of INH induced cell death, being necrosis higher in uninfected cells and apoptosis higher in infected cells. Moreover, low concentration of INH decreased cellular proliferation compared to untreated cells. Surprisingly, granulomas treated with higher concentration of INH displayed no changes on both cell death and proliferation.

Conclusions: These findings suggest that low concentration of INH could influence cell proliferation on granuloma-like structures. Further experiments to investigate whether lymphocytes are specifically involved in proliferative responses to INH are in progress.



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CRUDE ETHANOLIC EXTRACT OF *Piper arboreum* AUB. (PIPERACEAE) DECREASES THE NITRIC OXIDE PRODUCTION BY PERITONEAL PHAGOCYTES

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Introduction: Several species of genus *Piper* have been used in alternative medicine to treat vaginitis, gynecological and intestinal disorders. Furthermore other species of the large family *Piperaceae* have been identified as a source of several substances with different properties: psychotropic, antioxidant, antimicrobial and cytotoxic effects. However, the influence of plant's extract in the immune system modulation remains to be unexplored. In the present study we determine the influence of ethanol extract of leaves of *P. arboreum* on the peritoneal phagocytic cell activity as well as on the lymphoproliferative activity of spleen cells.

Methods and Results: The ethanolic extract of leaves was obtained by maceration for three weeks at room's temperature. After filtering, the solvent was evaporated under reduced pressure. The first dilution of the extract was performed in dimethyl sulfoxide followed by subsequent dilution in sterile phosphate buffered saline solution. Swiss mice were killed by carbon dioxide asphyxia and peritoneal cells and spleen were collected. Peritoneal phagocytes and spleen cells were cultivated in vitro and challenged with the crude extracts in different concentrations (0.391, 0.196 and 0.097 mg/ml). After 24 hours, the peritoneal phagocytes were submitted to evaluation of peroxide hydrogen (H_2O_2) and nitric oxide (NO) production and the spleen cells to lymphoproliferation analyses. Our results demonstrated that the concentration of 0.097 mg/ml triggered lymphoproliferation of spleen cells and the dose below of 0.196 mg/ml impaired the NO release. No differences were observed in the H_2O_2 production.

Conclusion: The inhibition of NO production by crude ethanolic extract of *P. arboreum* highlights its potential as an interesting option in immunotherapy treatment.

REACTIVE OXYGEN SPECIES-DEPENDENT INFLAMMASOME ACTIVATION MEDIATES IRINOTECAN-INDUCED MUCOSITIS THROUGH THE CONTROL OF IL-1 β AND IL-18 RELEASE.

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Introduction: Irinotecan is a chemotherapeutic utilized during treatment of several solid tumours, but this treatment is associated with several side effects, as mucositis. During mucositis occurs released of cytokines, as IL-1 β , and ROS. Recently, has been shown that ROS may promote inflammation by activating the inflammasomes leading to caspase-1 activation and subsequent cleavage of pro-IL-1 β and pro-IL-18. Interestingly, the treatment with the IL-1 receptor antagonist decreases the severity of mucositis. However the mechanisms involved in such protection and the pathways that mediate IL-1 production are still unknown. Thus, the aim of this study was to assess the role of inflammasome in Irinotecan-induced mucositis. **Methods:** To induce mucositis mice received saline or Irinotecan (75 mg/kg) was administered (i.p.), once a day, for four consecutive days. It was utilized mice WT, ASC^{-/-}, ICE^{-/-}, IL-18^{-/-}, GP91phox^{-/-}. Seven days after the beginning of the Irinotecan treatment, mice were euthanized by cervical dislocation. For the evaluation of the role of IL-1 receptor, WT or IL-18^{-/-} mice were treated (s.c.) with IL-1Ra (4 mg/kg) or saline, 8 hours before the first dose of Irinotecan. Then, mice were treated every 8 hours for the following 7 days, during all experimental period. For NOX inhibition, WT mice received one i.p injection of Apocynin (20mg/kg) 24 hours before the first dose of Irinotecan and then a daily i.p. injection of Apocynin along all the experimental period. Ileum was removed to analyze of cytokines (ELISA), influx of neutrophils (MPO), burst oxidative (TBARS and GSH), analyze histological and Western Blotting. **Results and conclusion:** Mice ASC^{-/-} and ICE^{-/-} presented less intestinal lesions, less MPO and less production of IL-1 β and IL-18. In addition, mice IL-18^{-/-} and WT mice treated with IL-1Ra was characterized by less MPO and less intestinal lesion. In addition, treatment



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with IL-1ra decreases the levels of IL-18 in WT mice and *IL-18^{-/-}* mice presented less production of IL-1 β . The animals WT that received Irinotecan showed

increase TBARS and increase of the expression of caspases-1, while *gp91^{phox-/-}* or Apocynin-treated mice presented decreased this parameters ($p < 0,05$). These results demonstrate the participation of inflammasome in the development of Irinotecan-induced mucositis and show that ROS derived from NADPH oxidase (NOX) control caspase-1 activation during mucositis. Approved by CETEA, protocol 113/11.

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CHLOROGENIC ACID WORSEN INTESTINAL INFLAMMATION IN A CHRONIC DSS-INDUCED COLITIS

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The inflammatory bowel diseases (IBD) are defined as chronic inflammatory diseases causing long-term and sometimes irreversible damage to the gastrointestinal structure and function. Although there is a large number of therapy trials in IBD, an effective therapeutic approach remains to be determined. Therefore, the aim of our work was to investigate the effects of chlorogenic acid (CGA), one of the most abundant polyphenol compounds in human diet, which has anti-inflammatory and antioxidant properties, in a murine model of chronic DSS-induced colitis.

Balb/C male mice, were divided in 3 groups: Naive (N), DSS+Vehicle (V) and DSS+CGA (CGA). Chronic colitis was induced by the addition of DSS 5% (wt/vol) to autoclaved drinking water for 5 days following normal autoclaved drinking water for 11 days. This cycle was repeated twice with DSS 2.5%. In the last 11 days of the 3rd cycle, the V group received vehicle solution (carboxymethylcellulose–2mg/mL) and CGA group received CGA solution (50mg/kg); both oral treatment.

We observed a decrease of the % of body weight and colon length, associated to an increase in the colon length/weight ratio in the groups V and CGA when compared to group N ($P < 0.05$). Additionally, we observed that CGA was able to increase these effects when compared to group V ($P < 0.05$). No difference was found in the total cellularity of bone marrow, but we observed a decrease of total leukocyte in the blood count in the groups V and CGA compared to N group.

Altogether these results show that CGA during the chronic DSS-induced colitis was able to worsen the colitis. Currently more studies are in progress in order to understand this phenomenon.



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EVALUATION OF THE ANTIOXIDANT ACTIVITY OF 3-PHENYLCOUMARIN DERIVATIVES IN THE OXIDATIVE METABOLISM OF HUMAN NEUTROPHILS STIMULATED WITH IMMUNE COMPLEXES.

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Introduction: Reactive oxygen species (ROS) production by neutrophils, which is an essential mechanism of innate immunity against infectious agents, can be triggered by immune complexes (IC). In some inflammatory diseases there is IC deposition, which triggers massive neutrophils recruitment and activation. In these situations, there are local disorders provoked by the oxidant and lytic compounds released by neutrophils. The present work reports on the evaluation of the antioxidant potential of ten 3-phenylcoumarin derivatives in the neutrophil oxidative metabolism stimulated with IC, through the luminol- and lucigenin-dependent chemiluminescence assays. **Methods and Results:** Samples cytotoxicity was evaluated by the trypan blue exclusion assay, lactate dehydrogenase activity measurement, and ability to scavenge HOCl and inhibit NADPH-oxidase and myeloperoxidase (MPO) activity. All the samples inhibited QL-lum and QLuc, being the inhibitory effect of five of them higher than or equal to 50%. This biological effect was not mediated by 3-phenylcoumarins cytotoxicity under the assessed conditions. Compound **C13** (6,7-dihydroxy-3-[3',4'-methylenedioxyphenyl]-coumarin) was the most effective QLuc inhibitor among the set of tested phenylcoumarins. CLuc inhibition by phenylcoumarins seems to be independent of NADPH-oxidase activity inhibition, since these compounds had a slight inhibitory effect on O₂ consumption by IC-stimulated neutrophils. On the other hand, compounds **C13** and **C24** (6,7-dihydroxy-3-[3',4'-dihydroxyphenyl]-coumarin) had the highest inhibitory effects on CLum, with the tested samples having similar IC₅₀ values. These compounds were also the most active in terms of MPO activity inhibition and HOCl scavenging. **Conclusion:** Our results show that the antioxidant activity of 3-phenylcoumarin derivatives in the oxidative metabolism of human neutrophils is related to the structural characteristics of these molecules. Among the evaluated set of compounds, **C13** and **C24** are the most promising samples for use as



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prototypes of molecules with therapeutic application in the modulation of neutrophil function. **Key words:** neutrophils, immune complexes, reactive oxygen species, phenylcoumarins, antioxidant.

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Baccharis dracunculifolia EXTRACT MODULATES REACTIVE OXYGEN SPECIES PRODUCTION WITHOUT MODIFICATION OF THE MAIN EFFECTOR FUNCTIONS OF HUMAN NEUTROPHILS

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Introduction: Reactive oxygen species production (ROS) by neutrophils is an important process in the defense against microorganisms, but ROS overproduction contributes to the pathogenesis of various diseases. In this sense, administration of antioxidant compounds may be beneficial to patients. The *Baccharis dracunculifolia* D.C. (Asteraceae) is a native Brazilian plant, and its extract is used in the treatment of inflammatory processes in folk medicine. This study aimed to evaluate the seasonality effect on the *B. dracunculifolia* (Bd) antioxidant activity in stimulated neutrophils and verify the effect of this plant on the main effector functions of these cells. **Methods and Results:** Extracts of Bd harvested on a monthly basis for 14 months (from Jun/06 to Jul/07) were analyzed by HPLC and evaluated for their ability to inhibit the luminol- and lucigenin-enhanced chemiluminescence (lumCL and lucCL, respectively). The toxicity of the most active extract (May/07) was verified by trypan blue dye exclusion and lactate dehydrogenase (LDH) release assays. We also assessed the effect of the most active extract on neutrophils phagocytosis and microbicidal activity. All the samples inhibited the lumCL and lucCL in a concentration-dependent manner. The sample of May/07 presented the highest activity (IC₅₀= 8.1 ± 1.1 µg/mL for lumCL and 6.4 ± 1.9 µg/mL for lucCL), and it was not related to the cytotoxicity at the studied concentrations. Also, this extract did not alter the ability of cells to phagocytize and destroy pathogens. **Conclusion:** Bd has a modulatory antioxidant activity against ROS produced by stimulated neutrophils in a non-toxic way, and this effect does not



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alter the main neutrophils effector functions, like phagocytosis and microbicidal activity. These results emphasize the importance of the study of natural products for the discovery of potential therapeutic agents.

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APPLICATION OF GALANGIN AS ANTI-INFLAMMATORY COMPOUND THROUGH OXIDATIVE METABOLISM MODULATION AND REDUCTION OF INTEGRIN CR3 EXPRESSION IN HUMAN NEUTROPHILS

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Introduction: The activity of immune system cells contributes to both protection and development of disease in the host. Leukocyte infiltration is a common finding in most inflammatory diseases. Integrin CR3 and Fc gamma (F γ) play an important role in this process, causing tissue damage, due to intense ROS production. Therefore, introduction of endogenous compounds that can modulate neutrophilic function could be beneficial for the treatment of inflammatory diseases. **Objectives:** Evaluation of the potential of the flavanol galangin to modulate the oxidative burst of human neutrophils stimulated by Fc γ and CR receptors and the expression these receptors on membrane. **Methods and Results:** Human neutrophils isolated by the gelatin method were employed. These cells were stimulated with different immune complexes in the presence of galangin or not, and the production of reactive oxygen species (ROS) was analyzed by luminol enhanced chemiluminescence assay. The results showed that galangin inhibited neutrophil ROS production and interrupted the cooperation of Fc γ and CR receptors. This effect was dependent on phagocytosis, as observed in experiments with other soluble activators such as calcium ionophore and PMA. Fc γ and CR3 expression on the membrane of neutrophils treated with galangin was also evaluated by using specific antibodies. The results showed that this compound was only able to promote reduction in CR3 expression on surface human neutrophils. This effect presented by galangin was not related to cell death or antioxidant action, as evaluated by trypan blue exclusion and DPPH assay, respectively. **Conclusion:** Galangin was efficient in modulating ROS production in human neutrophils stimulated via Fc gamma and CR receptor. This effect may be related to



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interference in CR3 expression. Together, these results showed that galangin has anti-inflammatory activity and could possibly reduce tissue damage in animal models of inflammatory disease.

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IMMUNOMODULATORY EFFECT OF THE CAROTENOID FUCOXANTHIN ASSOCIATED WITH VITAMIN C IN HUMAN NEUTROPHILS FUNCTION

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Introduction

Neutrophils provide the first line of defense of the innate immune system by phagocytosing, killing, and digesting bacteria and fungi. During these process neutrophils produce reactive oxygen species (ROS) which in excess can corroborate to cellular and tissue injuries. Fucoxanthin (Fc) is a carotenoid that has been studied for its antioxidant and anti-inflammatory actions. Vitamin C (Vit.C) also demonstrates a potent antioxidant action. The aim of the present study was to evaluate the effect of the Fc (2 μ M) associated with Vit.C (100 μ M) on oxidative and functional properties of human neutrophils.

Methods and Results

Neutrophils were obtained through the collection of human peripheral blood. After isolation, cells (1×10^6) were cultivated and treated freshly or up to 18 h with Fc (2 μ M) and/or Vit.C (100 μ M). The following evaluations were performed: phagocytic capacity, cytokines release (TNF- α , IL-6/ELISA), production of ROS: superoxide anion (dihydroethidium-DHE and lucigenin probe), hydrogen peroxide (DCFH-DA probe), MPO activity and HOCl release, antioxidant enzyme activities (SOD, Cat, GPx and GR), activity of G6PDH and GSH/GSSG ratio. All the experiments were performed in triplicate ($n > 4$). Fc, Vit.C and its association caused an increase in phagocytosis of neutrophils (34, 47 and 39%, respectively). The treatments showed a decreased in TNF- α and IL-6 release. Superoxide production assessed by DHE probe was decreased by Fc (20%), Vit.C (61%) and Fc+Vit.C (65%). Lucigenin assay also demonstrated decrease after Fc (57%), Vit.C (18%) and Fc+Vit.C (54%) treatments. The production of H₂O₂ was decreased in neutrophils treated with Fc, Vit.C and by the combination of both antioxidants. Fc reduced MPO activity, whereas Vit.C and Fc+Vit.C showed a more pronounced effect. Neutrophils' treatment with Fc, Vit.C and Fc+Vit.C decreased HOCl release. Fc decreased SOD (19%), Cat (36%) and GPx (34%), whereas Vit.C increased GR (221%). GSH/GSSG ratio was only altered by Vit.C treatment. Fc and Fc+Vit.C increased the G6PDH activity (24%).

Conclusion

These results demonstrate that Fc and Vit.C modulate functional properties of human neutrophils as shown by reduced ROS production and inflammatory cytokines release. Association of Fc and Vit.C has a better antioxidant activity when compared to Fc alone. However, Fc *per se* modulates the antioxidant enzymes in an independent way of Vit.C.



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ELLAGIC ACID PROMOTE THE RESOLUTION OF ALLERGIC AIRWAYS RESPONSES

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Introduction: Most patients with asthma have symptoms that are readily controllable by standard asthma therapies, however, 5-10% of asthmatic individuals have poorly controlled disease. Plant-derived secondary metabolites are the basis for many drugs currently used to treat pathologic conditions. Ellagic acid demonstrates anti-inflammatory, anti-oxidant and anti-allergic activity.

Methods and Results: BALB/c female mice were sensitized by subcutaneous injection (s.c.) on protocol days 0 and 7 with 10 µg ovalbumin (OVA) plus 1 mg aluminum hydroxide in 0.2 ml saline. On protocol days 14, 15, 16 and 17, mice were intranasal (i.n.) challenged with 10 µg OVA in 50µL saline. Mice received ellagic acid (10 mg/kg; orally) or dexamethasone (1 mg/kg, s.c.) or vehicle (water, orally) 30 min before OVA (i.n.) challenge on days 14, 15, 16 and 17 (preventive treatment) or after the last OVA challenge on protocol days 18, 19 and 20 (therapeutic treatment). The analyses in the bronchoalveolar lavage fluid (BALF) and lung were performed during the resolution phase on days 21 and/or 25. During resolution (day 21), the total leukocytes and eosinophils numbers were increased in BALF of vehicle-treated mice compared to control. Ellagic acid or dexamethasone (DEXA)-treated mice displayed decrease in total leukocytes and eosinophils (BALF) as in preventive as in therapeutic treatment compared to vehicle. Lung histopathology (day 21) demonstrated that ellagic acid and DEXA facilitated the resolution of leukocyte infiltration. These effects could be associated to reduction of the P-selectin expression in the lung. The statistical significance of differences was assessed by the Student's t-test and one-way analysis of variance (ANOVA). Data (mean and s.e.m.) are representative of two or more independent experiments with four mice.



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Conclusion: Together, these findings identify ellagic acid as potential pro-resolving therapeutic agent for asthmatic responses.

Financial support: FAPEMIG; CNPq; UFTM; FUNEPU; FAPESP

IMMUNOMODULATORY TREATMENT WITH THALIDOMIDE IN EXPERIMENTAL LEPTOSPIROSIS

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Introduction: Thalidomide is a well known inhibitor of tumor necrosis factor alpha and its immunomodulatory effects have been explored in autoimmune diseases and cancer. Oral administration of thalidomide induced prolonged survival of rats in an experimental model of sepsis by multidrug-resistant *Pseudomonas aeruginosa*. The effects of thalidomide as an adjuvant therapy to antibiotics in experimental leptospirosis have not been investigated. **Methods and Results:** Hamsters were infected by *Leptospira interrogans* strain L1130 and groups were assigned based on no treatment (NONE), thalidomide only (TAL), ampicillin only (AMP) or both (BOTH). Thalidomide was administered via a gastric tube: 50 mg/kg in linseed oil 2 ml/kg for three days. Ampicillin was administered intramuscularly 100 mg/kg/bid for six days. Treatment started two days (experiment 1) and three days (experiment 2) after onset of clinical signs of disease. Experiment 1: all hamsters from groups AMP and BOTH survived (n=8) while all hamsters from groups NONE (n=6) and TAL (n=8) died. Thus, the early start of antimicrobial therapy resulted in universal survival and precluded analysis of thalidomide as an adjuvant treatment option. TAL group, however, had longer interval between infection and death (median 11 vs 10 days) and lower frequency of pulmonary hemorrhages (5/8) when compared to NONE (6/6). Leptospiral load in kidney, liver and lung samples were higher in NONE when compared to other treatment groups. Experiment 2: lethal outcome occurred in 6/6 hamsters of NONE, 8/8 of TAL, and 6/8 in AMP and BOTH groups (median days to death: 9.0 vs 9.5, respectively). In this experiment, thalidomide had no benefit on survival when compared to hamsters treated with ampicillin alone. Leptospiral load in kidney, liver and lung samples were again higher in the NONE group. **Conclusion:** Thalidomide had minimal impact on



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survival in the hamster model of late treatment of experimental leptospirosis.

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INCREASED SUSCEPTIBILITY TO APOPTOSIS OF CD4 AND CD8 T CELLS PERIPHERAL AFTER STIMULATION OF VARIOUS ANESTHETIC

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Introduction: The surgeries are often associated with a temporary decrease of lymphocyte but the causes of the drop are not yet know. The use of any anesthetic has been associated with apoptosis of lymphocytes but in dogs no study was conducted. The effect of anesthetics isoflurane, sevoflurane and midazolam in apoptosis of lymphocytes from dogs was measured in vitro.

Methods and Results: A total of seven healthy dogs without clinical signs of disease were selected. Peripheral blood was immediately processed for isolation of mononuclear cells. Quantification of CD4 and CD8 in apoptosis was measured after incubation of cells with different anesthetics for 1h at 37 °C and 5% CO₂. The cell staining for CD4 and CD8 was performed using monoclonal antibodies conjugated to fluorochromes, apoptosis was measured using the kit Guava Nexin ® Assay. Data were acquired in the cytometer EasyCyteMini and analyzed with the Cytosoft ® software. The results were compared using the Mann-Whitney test, with significance level of 5%. The results indicated that the CD4 T cell stimulated in vitro by the anesthetic isoflurane associated with isoflurane and midazolam showed a significant increase in the level of apoptosis compared with the observed at baseline ($p < 0.05$). It was observed that the CD8 T cell stimulated in vitro by anesthetics isoflurane and midazolam associated with isoflurane or sevoflurane showed significant increase in apoptosis from baseline ($p < 0.05$).

Conclusion: The use of certain anesthetics can cause high levels of apoptosis of CD4 and CD8 T cell, impairing the post-operative recovery. The knowledge about the induction of apoptosis by anesthetics may allow future interventions to anesthetic protocols and reduce depletion of lymphocytes in patients with immune suppression.

Financial Support: CNPq and FAPESP.

Effect of Silicon treatment on the *Artemisia annua* glandular trichome and its artemisinin content, and the role of the plant tea infusion in the control of *Toxoplasma gondii* intracellular replication

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ABSTRACT

Introduction: Toxoplasmosis is a zoonotic disease whose traditional treatment shows adverse effects leading to the research of low-toxicity compounds as artemisinin, its derivatives and *Artemisia annua* infusion. This study investigated the effect of Silicon (Si) in soil on *A. annua* plant physiology, its role on artemisinin content and the role of tea infusion on *T. gondii* replication.

Methods and Results: The experimental design consisted of five treatments (0, 200, 400, 800 and 1600 kg/ha). Analysis of foliar macronutrients showed a significant increase only for Nitrogen at the highest Si concentration. The 400 kg/ha Si treatment induced the highest total glandular trichome area related with the intact glandular trichomes, as observed by scanning electron microscopy. Artemisinin content in plant leaves and tea infusion was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Proliferation assays showed that both cell treatments with tea infusion (with 400 kg/ha Si and without Si) after infection decreased parasite proliferation in a dose-dependent manner. Also, when cell treatment was performed along with the infection, there was inhibition of parasite growth for both treatments, though more evident with the infusion treatment without Si.

Conclusion: In conclusion, the soil with Si had positive effect on the glandular trichome areas and artemisinin contents, but this outcome was not associated



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with a better efficacy of *A. annua* tea infusion on *T. gondii* replication. These findings suggest that other components rather than artemisinin could be contributing to this effect, as flavonoids present in its leaves that may act in synergism with the artemisinin and improving its efficacy.

Financial support: CAPES, CNPq and FAPEMIG.

EFFECT OF LOW CONCENTRATIONS OF ANTINEOPLASTIC AGENTS ON HUMAN LYMPHOCYTES OF PERIPHERAL BLOOD

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Introduction: Previous studies of our group have shown that low concentrations (noncytotoxic) of antineoplastic agents positively modulate the dendritic cells (DC), favoring their in vitro maturation and improving their antigen presentation function. The effects on colorectal cancer cells were also investigated, and it was observed increased immunogenicity, as well as susceptibility to cytotoxic T lymphocytes. However, it has not been evaluated the effects of such low dose of chemotherapeutics on peripheral blood lymphocytes, key cells for antitumor resistance. Thereby, this study aims to investigate the effect of 5-fluorouracil (5-FU) and azacitidine (AZA) at minimum effective (MEC) and noncytotoxic (NTC) on lymphocytes of normal individuals.

Methods and Results: For this analysis cytotoxicity and proliferation assays were performed. Lymphocytes of 8 individuals (n=8) were treated with drugs at working concentrations in the presence or absence of mitogen, as well as apoptosis assays and mixed lymphocyte reaction. The production of IL-10 and IFN- γ was also analyzed in order to evaluate the activity of T cells. Statistical analyzes were performed using analysis of variance (ANOVA) followed by Tukey test for multiple comparisons between groups. Differences were considered significant when the probability of error was lower than 5% ($p \leq 0.05$). The results of cytotoxicity and spontaneous proliferation did not show any difference compared with controls, when cell proliferation was induced by mitogen, chemotherapeutics at MEC significantly reduced the cell number (5-FU: $0,410 \pm 0,088$ and AZA: $0,757 \pm 0,233$; $p < 0,05$). The apoptosis assay also has shown no difference with control groups. Neither the alloreactivity nor the cytokine production were affected by the treatment. **Conclusion:** Our data indicate that administration of the drug has no toxic activity on immune cells, and thereby reinforces the feasibility of the combination of metronomic chemotherapy and immunotherapy in the treatment of colorectal cancer.

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PTX3 molecule exacerbates the MSU-induced inflammation in an experimental model of gout

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Introduction: Gout is an auto inflammatory disorder associated with deposition of monosodium urate (MSU) crystals in joints and periarticular tissues. MSU crystals provoke an intense inflammatory response via NLRP3 inflammasome activation, leading to IL-1 β maturation and release, contributing to chemokine-related neutrophil production, neutrophil recruitment and pain. Pentraxins are a superfamily of acute phase proteins, including the long pentraxin PTX3, a molecule acting as the humoral arm of innate immunity. The objective of this study was to investigate the role of PTX3 in experimental model of gout.

Methods and Results: The mouse model of gout was induced by intra-articular injection of MSU into the knee. To study the participation of PTX3 in this model, mice deficient for PTX3 (PTX3^{-/-}), mice that overexpressed PTX3 (TG2) and their respectively wild type (WT) littermates were used (C57/Bl6 and CD1 - 5-6 mice per group). 6 h and 15 h after MSU injection, mice were culled for the inflammatory parameters assessment. Injection of MSU was associated to an increase of PTX3 detection in the knee joint (6 h - V: 10.77 \pm 2.65, MSU: 20.62 \pm 1.98, 15 h - V: 9.77 \pm 1.11, MSU: 35.55 \pm 2.12). PTX3^{-/-} mice had a reduced inflammatory response followed MSU injection (100 μ g; 15 h), including reduction in neutrophil infiltration into the articular cavity (V: 6.44 \pm 2.78; WT: 37.25 \pm 11.51; PTX3^{-/-}: 6.33 \pm 2.33) and IL-1 β (V: 1.46 \pm 0.13; WT: 70.03 \pm 11.50; PTX3^{-/-}: 15.93 \pm 4.54) and CXCL1 (V: 31.54 \pm 6.65; WT: 98.99 \pm 27.47; PTX3^{-/-}: 49.22 \pm 13.33) production compared to WT mice. In TG2 mice, injection of MSU (30 μ g; 15 h) was associated to the worsening of inflammation compared to WT mice, with an increase of neutrophil accumulation into the knee (V: 0.57 \pm 0.29; WT: 2.71 \pm 1.03; TG2: 12.10 \pm 2.00) and the production of IL-1 β (V: 0; WT: 17.89 \pm 9.77; TG2: 51.09 \pm 19.39) and CXCL1 (V: 11.77 \pm 9.06; WT: 19.30 \pm 9.05; TG2: 9.37 \pm 20.24). Also, TG2 mice had an increased in the hypernociceptive response compared to WT ones (WT: 12.38 \pm 0.30; TG2: 10.04 \pm 0.50). The



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concomitant injection of MSU and PTX3 amplified the cleaved of caspase-1 in synovial tissue as compared to single MSU injection. **Conclusion:** These results indicate that PTX3 have an important contribution to the MSU-induced inflammatory response *in vivo*. Mechanistically, PTX3 facilitates the recognition of MSU by synovial cells, increasing the capase-1 cleavage and enhancing the MSU-associated inflammation.

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INFLUENCE OF ISONIAZID TREATMENT DURING *Mycobacterium tuberculosis* -MACROPHAGE INTERACTIONS

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Introduction: Tuberculosis (TB), caused by intracellular pathogen *Mycobacterium tuberculosis* (Mtb), is one of the most devastating infectious diseases worldwide. One of the main anti-TB antibiotic used is isoniazid (INH), which inhibits mycolic acid synthesis, an important bacteria's cell wall component. *In vitro* studies have suggested that INH-dependent bactericidal efficiency requires both a prolonged time of drug exposure as well as elevated concentrations in infected macrophages. In contrast, multi-centre clinical studies have shown that INH promotes an initial killing of 95% of bacteria during the first 2 days of treatment (Am J Resp Crit Care Med.156:895-900, 1997), pointing to an indirect effect of this drug, which is found at low concentrations (nM range) within the granuloma. To investigate the mechanisms by which INH inhibits Mtb survival during its interaction with macrophages, we have utilized an *in vitro* system in which the bacterium has been exposed to low concentrations of INH and macrophage infectivity as well as cellular activation experiments were performed.

Methods: The virulent Mtb laboratory strain H37Rv was exposed to low INH concentrations (0.01, 0.1 or 1.0 μ M) for 24h. Macrophages were then infected with pre-treated Mtb groups. Following washes, bacterial growth was determined after plating cellular extracts onto 7H10 medium and counting of colony forming units (CFU). TNF and NO (nitrite) concentrations were determined in macrophage supernatants by ELISA and Griess reaction, respectively.

Results: The doses used in this study did not alter extracellular Mtb growth. In contrast, infected macrophage cultures displayed decreased numbers of Mtb when compared to bacterial cultures, indicating that macrophages control Mtb growth. However, at early (4h) or late (24-96h) time points, no difference in CFU



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counting was observed between the untreated and treated infected macrophage groups. Consistently, both treated and untreated Mtb-infected macrophage groups displayed similar cellular activation phenotype as measured by means of TNF and NO levels.

Conclusions: These results suggest that pre exposure of bacteria to INH does not interfere in bacteria infectivity and intracellular survival, which does not explain the efficient INH-induced mycobacterial killing. It is still unknown the precise mechanism by which INH efficiently kills Mtb *in vivo*. Experiments to address this are in progress.

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ASSOCIATION OF CD39 EXPRESSION ON TREG CELLS WITH THERAPEUTIC EFFICACY OF METHOTREXATE FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints. The therapeutic approach of first choice for the RA treatment is the use of low doses of Methotrexate (MTX), an inhibitor of dihydrofolate reductase. However, a considerable number of RA patients is refractory to MTX treatment and the mechanism by which this phenomenon occurs has not been established. Data from the literature show that the antiinflammatory effect of MTX is associated with its ability to maintain high levels of extracellular adenosine (ADO) by a mechanism based on ATP degradation by ectonucleotidases CD39/ENTPD1 and CD73/ecto-5' nucleotidase, which are highly expressed on the surface of regulatory T (Treg) cells. **Objective:** To investigate the mechanisms of MTX resistance in RA patients, focusing in the biochemical activity and suppressive function of Treg cells. **Patients and Methods:** In this study, samples of peripheral blood from RA patients (n = 89) and healthy donors (n = 16) were collected. First, we characterized the groups of RA patients evaluating cytokine production on plasma and by stimulated T CD4⁺ cells. By flow cytometry, leukocyte populations of PBMC were phenotyped for the evaluation CD39 and CD73

expression on different cell types. The activity of ectonucleotidases on CD4⁺ T cells in the generation of extracellular ADO was assessed by high performance liquid chromatography (HPLC). **Results:** Inflammatory cytokines IL-1 β and TNF- α on plasma and the frequency of CD4⁺ T cells producing IL-17 and IFN- γ were significantly increased in unresponsive patients (UR-MTX) compared with responsive patients (R-MTX) and healthy subjects. In the phenotypic characterization of leukocytes, there was no difference in the percentage of CD4⁺T, CD8⁺T, B cells and dendritic cells between groups. However, we observed a significant increase in the percentage of Treg cells (CD4⁺CD25⁺FoxP3⁺) in R-MTX patients. Interestingly, while CD73 expression was found not changed, the percentage of Treg cells expressing CD39 was increased in R-MTX patients. Additionally, the dosage of extracellular ADO in the supernatant of Treg cells from patients UR-MTX was lower compared to other groups, since this group of patients presents a reduced CD39 expression on Treg surface. **Conclusion:** According to these findings the evaluation of CD39 expression on Treg cells might represent a possible biomarker of response to MTX for RA treatment.

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HYALURONIDASE LOADED IN PLGA MICROPARTICLES IS A PROMISE TOOL TO TREAT PULMONARY FIBROSIS

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Introduction: Hyaluronidases (HYALs) comprise a group of enzymes that degrade hyaluronic acid (HA). Our group demonstrated before that a single dose of bovine HYAL intranasally increased a heterogeneous population of mononuclear cells with an MSC-like phenotype in BALF and reduced bleomycin-induced fibrosis. Considering that HYAL could be degraded along the treatment, our purpose was to load the enzyme in microparticles (MP) of Poly (d,l-lactide-coglycolide) [PLGA] and evaluated the potential to treat pulmonary fibrosis, for which very few effective therapies exist.

Methods: Unloaded or HYAL-loaded MP were prepared using emulsion/solvent evaporation and double emulsion/solvent evaporation methods. Size distribution, zeta potential, morphology and encapsulation efficiency of the HYAL in MPs were determined. C57Bl6 mice were exposed to 4 U/kg of bleomycin intratracheally, and 7 days later treated intranasally with 16 U of soluble HYAL, HYAL-loaded-MP or unloaded-MP. Bronchoalveolar fluid (BALF) cells were recovered 96h later and the cells enumerated. Histological analyzes and collagen content were performed using H&E and picrosirius staining.

Results: HYAL were efficiently entrapped in MP. HYAL-loaded MP presented an appropriated zeta potential, and average size and morphology that allowed their pulmonary delivery. Preliminary data showed that HYAL-loaded MP led to a reduction in neutrophil recruitment following bleomycin, greater than soluble HYAL. Unload-MP did not show effect in the fibrosis induced by bleomycin. Histopathological findings showed that the treatment with HYAL-loaded MP is more efficient than soluble HYAL to reduce bleomycin-induced fibrosis and collagen deposition.

Conclusion: The HYAL-loaded MP presented characteristics that make it a potential delivery system to treat pulmonary fibrosis.



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MODULATION OF CYTOKINES PRODUCTION IN ARDS INDUCED BY CLP: LEUKOTRIENES PARTICIPATION

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Introdução: Sepsis is a systemic inflammatory response to infections and has an increased mortality and morbidity in immunocompromised patients. It is the major cause of death in ICU and is among the main causes of death worldwide. About 40% of septic patients develop acute respiratory distress syndrome (ARDS). ARDS is an inflammatory syndrome characterized by increased inflammation and permeability, clinically manifested by refractory hypoxemia, loss of lung compliance, infiltrates on chest radiographs and dyspnea and tachypnea. There is a high production of pro- and anti-inflammatory mediators in sepsis and ARDS such as protein mediators and lipid mediators. In this work we investigated leukotrienes (LTs), which are lipid mediators produced from arachidonic acid by the enzyme 5-lipoxygenase (5-LO) generating LTB₄ and cysteinyl-LTs. The LTs are found in tissues in inflammatory conditions, and have its biological actions through its receptors, BLT (LTB₄) and cys-LT (cysteinyl-LT). It was observed that 5-LO ^{-/-} mice are more resistant to sepsis, and presents a lung protection (histology, morphometry and elastance) and a modulation of cytokine production. The objective of this study is to evaluate the participation of each classes of LTs in modulating the cytokine profile in ARDS induced by sepsis.

Methods and Results: For this purpose we pre-treated C57 black/6 mice with BLT1 and Cys-LT1 antagonists before CLP surgery (where the cecum is ligated and punctured to induce sepsis). After 16 hours, the cytokines from plasma and lung parenchyma were evaluated. The production of Interleukin (IL) -1 β and TNF- α (Tumor Necrose Factor) was higher in plasma and lung of CLP pre-treated with the antagonists group compared to untreated animals after CLP. The production of IL-6, MCP-1 (Macrophage Chemotactic Protein) and KC (keratinocyte-derived chemokine) in the lung parenchyma and plasma as well as the neutrophilic infiltration in the lung tissue showed no difference between CLP groups administered or not with the antagonists. Whereas the release of IL-10 was increased in plasma and lung parenchyma of the CLP mice treated with the antagonists compared with untreated CLP group.

Conclusion: It was observed that the use of antagonists BLT1 and Cys-LT1 appears to modulate the release of IL-1 β , TNF- α , and IL-10 when compared to untreated CLP group, whereas there was no difference CLP treated and



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untreated CLP groups for the release of IL-6, MCP-1 and KC and neutrophil recruitment to lung.

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MICROPARTICLES LOADING PROSTAGLANDIN-D₂ AS THERAPEUTIC STRATEGY TO THE TREATMENT OF HISTOPLASMOSIS

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Introduction: The prostaglandins (PGs) are known mainly for their roles in inflammatory processes. PGD₂ and PGE₂ have potent antiinflammatory and inflammatory effects dependent on stimulus. Although several studies have examined the participation of PGE₂ in the pathogenesis of pulmonary diseases, few studies determined the role of PGD₂ in infections. Histoplasmosis is characterized by suppurative inflammatory reactions and the roles of different PGs classes are not demonstrated. Therefore, these compounds possess poor hydro solubility and chemical instability, not allowing their *in vivo* administration. Microparticles (MS) improve stability and sustained release of substances and target their deliveries. Our purposes were to develop MS-loading PGD₂ or PGE₂ and to investigate the effects of their administration in mice infected with *H.capsulatum*.

Methods and Results: Initially, we prepare PLGA [poly-(D,L-lactide-co-glycolide)] (50:50) MS-loading PGD₂ or PGE₂ by emulsification-solvent evaporation technique. Analyses revealed that all MS presented spherical shape, with regular surfaces, mean size under 10 µm, negative zeta potential with the capacity to preserve biological activity of the lipid mediators. C57Bl6 mice were infected intratracheally with sublethal inoculum of *Histoplasma capsulatum* (5x10⁵ yeast/100 µl) and treated intranasally with MS-PGD₂, MS-PGE₂ or MS-unloaded at 1, 4, 7 and 10 days post infection. A group of infected mice received celecoxib (1mg/kg) orally concomitant with MS-administration. Our results showed that neutrophils recruitment decreased after all treatments, except post celecoxib plus MS-PGE₂. Mononuclear cells recruitment decreased post celecoxib, MS-PGD₂ or soluble PGD₂ treatment. The lung histological sections showed infiltrating cells reduced after MS-PGD₂ administration. Cytokines production as IL-6, KC and TNF-α decreased after MS-PGD₂ treatment and IL-10 and nitrite production increased after MS-PGE₂ treatment. The number of yeasts reduced in lung and spleen after treatment with celecoxib, celecoxib plus MS-PGD₂ or MS-PGD₂.

Conclusion: PGD₂ is a key immunoregulatory lipid mediator in the protection against *H. capsulatum* infection. MS-PGD₂ could be an alternative therapy to treat histoplasmosis.



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A DEFECT IN GRANULOPOIESIS IN PERFORIN-DEFICIENT MICE

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Introduction: Perforin (Pf) is a major component in granules of cytotoxic T lymphocytes and natural killer cells, important for their cytolytic action against tumor cells and intracellular pathogens. The possible role of Pf in granulopoiesis remains unexplored. We evaluated the effects of Pf deficiency in steady-state and stimulated murine granulopoiesis.

Methods and results: We enumerated total nucleated cells, neutrophils and eosinophils in freshly harvested (Day 0) BM, or in BM cultured with IL-5 1 ng/ml for 7 days (eosinopoiesis) or GM-CSF 0,1-2 ng/ml for 6 days (neutropoiesis). Where indicated, BM cells were exposed to dexamethasone (Dex), a known enhancer of granulopoiesis, *in vivo* (5mg/kg, i.p., 24 h before BM harvest) or *in vitro* (10^{-7} M). Steady-state bone marrow (BM) cellularity (Mean \pm SEM) differed significantly between Pf-deficient (Pf-KO, $27,19 \times 10^6 \pm 1,25$ femoral BM nucleated cells; n=4) mice and wild-type (WT, $40,79 \times 10^6 \pm 1,89$, n=5) controls (p=0,001). *In vitro*, Dex upregulated eosinopoiesis in WT (IL-5, $9,3 \times 10^4 \pm 1,7$; IL-5+Dex 10^{-7} M, $25,2 \times 10^4 \pm 1,5$; n=5; p<0,001), but not Pf-KO-deficient BM. By contrast, GM-CSF-stimulated (0,1ng/ml) neutropoiesis was upregulated by Dex in WT (from $3,8 \times 10^4 \pm 1,4$ to $41 \times 10^4 \pm 8,2$; n=3; p=0,011) and PfKO (from $3,4 \times 10^4 \pm 0,4$ to $92 \times 10^4 \pm 19,4$; n=3; p=0,010). Dex upregulated both eosinopoiesis (from $18,4 \times 10^5 \pm 1$ to $50,1 \pm 4,8$ eosinophils in femoral BM; n=3, p=0,003) and neutropoiesis (from $14,5 \times 10^6 \pm 17,6$ to $21,7 \times 10^6 \pm 7,50$ neutrophils in femoral BM; n=3, p=0,019) *in vivo* in BM of WT, but not Pf-KO mice. Dex i.p. induced comparable thymic involution in both strains, showing that Pf-KO are not glucocorticoid-insensitive, and that Dex reached comparable systemic levels. Dex did not increase eosinophil numbers in spleen, unlike BM.

Conclusion: These data document a hitherto undescribed defect in *in vivo* and *in vitro* BM granulopoiesis in PfKO mice. To our knowledge, this is the first demonstration that a glucocorticoid effect *in vivo* is prevented by Pf deficiency, and should prompt future evaluation of the role of Pf in glucocorticoid immunomodulation.

Financial support: CNPq, FAPERJ, CAPES.

LUNG MORPHOLOGICAL ALTERATIONS IN OVALBUMIN-SENSITIZED MICE: EFFECT OF COUMARIN TREATMENT

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³Fundação Oswaldo Cruz

Introduction: Thousands of people in Brazil who suffer from allergic airway diseases. Asthma is a chronic respiratory disease characterized by airway inflammation and airway hyperresponsiveness (AHR). One strategy to treat allergic diseases is the development of new drugs. Coumarin are compounds derived from plants and are known to have antiallergic, anti-inflammatory, immunomodulatory and antioxidant properties. The aim of this study was to evaluate whether the coumarin (3- Chlorocoumarin) treatment was able to improve lung morphological changes in a model of allergic airway diseases.

Methods and Results: At weekly intervals swiss mice (n=7) were subcutaneously (sc) sensitized twice with ovalbumin/Alum and challenged twice with OVA given intranasally mice. Oral treatment of coumarin (30 mg/kg) was performed 1 h before each OVA-sensitization and OVA challenge. Bronchoalveolar lavage fluid (BAL) was assessed for total cell counts, and lung tissues were examined for cell infiltration (HE stained) and mucus hypersecretion (PAS Stained). Animals treated with coumarin decreased in the number of total cells (157±23) in BAL when compared with OVA group (860±240). In the analysis of the lungs, marked histopathology abnormalities were found in OVA group characterized by bronchostenosis, epithelial hyperplasia, airway wall edema, inflammatory cells in the bronchial and vascular regions and the production of mucus plugging. However in the coumarin group these lung abnormalities were attenuated. In animals treated with the standard drug, dexamethasone, was also observed a significant reduction in the inflammatory BAL (64 ± 16), and a restoration of the morphology-related lung inflammation and mucus production. **Conclusion:** The present data demonstrated the coumarin enhances lung morphological changes in ovalbumin-sensitized mice and suggesting an immunomodulatory effect in model of allergic airway disease.

Financial support: PROPESQ/UFRN

MODULATORY ACTIVITY OF A TERPENOID FROM THE FRUITS OF *ERYTHROXYLUM PASSERINUM* IN A MODEL OF ASTROCYTIC CELLS

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Introduction: Many plants from the genus *Erithroxylum* (Rutaceae) are endemic of the Brazil, mainly present in Restinga and coast zone. Ethnopharmacological studies in the state of Bahia reported cases of intoxication in humans after consumption of fruits from *E. passerinum*, characterized by euphoria and nausea, but little is known about its active compounds in CNC. Astrocytes are present a fundamental role in the control of homeostasis of CNS. As immune-competent cells they are implicated in the neuroimmune interactions and response to CNS damages. This study investigated the cytotoxicity and modulatory activity of a terpene (AEP1), isolated from fruits of *E. passerinum*, in the model of human astrocytic cells, the human glioblastoma derived cell line GL-15.

Methods and Results: As determined by MTT and trypan blue exclusion tests AEP1 exhibited low cytotoxic effect, inducing reduction on mitochondrial metabolism and cell viability only at 250mg/mL, after 48 h treatment. Chromatin staining and ultrastructural analysis demonstrated that AEP1 induced abnormal reniform and lobulated nucleus, and DNA fragmentation, observed by the Comet test, characterizing apoptosis. Moreover, the majority of remaining adherent cells exposed to 250mg/mL AEP1 presented a more condensed cell body with thin cellular process, as observed by ICQ for α - tubulin. Moreover, CBA performed to cytokines IL-12p70, INFg, IL-2, IL-10, IL-8, IL-6, IL-4, IL-5, IL-11b, TNFa and TNFb with the medium of cultures showed that in control conditions GL-15 cells secreted at higher levels cytokines from Th2 profile (IL-4, IL-5). However, treatment with 250 μ g/mL AEP1 induced a change on profile of cytokines secretion with a significant decrease on secretion of INFg and IL-5.

Conclusion: These results indicate that terpene AEP1 interferes on biology of GL-15 astrocytic cells and may be regarded as a compound active to CNS cells probably related to symptoms observed in human after consumption of *E. passerinum* fruits.

Financial support: CNPq, CAPES and FAPESB.

CONSTRUCTION AND EVALUATION OF ANTIFUNGAL PROPERTIES OF CHIMERIC PROTEINS AGAINST THE FUNGAL CELL WALL GLUCANS

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Introduction: Fungal infections have emerged as a serious problem in public health. Current therapeutic strategies are mostly clinically ineffective against the majority of fungal pathogens. Therefore, studies are needed to develop new efficient strategies for the treatment of mycoses. Passive immunization with monoclonal antibodies can help in the elimination of certain infectious processes caused by intracellular fungi. Using the lectin properties of dectin-1 and WGA, with recognized affinity against common fungal structures, respectively, β -glucan and chitin, we have fused their cDNA sequences to the effector part of the immunoglobulin IgG2a, in order to construct chimeric proteins (lectin-Fc) and evaluate the protective role against various fungal pathogens. Thus, we propose the development and characterization of “pan” antifungal lectin-Fc chimera as new potential therapeutic strategies to target universal structures of the fungal cell wall.

Methods and Results: RNA from mouse dendritic cells, IgG2a producing hybridomas and *Triticum aestivum* were prepared using RiboPure™-Yeast Kit according to the manufacturer's instructions. cDNA was synthesized using oligo-dT primer and First Strand cDNA Synthesis kit. The cDNA encoding the various regions was then amplified PCR using specific 5' and 3' primers designed for each fragment. For construction of chimeras, the products were fused by PCR overlapping. Purified PCR products were then ligated into pSecTag. The CHO cells were transfected with pSecTag dectin-Fc and WGA-Fc by electroporation. Stable transfectants were selected by culturing the cells in DMEM/10%FCS with zeocin. ELISA analysis was used to assess the productivity of the secreted chimeric protein by plates sensitized with β -glucan (laminarin) and chitin. Assays were performed to determine the dissociation constant of chimeras relative to the native dectin or WGA and revealed only a slight binding difference. Laminarin or chitin recognition was abrogated upon chemical hydrolysis of glucans. Immunofluorescence was performed to evaluate the binding of the chimeras to fungi and showed labeling of cell walls respectively for both constructs.

Conclusion: The initial analysis showed that the chimeras have the potential to bind the ligands. Complementary tests *in vitro* and *in vivo* should be performed to ensure the protective function of the chimera.



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THE COMPLEX KETOPROFEN-LANTHANUM TREATMENT INCREASES THE SURVIVAL OF EHRLICH SOLID TUMOR-BEARING MICE. EVALUATION OF THE PERITONEAL PHAGOCYtic CELL ACTIVITY.

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Introduction: Metal complexes with potential biological targets have been explored to search for new drugs. Complexes of lanthanides, a group that comprises fifteen metallic chemical elements, with ketoprofen, an analgesic anti-inflammatory steroid, had been described as potential drugs for cancer therapy. In this study, we investigated the influence of the complex ketoprofen-lanthanum (III) [La (Ket) 3.1H₂O) in the survival of solid Ehrlich tumor-bearing mice (BTM) as well as in the peritoneal phagocytic cells activity.

Methods and Results: Swiss mice were divided in four groups: Group BTM-La(Ket), composed of BTM which were submitted to La(Ket) treatment (58.56 mg / kg body weight); Group BTM, composed of mice which were submitted to tumor implantation by inoculation with 1.0×10^7 Ehrlich tumor cells via subcutaneous rout in the back of the animals; Group La(Ket), composed for free-BTM which were submitted to La(Ket) treatment; and Group CTL, composed by naïve mice which were submitted to placebo- vehicle The complex was administered by gavages for three times: on day 7, 11 and 14 after tumor implantation. On day 16 day, the mice were evaluate according to survival rate Furthermore, the peritoneal cells were harvested and submitted to adherent cells culture to determine the production of H₂O₂, NO and IL-10 The results showed that mice from the group BTM-La(Ket) exhibited less mortality than animals from BTM group. The peritoneal phagocytic cells from the group BTM-La(Ket) exhibited lower levels of NO and increased production of IL-10 and H₂O₂.

Conclusion: These results suggest a promising role of this complex as an option in the treatment of solid tumor.

ANTI-INFLAMMATORY EFFECT OF HYDROALCOHOLIC EXTRACT FROM *Pseudobombax marginatum* INNER BARK.

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Introduction: *Pseudobombax marginatum*, “Embiratanha”, is a tree that spreads over several biomes. In the potiguar’s Caatinga, its inner bark is used by population mainly for avoiding pain and inflammation (Rev. Bra. Pl. Med. 12:31-42, 2010). Throughout this study we intend to elucidate some therapeutic properties of the *P. marginatum* extract as an alternative in the inflammation treatment.

Methods and results: It was carried out a secondary metabolites hydroalcoholic extraction from the inner bark. Tannins, flavonols, flavanones, flavanonóis, xanthonés and free steroids were then detected by a qualitative prospection. To evaluate the *P. marginatum* hydroalcoholic extract effect on inflammation we used the paw edema and peritonitis induced by 1% carrageenan animal’s models and also myeloperoxidase assay. *Wistar* rats and *Swiss* mice male were separated into groups of six individuals and orally pre-treatment with the extract (30, 100 and 300 mg/kg), saline (vehicle) or subcutaneous dexamethasone (2 mg/kg – control). In the paw edema model, after the 2nd hour of evaluation it was noticed that the animals treated with extract (100 and 300 mg/kg) exhibited a different edema profile when compared with the one observed in animals treated with vehicle. Through the area under the curve test it was possible to notice that the 100 mg/kg dose ($p < 0,001$) caused a 48% inhibition in the edema’s volume ($1,9 \pm 0,3$ ml) when compared with vehicle ($3,7 \pm 0,2$ ml). The myeloperoxidase assay was carried out using the paw’s tissue from the animals used in the previous test. The enzyme activity was found to be decreased ($p < 0,001$) in the animals’ tissue that were pre-treated with the 100 and 300 mg/kg extract doses, $21,7 \pm 5,62$ and $20,5 \pm 8,3$ UMPO/mg tissue, respectively, in comparison with the ones treated with vehicle ($53,2 \pm 11,38$). In the peritonitis model, the groups treated with the extract showed a lower polymorphonuclear cells migration ($p < 0,001$) to the peritoneal cavity, $3,1 \pm 0,25$; $2,8 \pm 0,27$ and $2,2 \pm 0,16$ for 30, 100 and 300 mg/kg doses, respectively, in comparison with the ones treated with vehicle ($7,6 \pm 0,93$). The overall leukocyte migration inhibition was 56% for the 300mg/kg group and 53% for dexamethasone.



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Conclusion: The *P. marginatum* 100 and 300 mg/kg extract doses have an anti-inflammatory effect. However, further investigations are necessary to elucidate the mechanisms and mediators involved in this response.

Financial support: CAPES, UERN and UFS.

IMMUNOMODULATORY ACTION OF SECONDARY METABOLITE 5-HIDROXI-2-HIDROXIMETIL-Y-PYRONE FROM *Aspergillus flavus*.

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Introduction: The mycelial fungus *Aspergillus flavus*, when cultivated at specific media, are able to produce secondary metabolites with industrial interest, like 5-hidroxi-2-hidroximetil-y-pyrone (HMP), also known as Kojic Acid, a drug widely used for cosmetic purposes. Although HMP has been demonstrated to act as free radical scavenges (Inflammation. 1991; 15(4):303-15), and more recently as an inducer of macrophage activation (Cell Biol Int. 2011; 35(4):335-43), little is known about its immunological functions. In this work we evaluated the action of this molecule on mouse lymph node cells (LNC). **Methods and Results:** Lymph nodes collected from different sites of BALB/c mice were crushed, passed thru a nylon mesh in RPMI1640 10% BFS, pooled and centrifuged. Cell pellets were re-suspended in RPMI and 10^6 cells were distributed in each of a 24-well cell culture plate. HMP was then added to the culture at a concentration of 1000 μ M with or without Concanavalin A (Con A) (5 μ g/mL). Cell-free supernatants were collected after 24 h of incubation and stored at -20°C until determination of IFN-g titration by ELISA. HMP completely inhibited the synthesis of IFN-g by Con A on LNC (control: 51.8 pg/mL; Con A: 961 pg/mL; HMP: 84.3 pg/mL; HMP plus Con A: 92.1 pg/mL), suggesting a possible immunomodulatory action. It has been shown that HMP reduces NF- κ B on Keratinocytes (J Dermatol Sci. 2003 May; 31(3):193-201), enhances liver tumor-promoting activity (Toxicol Sci. 2004 Sep; 81(1):43-9. Epub 2004 Jun 16), and possesses thyroid tumorigenic activity due to a non-genotoxic activity (J Toxicol Sci. 2001 May; 26(2):85-94). The inhibition of IFN-g secretion by LNC may be one of the mechanisms involved on tumorigenic function of KA. **Conclusion:** HMP strongly inhibited INF-g secretion stimulated by Con A on LNC *in vitro*. Further studies are needed to confirm the immunomodulatory function of HMP and to elucidate its possible participation on tumorigenesis.

Financial support: CNPQ, PIBIC, UFPA.

INHIBITION OF PROTEASE ACTIVITY IS ASSOCIATED WITH RESOLUTION OF ACUTE INFLAMMATION BY DECREASING ANNEXIN-A1 CLEAVAGE

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Introduction: Annexin-A1 (AnxA1) is a glucocorticoid (GC)-induced protein of 37KDa that has been shown mediate several GC functions. The 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, generating the 33kDa isoform of unknown properties. In this study we investigated the dynamics of AnxA1 expression and the role of serine protease inhibitors during resolution of LPS-induced neutrophilic inflammation.

Methods: BALB/C mice were challenged by i.pl. (intrapleural) administration of LPS (250ng/cavity) or PBS. The cells present in the pleural cavity were harvested at different times of LPS challenge or after 4h of treatment with a pan-inhibitor of serine proteases (PMSF) or a specific neutrophil elastase inhibitor (sivelestat) and processed for viable and apoptotic leukocyte count and western blot analysis.

Results: The injection of LPS induced a time-dependent influx of neutrophils into the pleural cavity of mice which was maximal at 8-24h, diminished at 48h and completely resolved at 72h. Intact AnxA1 was detected in PBS-challenged mice and cleavage was maximal during the times of neutrophil recruitment. Intact AnxA1 expression was recovered during the resolution phase of this inflammatory response. Similarly the endogenous elastase activity peaked at 8h and decreased at 48h. Treatment of mice with a pan-inhibitor of serine proteases, PMSF (30mg/kg, i.p.), 4h after LPS challenge decreased neutrophils numbers into the pleural cavity and inhibited elastase activity. Administration of a specific neutrophil elastase inhibitor, sivelestat (5mg/kg, i.p.), 4h after LPS challenge decreased the number of neutrophils (mean \pm SEM $\times 10^5$ neutrophils/cavity; PBS: 0.28 ± 0.25 ; PBS+sivelestat: 0.62 ± 0.33 ; LPS+vehicle: 23.08 ± 7.40 ; LPS \pm sivelestat: 7.04 ± 1.4 ; $n=5$; $P<0.05$) and elastase activity. Such effects of elastase inhibition were associated with increased numbers of neutrophils with apoptotic morphology, caspase-3 cleavage and decreasing cleavage of AnxA1 (37KDa).

Conclusion: Our results showed that intact AnxA1 plays an important role in the signaling events leading to resolution of neutrophilic inflammation and inhibition of endogenous AnxA1 cleavage may be an alternative treatment to



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acute inflammation.

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DOES TREATMENT WITH LITHIUM CARBONATE MODULATE EXPRESSION OF GENES RELATED TO WNT/B-CATENIN PATHWAY IN VIVO? STUDY IN PBMC CELLS OF BIPOLAR PATIENTS

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Introduction: The Wnt/ β -catenin signaling pathway regulates several functions in vertebrates, such as cellular differentiation, proliferation and survival. In previous studies led by our group, increased HIG2 expression (a product of the Wnt/ β -catenin pathway) was observed in CD4+ T cells of LTNP HIV+ patients (a group of HIV infected patients who do not progress to AIDS with no use of antiretroviral drugs). We observed that in vitro treatment of peripheral blood mononuclear cells (PBMC) with lithium salts activated this pathway and consequently enhanced HIG2 expression. We sought to test whether oral administration of lithium salts could increase expression of key members and targets of the Wnt/ β -catenin pathway. **Methods and results:** We also used as tool a group of patients recently diagnosed with bipolar disorder (BD), type I or II, prior to and two months after lithium therapy. Our group analyzed the modulation of downstream genes of the canonical Wnt pathway such as Bcl-xl, cyclin D1, HIG2, TCF4, LEF-1 and the target GSK-3 β in PBMC by Real-Time RT-PCR and compared the expression of these genes between five bipolar patients pre- and post-treatment with lithium carbonate, and twelve healthy control subjects using the $2^{-\Delta Ct}$ method and Kruskal-Wallis test with Dunn's post-test for statistical analysis. We observed significant differences between bipolar patients who were under lithium treatment and control subjects (GSK-3 β , TCF4 and HIG2). In addition, we found significant differences were observed in GSK-3 β , TCF4 and cyclin D1 expression between pre-treated bipolar patients and control subjects, showing that bipolar disorder per se has an effect on Wnt/ β -catenin signaling. **Conclusion:** Both lithium treatment-related and bipolar disorder-specific gene expression changes were observed in human PBMC.

Financial support: CNPQ, FAPESP and Fundap.

ALLIUM CEPA L THERAPEUTIC POTENTIAL ON IL-13-INDUCED HYPER-REACTIVITY OF MURINE TRACHEAL SMOOTH MUSCLE

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Introduction: Muscle cells are potentially linked with many features of asthma, including the production of cytokines and inflammatory mediators involved in tissue remodeling. Interleukin-13 has been implicated as a key cytokine in the pathogenesis of allergy and asthma. *Allium cepa* L. is largely used by folk medicine to treat asthma. In this study we explored the *in vitro* IL-13-induced hyper-reactivity of murine tracheal smooth muscle and the bronchodilator potential of *Allium cepa* L. (*AcE*) methanolic extract. **Methods and Results:** AJ mice were euthanized, trachea was rapidly removed and were placed individually in each well of a 48-well plate and incubated at 37°C in the presence or absence of IL-13 at 10ng/mL in supplemented DMEM for 24h. Trachea smooth muscle reactivity was assessed in organ bath containing 10 ml Krebs- bicarbonate's solution, maintained at 37°C and gassed with a 95% O₂ + 5% CO₂ mixture (pH 7.4). Epithelium integrity was assessed qualitatively by the degree of relaxation caused by bradykinin (10⁻⁶ M) in the presence of contractile tone induced by carbachol (10⁻⁶ M). Cumulatively increasing concentrations of *AcE* were added. Incubation of murine tracheal rings with IL-13 for 24 h potentiated carbachol-induced contractions of murine tracheal smooth muscle by IL-13. The concentration response curve to carbachol was significantly higher in rings pretreated with IL-13 compared with controls. Furthermore, *AcE* concentration-dependently relaxed tracheal contractions induced by carbachol pretreated with IL-13 previously. **Conclusion:** These data indicate that IL-13 contributes to tracheal smooth muscle hyper-responsiveness and suggest that *Allium cepa* L. have potential as anti-asthmatic drug by its bronchodilator property.

Financial support: FAPESB / CAPES

CAFFEIC ACID MODULATES INFLAMMATORY RESPONSES ON LIPOPOLYSACCHARIDE-STIMULATED CELLS

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Introduction: Caffeic acid - 3,4-dihydroxycinnamic acid (CA), a major representative of naturally occurring phenolic compounds, frequently occurs in fruits, grains and dietary supplements for human consumption and is also found in several traditional herbs. Recent studies described its antioxidant, anticancer, anti-inflammatory and immunomodulatory activities. The objective of the present study was to evaluate the anti-inflammatory effect of CA in lipopolysaccharide (LPS)-stimulated cell culture.

Methods and Results: Spleen cells of BALB/c mice were stimulated with 20 µg/mL of LPS with or without CA at a 5 µg/mL. After 72h of culture, the supernatants were collected and IFN-γ levels were determined by ELISA. Cell proliferation was estimated based on MTT-tetrazolium method. In order to evaluate nitric oxid production, BALB/c mice were inoculated with 20µg/animal of LPS, i.p.. After 72h of injection, peritoneal lavage was performed to obtain the macrophages. The macrophages were then pretreated with CA at different concentrations (1, 10 e 100µM) and incubated for 1 h, thereafter, LPS (5 µg/mL) was added followed by further incubation of 24 h. Nitric oxide production was determined by Griess' reaction in macrophage cultures stimulated with 5 µg/mL of LPS. Our results have shown that LPS induced spleen cell proliferation ($p<0.001$), increased IFN-γ levels by spleen cells ($p<0.05$) and NO production by macrophages ($p<0.001$). CA reduced LPS-induced IFN-γ production at 5µg/mL ($p<0.05$), but did not affect cell viability. CA at 100 and 10 µM reduced the LPS-induced NO production ($p<0.01$ and $p<0.001$, respectively) as well.



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Conclusion: Caffeic acid modulates the release of nitric oxide and IFN- γ in lipopolysaccharide-stimulated cells *in vitro*.

Financial support: CAPES/ CNPq

THE COMPLEX KETOPROFEN-LANTHANUM TREATMENT DECREASES THE EDEMA FORMATION

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Introduction: Metal complexes with potential biological targets have been explored to discovering new drugs. Complexes of lanthanides, a group that comprises fifteen metallic chemical elements, with ketoprofen, an analgesic anti-inflammatory steroid, had been described as potential drugs for cancer therapy. As to there is no information about the possible anti-inflammatory potencialization of this complex, in the present study we investigated the influence of complex ketoprofen-lanthanum (III) [La (Ket) 3.1H₂O] on the skin tissue inflammatory response as well as on the cytotoxicity activity of the spleen cells.

Methods and Results: Swiss mice were divided in three groups: Group La(Ket), composed of mice which were submitted to La(Ket) treatment (58.56 mg/kg body weight); Group Ket, composed of mice which were submitted to ketoprofen treatment (50 mg/kg body weight) and Group CTRL, composed by naïve mice which were submitted to placebo-vehicle (dimethyl sulfoxide, 1.0 ml/kg body weight). The substances were administered in a single dose by gavage. After 60 minutes, the mice were submitted to inoculation of 2.5% formalin into footpad. Six hours later, the edema formation were measured; after, the mice were killed and the inoculated footpads were submitted to histological analyses and the spleen was processed to *in vitro* cell culture cytotoxic assay. Our results demonstrated that the La(Ket) treatment decreases the edema size in the formalin-inoculated footpad in comparison to Ket treatment. The histopathological analyses showed no differences in the inflammatory cell distribution. Furthermore, no cytotoxic effect was observed in spleen cell culture.

Conclusion: This study shows that although the metal lanthanum does not potentiate the action of the ketoprofen as to inflammatory cell distribution, the complex [La (Ket) 3.1H₂O] affects the fluid release from the vessels and thus it reduces the skin edema formation during formalin inoculation.

STUDY OF THE EFFECT OF ORGANIC LECTÍNICA FRACTION OBTAINED FROM LATEX *EUPHORBIA TIRUCALLI* IN TUMOR CELLS OF EHRLICH

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Introduction: According to World Health Organization, cancer is the second leading cause of death in most developed countries and according to the National Cancer Institute, breast cancer is the most common in the world, accounting for 22% of new cases per year. *Euphorbia tirucalli* is a frequently mentioned medicinal plant in traditional medicine for its use as an antitumor agent. This study regards the investigation of the biological activities of the latex of *E. tirucalli*, purification and evaluation of its potential antitumor fraction.

Methods: Initially, the *E. tirucalli*'s látex was collected and tested the hemagglutination and its inhibition to different carbohydrates in order to identify the lectin activity. After purification by affinity chromatography using D-galactose column (for isolation of lectins), it was obtained two fractions: binding and void. Afterwards, the biological effects of crude extract was tested in Ehrlich tumor cells and HeLa.

Results: We observed that crude extract causes hemolysis in human blood during the hemagglutination assay, indicating a cytotoxic potential of this product. From the SDS-PAGE, we observed the protein profile of the crude extract and the efficiency of purification of the binding fraction, also evidenced in hemagglutination assays to chromatography and the subsequent reduction of the hemolytic effect, first observed in the crude extract. In immunofluorescence slides, it was found that the binder fraction interacts with receptors present on the surface of tumor cells in question, as well as the lymphocytes. Although the binder fraction interacts with the tumor cells member, there is no cytotoxic effects.

Conclusion: This method showed to be efficient in the purification process of lectínica profile activity of the latex of *E. tirucalli* eliminating the toxic effects



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attributed to the crude extract. The isolation of the binder fraction causes the elimination of adverse effects possible in the presence of the crude extract and also interferes with their interaction with cell surface receptors of tumor cells.



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THE POLYAMINE ANALOGUE GC7 INHIBITS TNF- α SECRETION BY MACROPHAGES

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Introduction: The compound N1-guanyl-1,7-diaminoheptane (GC7) is a spermidine analogue known to interfere with polyamines metabolism and to inhibit the formation of the hypusine residue in the protein eIF5A. The eukaryotic translation initiation factor 5A (eIF5A) is an essential and highly conserved protein and the only protein that contains the amino acid hypusine. The hypusine residue is formed by a posttranslational modification that involves two steps: initially the enzyme deoxyhypusine synthase (DHS) transfers the aminobutyl moiety from the polyamine spermidine to the amino group of a specific eIF5A lysine residue (K50 in mouse) to form deoxyhypusine; in the second step, the deoxyhypusine hydroxylase enzyme (DOHH) adds a hydroxyl group to deoxyhypusine. Recently, eIF5A and DHS has been identified as targets for reducing inflammation and related cellular stresses in a variety of cell types. Therefore, we decided to test anti-inflammatory effect of GC7 on lipopolysaccharide-stimulated RAW 264.7 macrophages as an *in vitro* model for the study of inflammation.

Methods and Results: As expected, GC7 caused an important reduction of hypusine levels in eIF5A from RAW 264.7 cells, which dramatically affected cell proliferation. On the other hand, there is no effect on cell viability upon treatment with GC7. Interestingly, the GC7 treatment inhibited the tumor necrosis factor-alpha (TNF- α) secretion by RAW 264.7 cells stimulated with LPS), which is a marked evidence of anti-inflammatory effect (TNF- α [ng/mL] - Medium: N.D.; LPS: 138 ± 6.7 ; LPS + 50 μ M GC7: 36.7 ± 1.38 ; LPS + 100 μ M GC7: 22.75 ± 3.01 ; LPS + 150 μ M GC7: $22.66 \pm 14,42$).

Conclusion: These results demonstrate a direct anti-inflammatory effect of GC7 in macrophages and we are now testing for other inflammatory markers and also the mechanisms underlying the inhibition of TNF- α production. The studies using RAW 264.7 cells may contribute to the understanding of eIF5A role in the immune response.



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IMMUNOMODULATORY EFFECTS OF *CYMBOPOGON CITRATUS* IN A MURINE MODEL OF *BLOMIA TROPICALIS*-MEDIATED ALLERGY

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Introduction: Asthma is an allergic inflammatory disorder characterized by immediate hypersensitive reactions and is considered a current public health problem. Bronchodilators and corticosteroids are conventional drugs used for asthma treatment, which are associated with negative side effects. Ethnopharmacological survey conducted in the city of Salvador (BA) found that the specie *Cymbopogon citratus* L. is one of the most popular herb used in herbal medicine to treat allergic diseases. The aim of this study was to investigate the immunomodulatory properties of *C. citratus* L. in a *Blomia tropicalis* (Bt) – an induction allergy model. **Methods:** The allergic process was induced in A/J mice by the administration of *Blomia tropicalis* antigen (Bt) (100µg/animal), subcutaneously, in 4mg/ml of [AL(OH)³] on days 0 and 7. After the last sensitization, the animals received four intranasal challenges (10µg/animal) at intervals of one per day. Mice were orally treated with hexane extract of *Cymbopogon citratus* L at 60mg/kg (CcHE1), 120mg/kg (CcHE2), 180mg/kg (CcHE3) and 240mg/kg (CcHE4) or dexamethasone (dose) from the 8th until the 14th day. Twenty-four hours after the last challenge, the animals were euthanized. **Results:** Administration of Bt to A/J animals produced typical allergic markers in mice, such as cell migration and cytokines in bronchial alveolar lavage (BAL). The treatment significantly reduced the total number of inflammatory cells (p<0.001) and eosinophils in the BAL in all doses tested. The



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EPO levels were even lower than dexametasone ($p < 0.01$) when animals were treated with (CcHE2) and (CcHE3). IL-4 levels decreased ($p < 0.05$) in animals treated with (CcHE2) and (CcHE3). The IgE levels were reduced in animals treated with (CcHE1) $p < 0.05$. **Conclusions:** The results suggest that *C. citratus* L. has immunomodulatory properties in our allergy model of asthma and that this herb can be a promising source for the development of new drugs for the treatment of asthma and other allergies. New experiments are in progress in our laboratory and in others, investigating the molecules present in the *C. citratus* responsible for the observed effects and to try to further elucidate the mechanism of action of these compounds.

Financial support: FAPESB

EVIDENCE OF ANTI-INFLAMMATORY EFFECTS *ROSMARINUS OFFICINALIS* L. IN IN VIVO EXPERIMENTS.

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Introduction: *R. officinalis* L. is useful to treat inflammatory diseases. **Objective:** The aim of this study was to evaluate the anti-inflammatory effects of the fraction Ethyl acetate (EA) of *R. officinalis*, it's derived and isolated compounds: Betulinic acid (BA), Carnosol (CA) and Rosmarinic acid (RA), upon leukocytes, exudation and interleukin-17 (IL-17) and interleukin-10 (IL-10), using a murine model of pleurisy induced by carrageenan (Cg). **Methods and Results:** Dried *R. officinalis* aerial parts were underwent extraction through the steam technique to remove the essential oils. This substance was macerated at room temperature to obtain the CE without oil, which was partitioned with ethyl acetate solution to obtain the EA fraction, which was separated, purified, yielding the isolated compounds: BA, CA and RA. Swiss mice were used though the experiments in accordance with methodology described in *Brit.J.Pharmacol.183.811-19.1996*. Different groups of animals were treated with EA (10-50mg/kg), BA (1-10mg/kg), CA (1-10mg/kg) or RA (2.5-10 mg/kg) administered by intraperitoneal route, 0.5h before Cg (1%) injection administered by intrapleural route. The inflammatory response was analyzed after 4h. In another experiments animals were treated (0.5h) with EA (25 mg/kg), BA (2,5 mg/kg), CA (2,5 mg/kg) or RA (5 mg/kg) for analysis, IL-17 and IL-10. The inflammation were analyzed after 4h. Statistical differences between groups were determined by ANOVA complemented with Dunnett's test. Values of $p < 0.05$ were considered significant. EA (25-50 mg/kg) BA (2,5-10 mg/kg), CA (2,5-10 mg/kg) and RA (2.5-10 mg/kg) inhibited leukocytes (EA:32.0±4.1% to 32.8±2.6%; BA:20.6±2.1% to 44.3±3.6%; CA:37.2±6.5% to 45.3±4.6%; RA:22.2±1.7% to 34.8±3.9%), neutrophils (EA:31.8±4.6% to 32.5±2.9%; BA:19.9±2.4% to 43.6±3.9%; CA:35.8±6.2% to 44.6±4.5%; RA:21.1±2.2% to 35.3±3.3%) and exudation (EA:20.1±1.4% to 22.3±2.7%; BA:13.6±3.8% to 22.8±5.7%; CA: 20.0±8.8% to 22.8±3.8%; RA:19.4±3.4% to 14.8±2.1%) ($p < 0.05$). EA (25 mg/kg), BA (2,5 mg/kg), CA (2,5 mg/kg) and RA (5 mg/kg) inhibited IL-17 levels by 55.1±10.5%; 54.2±4.3%; 52.0±4.1% and 58.2±4.8%,



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and increased IL-10 levels by $57.2 \pm 1.1\%$; $73.9 \pm 4.3\%$; $72.5 \pm 2.3\%$ and $67.1 \pm 3.9\%$, respectively ($p < 0.05$). **Conclusion:** *R. officinalis* showed important anti-inflammatory activity by inhibiting leukocytes and exudation. These effects were associated with decrease of IL-17 levels and increase of IL-10.

Financial support: CAPES

EFFECT OF SINVASTATIN IN EXPERIMENTAL MODEL OF SEPSIS

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(1)Oswaldo Cruz Foundation

INTRODUCTION: Sepsis is a severe medical condition characterized by systemic inflammatory response secondary to infection, which frequently progress to multiple organ dysfunction and death. It is currently the leading cause of death in intensive care units worldwide. Several therapies were tested in the prevention and treatment of sepsis, without good results. Cohort studies have suggested that statins users were protected to the development and mortality of sepsis. (Ann Pharmacother, 2007). Statins act by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and in addition, they are emerging as potent inhibitors of inflammatory processes. In this study we aimed to evaluate the effects of simvastatin in clinical relevant model of sepsis in rodents (Cecal Ligation and Puncture-CLP). We analyzed inflammatory parameters, such as cell migration, cell activation, bacterial elimination and nitric oxide production. Organ damage was also analyzed. **Methods:** Swiss mice were submitted to Cecal Ligation and Puncture (CLP) and 6 h later they were treated with simvastatin (2mg/kg – iv). 24 h after onset of sepsis, cardiac puncture was performed to collect plasma and peritoneal lavage was carried out. Cytokines production was measured by ELISA. Nitric oxide was quantified by Griess method. *In vitro* studies were performed using peritoneal macrophages. **Results:** Our results showed that simvastatin was able to improve renal function by decreasing the levels of creatinine (0,6500 μ g/ml-SEM \pm 0,1165- to 0,4143 μ g/ml-SEM \pm 0,06335) in the blood of animals submitted to CLP. 24 hours after surgery, the drug was able to reduce the number of neutrophils (59,11e⁵-SEM \pm 11,40 to 35,85 e⁵-SEM \pm 5,853), TNF- α , MIF, IL-6 and IL1 β levels and lipid bodies formation (26,59LB-SEM \pm 0,5958 to 13,79LB- SEM \pm 0,4910) in the peritoneum. Simvastatin treatment decreased the number of Colony Forming Units (18,85CFU SEM \pm 4,043 to 8,817CFU-SEM \pm 2,706) at the site of inflammation as well as *in vitro*. The treatment with simvastatin caused a decrease in nitric oxide levels in the bloodstream (31,79 μ MSEM \pm 13,65 to 4,507 μ MSEM \pm 1,321). **Conclusion:** Our study indicates that simvastatin improved bacterial clearance and inflammatory profile in sepsis, with effects on main determinants of the pathophysiology of sepsis, which makes this drug a potential candidate to an adjunctive therapy for sepsis.

Financial Support: CNPq, FAPERJ, FIOCRUZ

HIGH FIBER DIET REDUCES INFLAMMATORY RESPONSE AND PROTECT MICE FROM DSS-INDUCED COLITIS

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Introduction: Soluble fiber obtained from diet is fermented in the gut by the microbiota leading to short chain fatty acids (SCFA) production. These products have important role on the modulation of the immune system, acting on specific G-protein-coupled receptors on immune cells. Colitis, an important inflammatory bowel disease with unknown origin, affects the large intestine, presenting an important impact on quality of life of patients. The aim of this study was to evaluate the effect of a high fiber (HF) diet on the inflammatory response in a murine model of colitis.

Method and results: Female BALB/c mice were fed with chow or HF diet for 40 days prior to colitis induction. Acute colitis was induced by adding dextran sulphate sodium (DSS) to drinking water for 7 days (control received water). In DSS-induced colitis, HF diet reduced mononuclear and polymorphonuclear cells on the 7th day when compared to chow fed mice. Also, HF diet was important in the reduction of myeloperoxidase activity (C:1.59±0.25; HF:0.60±0.35 - relative units), CXCL1 (C:2,641±745.0; HF:1,411±305.8) and CCL11 (C:2.33±0.57; HF:0.74±0.02 - pg/100 mg of tissue - ELISA) production in the large intestine when compared to chow fed mice. Also, HF diet had a great protection against colon length reduction as seen in chow fed mice (C:17.70%; HF:6.80% - related to their control animals). Corroborating the beneficial effect of HF diet on diminution the severity of DSS disease, mice fed with HF diet have 100% of survival compared to chow diet (40%). To evaluate the mechanisms associated to HF diet protection on colitis, we tested the effect of acetate, a metabolic product from fermentation of soluble fiber. Mice treated with sodium acetate (150mM; p.o.) during 5 days prior to colitis in combination or not with HF diet were effective in protection against weight loss, clinical and colon histological score (C:10.17±0.75; acetate:6.00±1.39; HF:7.2±0.70 HF+acetate:5.5±0.34) in DSS-induced colitis. However, acetate treatment alone did not reduce the mortality rate. The combination of HF diet with sodium acetate had the same protection against mortality as seen in HF diet alone.



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Conclusion: HF diet protects mice from DSS-induced acute colitis by reducing inflammation in the large intestine. These protective effects occur independently of acetate production.

Financial Support: Capes, CNPq and Fapemig.

HOUSE DUST MITE TRIGGERS A LUNG INFLAMMATORY RESPONSE RESISTANT TO DEXAMETHASONE IN A/J MICE

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Introduction: Asthma is a chronic inflammation of the lung airways caused by environmental factors in genetically predisposed individuals. Resistance to glucocorticoid, a pivotal problem in asthma therapy, remains poorly understood, which has been associated to the lack of animal models able to recapitulate changes that occur in the lung under conditions of glucocorticoid resistance. We sought to test here if A/J mice challenged with house dust mite (HDM), a clinically relevant allergen, would be a suitable model for studying corticosteroid resistance.

Methods and Results: Anesthetized A/J mice were instilled with HDM (1 mg/mL) intranasally (25 μ L) three times a week for three weeks. Oral dexamethasone (1 mg/kg) was administered once a day for 6 days, 1 h before challenge. Analyses of dexamethasone effects on inflammation and AHR were performed 24 h post-challenge. HDM challenged-A/J mice presented increase in total leukocytes recovered from the BALF 24 h after the last challenge (mean=23.7, SE= \pm 5.4, n=7) when compared to PBS control (mean=2.4, SE= \pm 0.26, n=5). This increase in total leukocyte was accounted for by elevation in the numbers of eosinophils (mean=13.8, SE= \pm 3.7, n=7), macrophages (mean=6.1, SE= \pm 1.4, n=7) and lymphocytes (mean=1.7, SE= \pm 0.3, n=7). Dexamethasone treatment reduced eosinophil (mean=4.5, SE= \pm 1.6, n=7) and lymphocyte BALF numbers (mean=0.5, SE= \pm 0.1, n=7). HDM challenge induced AHR 24 h after the last challenge when compared to PBS, and the corticoid treatment inhibited this AHR. Lung cytokine secretion was evaluated with ELISA commercial kits. Dexamethasone could not inhibit IL-4 lung secretion induced by HDM but reduced IL-10 production. Treg cells obtained from the bronchoalveolar lavage fluid (BALF) were analyzed by flow cytometry, by staining cells with anti-CD4 (FITC), anti-CD25 (APC) and anti-Foxp3 (PE). Dexamethasone reduced the number of Tregs (mean=6.4, SE= \pm 2.15, n=7) in the BALF when compared to HDM challenged mice (mean=17.5, SE= \pm 3.9, n=8).

Conclusion: These results show that HDM intranasal instillation induces lung inflammation, increase in lung cytokine secretion and Treg cell numbers as well as AHR in A/J mice. Although this experimental system presents several asthma features, a certain degree of refractoriness to dexamethasone is apparent, suggesting that this model may reproduce a corticosteroid-resistant asthma phenotype.



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CRUDE ETHANOLIC EXTRACT OF *Piper fuliginum* KUNTH (PIPERACEAE) DECREASES THE NITRIC OXIDE PRODUCTION BY PERITONEAL PHAGOCYTES

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Introduction: Several species of genus *Piper* have been used in alternative medicine to treat vaginitis, gynecological and intestinal disorders. Furthermore, other species of the large family *Piperaceae* have been identified as a source of several substances with different properties: psychotropic, antioxidant, antimicrobial and cytotoxic effects. However, the influence of plant extract in the immune system modulation remains to be explored. In the present study, we determined the influence of ethanol extract of leaves of *P. fuliginum* on the peritoneal phagocytic cell activity as well as on the lymphoproliferative activity of spleen cells.

Methods and Results: The ethanol extract from the aerial parts of *P. fuliginum* was obtained by maceration using ethanol as solvent and then filtered and dried. The first dilution of the extract was performed in dimethyl sulfoxide followed by subsequent dilution in sterile phosphate buffered saline solution. Swiss mice were killed by carbon dioxide asphyxia and peritoneal lavage and spleen were collected. Peritoneal phagocytes and spleen cells were cultivated *in vitro* and challenged with the crude extracts in different concentrations (0.391, 0.196 and 0.097 mg/ml). After 24 hours, the peritoneal phagocytes were submitted to evaluation of peroxide hydrogen (H₂O₂) and nitric oxide (NO) production and the spleen cells to lymphoproliferation analyses. Our results demonstrated that the concentration of 0.391 mg/ml impaired the NO release. No differences were observed in the H₂O₂ production and in the lymphoproliferation analyses.

Conclusion: The inhibition of NO production by crude ethanolic extract of *P. fuliginum* highlights its potential as an interesting option in immunotherapy treatment.

POTENT ANTI-INFLAMMATORY EFFECT OF PYRENOICINE A ISOLATED FROM *PENICILLIUM PAXILLI*

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Introduction. Marine microorganisms are a rich source of compounds with pharmacological activities. Recently we have isolated Pyrenocine A as a major compound from the crude extract obtained from the growth medium of a marine-derived fungus *Penicillium paxilli*. In the present investigation we evaluated the anti-inflammatory activity of Pyrenocine A, using a therapeutic approach.

Methodology and Results: RAW 264.7 cells were stimulated with LPS, CpG or Poly I:C (1 µg/mL) for 2h and treated with Pyrenocine A (0.78 µg/ml to 0.02 µg/ml) or vehicle for 24 h. The anti-inflammatory effect of Pyrenocine A was evaluated through the cytokines and nitrite levels, Mac-1 expression, B-cells proliferation and intracellular signaling pathway. The treatment with Pyrenocine A was able to inhibit the TNF-α and IL-1α production by RAW 264.7 cells stimulated with LPS. Beside cytokines, Pyrenocine A promoted the down-modulation of Mac-1 expression in RAW 264.7 cells when compared with LPS-stimulated cells (Mac-1 – MIF- LPS = 5,325; 0.78 µg/mL = 1,962; 0.39 µg/mL = 1,921; 0.19 µg/mL = 1,785; 0.09 µg/mL = 1,779). Moreover, we found that Pyrenocine A was able to inhibit NO production when RAW 264.7 cells were stimulated with CpG (NO [µM]: CpG :23.32 ± 2.39; CpG+ Pyrenocine A: 0.78 µg/mL: 9.843 ± 1.45*; 0.39 µg/mL: 13.18 ± 1.62*; 0.19 µg/mL: 14.60 ± 4.67), but not with Poly I:C (NO (µM) - Poly I:C: 33.35 ± 1.84; 0.78 µg/mL: 27.25 ± 2.25; 0.39 µg/mL: 31.41 ± 38.65; 0.19 µg/mL: 29.27 ± 3.52). We also found that Pyrenocine A promote intense suppression of B-cells proliferation stimulated with LPS (~70% inhibition when compared to LPS alone). **Conclusion.** Pyrenocine A was able to suppress cytokines production (TNF-α and IL-1α), the expression of molecules involved in the cell migration (Mac-1) and the of B-cells proliferation. Moreover, the results suggest that mechanism of action of Pyrenocine A is via MyD88-dependent signaling pathway, but not by TRIF-dependent signaling.



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CRUDE ETHANOLIC EXTRACT OF *Piper umbellata* L. (PIPERACEAE) DECREASE THE NITRIC OXIDE PRODUCTION BY PERITONEAL MACROPHAGES AND EXHIBITH LYMPHOPROLIFERATIVE ACTIVITY

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Introduction: *Piper umbellata* is a species of genus *Piper* that have been used in alternative medicine to treat some disorders in the liver and epilepsy. It has also been demonstrated anti-malaria and anti-inflammatory proprieties. However, the influence of plant extract in the immune system modulation remains to be explored. In the present study we determine the influence of ethanol extract of leaves of *P. umbellata* on the peritoneal phagocytic cell activity as well as on the lymphoproliferative activity of spleen cells.

Methods and Results: The ethanol extract from the aerial parts of *P. umbellata* was obtained by maceration using ethanol 100% as solvent and then filtered and dried. The first dilution of the extract was performed in dimethyl sulfoxide followed by subsequent dilution in sterile phosphate buffered saline solution. Swiss mice were killed by carbon dioxide asphyxia and peritoneal lavage and spleen were collected. Peritoneal phagocytes and spleen cells were cultivated *in vitro* and challenged with the crude extracts in different concentrations: 0,391, 0,196 e 0,097 mg/ml. After 24 hours, the peritoneal phagocytes were submitted to evaluation of peroxide hydrogen (H₂O₂) and nitric oxide (NO) production and the spleen cells to lymphoproliferation analyses. Our results demonstrated that the concentration of de 0,122 mg/ml decreased the NO release and increased the lymphoproliferation index. No differences were observed in the H₂O₂ production

Conclusion: The inhibition of NO production and lymphoproliferative activity by crude ethanolic extract of *P. umbellata* highlights its potential as an interesting option in immunotherapy treatment.

MONOACYLGLYCEROL LIPASE INHIBITION REDUCES LEUKOCYTE MIGRATION IN A MURINE MODEL OF ACUTE LUNG INJURY

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Introduction: Endocannabinoid signaling is terminated by enzymatic hydrolysis, a process that for 2-Arachidonoylglycerol (2-AG) is mediated by monoacylglycerol lipase (MAGL). The piperidine carbamate 4-nitrophenyl-4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate (JZL184), that inhibits the MAGL and presents high potency and selectivity. Thus, it increases 2-AG levels, an endocannabinoid that acts on CB1 and CB2 cannabinoid receptors. The aim of this work was to investigate the effects of MAGL inhibition in a murine model of acute lung injury (ALI) induced by LPS.

Methods and Results: Twenty five C57BL/6 male mice were used (4-8/group); mice were divided into 4 groups, vehicle+saline (V), vehicle+LPS (L), JZL184+saline (J) and JZL184+LPS (JL). The mice were treated with JZL184 (16mg/kg) or vehicle (0,1mL/10g i.p.) and after 60 min they received a LPS intranasal instillation (100µg/mL; 1µL/g) or sterile saline (1 µL/g). Forty eight hours later all mice were euthanized and blood, bone marrow and bronchoalveolar lavage fluid (BAL) were harvested. We observed that mice of L group showed an increase in the total number of cells in the BAL ($F(3,21)=23,19$; $p<0,0001$) especially neutrophils ($F(3,21)=5,888$; $p<0,05$) when compared with the V and J groups. The total number of cells ($F(3,21) = 23,19$; $p < 0,0001$) and of neutrophils ($F(3,21) = 5,888$; $p < 0,05$) in the BAL were decreased in mice of JL group. Additionally, we showed that the total number of cells and neutrophils in the blood were decreased in the L group ($F(3,21)=6,917$; $p<0,05$; $F(3,21)=9,236$; $p<0,05$, respectively) when compared to V and J groups, and that treatment with JZL184 didn't prevent these changes ($F(3,21)=6,917$; $p<0,001$; $F(3,21)=9,236$; $p<0,05$, respectively). However, we showed that the number of granulocytes in the bone marrow increased in the L group ($F(3,21)=3,8$; $p<0,05$) and that data of JL group were similar to those of the V and J groups. **Conclusion:** The present data displayed the migration of neutrophils from blood to lungs and from bone marrow to the blood in all mice. Altogether, we conclude that the ALI establishment is evident 48 hours after LPS nasal instillation and that increased levels of circulating 2-AG, through



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MAGL inhibition, has an anti-inflammatory effect in this ALI murine model.

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PROTEINASE ACTIVATED RECEPTOR-4 PLAYS A MAJOR ROLE IN THE RECRUITMENT OF NEUTROPHILS INDUCED BY TRYPSIN OR CARRAGEENAN IN PLEURISY IN MICE

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Introduction: The activation of proteinase-activated receptors (PARs) has been implicated in the development of important hallmarks of inflammation, like *in vivo* leukocyte recruitment. Serine proteases have been implicated in the development of inflammatory response because of their ability to selectively activate G-protein-coupled proteinase-activated receptors (PARs) through the proteolytic cleavage of a specific site on the N-terminal amino acid sequence of the receptor, revealing a tethered ligand that then binds to and activates the receptor. The activation of PARs has been implicated in the regulation of important hallmarks of inflammation, such as *in vivo* leukocyte recruitment. Here, we examined the effects of aprotinin, a potent trypsin proteinase and kallikrein-kinin system inhibitor, and the PAR-4 antagonist YPGKF-NH₂ (tcY-NH₂) on neutrophil recruitment in response to carrageenan and trypsin in the pleural cavity of mice. **Methods and Results:** BALB/c mice were intrapleurally injected with trypsin or PAR-4 activating peptide AY-NH₂, pretreated with aprotinin or tcY-NH₂ (1 !g/cavity) prior to an intrapleural injection of trypsin or carrageenan, or pretreated with leukotriene(LT)B₄ antagonist U75302 (3 !g/cavity) prior to a trypsin injection. The number of infiltrating neutrophils was evaluated at 4 h. PAR-4 activating peptide AY-NH₂ and Trypsin induced a neutrophil recruitment that was inhibited by aprotinin, tcY-NH₂ or U75302. Aprotinin and tcY-NH₂ also inhibited the neutrophil recruitment induced by carrageenan. **Conclusion.** These data suggest a key role for PAR-4 in mediating neutrophil recruitment in a pleurisy model in mice, specifically due to the activity of trypsin or trypsin-like enzymes. **Ethical Approval:** Animal (CETEA UFMG (05/11)).

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Effect of Resolvin D2 in bone-marrow macrophage infected with BCG

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Introduction: Resolvins are a family of lipid mediators with key roles in the resolution phase of inflammation, derived from Omega-3 fatty acids. Resolvin D2 (RvD2) is new member derived from the Omega 3, DHA, that has demonstrated anti-inflammatory effects in sepsis and colitis. However, its effect on macrophages response to intracellular pathogen is unknown. Then, the aim of this study was to analyze the effect of RvD2 on macrophages infected with BCG. **Methods and Results:** We treated bone marrow-macrophages (BMM) with RvD2 (10nM) and infected or not with BCG (MOI 0.5:1) for 24 hours. First we sought to investigate the role of RvD2 on the BMM. The BMM treated with RvD2 did not release cytokines as KC, IL-6, IL-1 β or IL-10, also had no effect on gene and protein expression of COX-2, and in the lipid body (LB) formation. However, increased expression of PPAR- γ was observed in the BMM treated with RvD2. When BMM was infected with BCG we observed an increase in IL-10, KC, IL-6 and IL-1 beta in relation to control. BCG induced gene and protein expression of COX-2, also increased the PPAR- γ gene expression and LB. BMM pretreated with RvD2 failed to inhibit BCG induced cytokines production. COX-2 induced by BCG was not modulated by RvD2, however, the PPAR- γ expression induced by BCG was modulated. RvD2 did not interfere in the LB formation by BCG. We also analyzed the BCG phagocytosis by the BMM. BMM were treated with RvD2 and infected with BCG for 2 hours. We observed a dramatic reduction in the phagocytic index with RvD2 treatment in relation to the BCG BMM. Also, there was a reduction in the infection rate. **Conclusions:** Our results suggest an immunomodulatory effect of RvD2, mostly on BMM phagocytosis capacity. Further studies are necessary to understand the role of



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this lipid mediator specialized in resolution in the control of intracellular pathogen infection.

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