01. Cellular and Molecular Pharmacology

01.001

Biological evaluation and toxicological profile of the new antibacterial prototypes against bovine mastitis. Silva AR[1], Nunes ELC[2], Silva GCCS[1], Cardoso EA[1], Santana SS[3], Gomez JAG[3], Vargas MD[3], Castro HC[2], Lione VOF[1] [1]UFRJ-LaBioFar – Fármacos e Medicamentos, [2]LABiEmol-UFF, [3]IQ-UFF – Química Inorgânica

Introduction: Brazil has the second largest consumer market for dairy products in Latin America. However, our country still has productivity indices dairy farming very unfavorable: on average, a Brazilian cow produces per day little more than four gallons of milk, about 7.5 times less than in the United States, or just the equivalent to 20% of what one French cow produces being the mastitis, a disease characterized by inflammation of the mammary gland, one of the factors that affect milk production. The aim of this study was to evaluate the biological, toxicological activity and mechanisms of action of 19 compounds naphthoquinones in strains bacterial isolated bovine mastitis, kindly provided by the Laboratory of Microbiology of Milk/Embrapa Gado de Leite de Juiz de Fora (MG). **Methods:** The antimicrobial activity was determined by Antimicrobial Susceptibility Test (AST). We used as positive control the vancomycin and as negative control DMSO. The following compounds were tested: SS4, SS7, SS8, SS14, SS27, SS31, SS37, SS38, SS42, SS44, JG57, JG58, JG59, JG68, JG85, JG92, JG94, JG100, JG576 at concentration 5 mg/mL. For tests of nephrotoxicity, Vero cells were used. Cells were plated in 96-well flat bottom (TPP) at density of $1x10^4$ cells per well, 24 hours before test. Cells were maintained in complete medium DMEM and exposed to different concentrations of active molecules: 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, 128 µg/mL, 256 µg/mL, 512 µg/mL in periods of 8 to 24 hours. After the incubation period, the MTT (SIGMA) viability assay was done using an ELISA reader Spectrum at a wavelength of 490 nm. The tests were performed in triplicate. **Results**: Of the 19 naphthoquinones compounds tested, five derivatives of the SS series showed antimicrobial activity (4, 7, 8, 27, 37) with emphasis on the SS27 molecule with bioactivity against gram positive and gram negative. The SS8 molecule was shown to be moderately toxic at the concentration of 16 µg/mL to 64 µg/mL in the 8h time. The SS7 molecule had slightly toxic profile in concentration of 8 µg/mL, after 24 hours of exposure, better than the drugs in clinical use. The compounds SS4 and SS27 showed severely toxic profile. **Discussion**: The level of toxicity of antimicrobial new prototypes is stage essential for establishing the feasibility of the safe use of these compounds in humans and animals. The *in vitro* method used showed biological, pharmacological and toxicological characterization, allowing to screening 5 naphthoquinones derivatives will guide the design and synthesis of new, more active and less toxic antimicrobial drugs that can be used safely in Preclinical and clinical phases. The results in the MTTT assay are promissory, especially; SS8 and SS7 molecules presented a profile of less toxicity like drugs already on the market. As considered toxic molecules SS4 and SS27 will be continue to be studied to investigate their hepatocitotocixity that direct testing *in silico*, suggesting structural modifications to enhance the effects of these molecules. The molecules SS4 and SS27 with toxic profile will continue to be studied to investigate their hepatotoxicity to guide testing *in silico*, suggesting structural modifications to enhance the effects of these molecules.

01.002

Evaluation of the intrinsic efficacy of N-phenylpiperazines as atypical antipsychotic candidates on D2L receptor. Pompeu TET[1], Hermans E[2], Menegatti R[3], Fraga CAM[4], Noël F[1]
[1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UCLouvain – Neuroscience, [3]UFG, [4]LASSBio-UFRJ

Introduction: The evaluation of the intrinsic efficacy of an antipsychotic candidate is important to predict its clinical efficacy since antagonism at the D2 receptor is a rule for all antipsychotics in use. On the other hand, partial agonism at this receptor has been claimed for aripiprazole. Using a system where the level of D2L expression is under control and two different binding buffers (containing either NaCl or NMDG), we evaluated the intrinsic efficacy of our antipsychotic lead compound, LASSBio-579, and its active *p*-OH metabolite (LQFM 037) together with clozapine and aripiprazole. **Methods:** HeLa cells – Tet-On-pTRE expressing D2L receptor were maintained in DMEM, with 10% FBS, 1% penicilin/streptomicin, 50 µg/mL of hygromycin B and 250 µg/ml of geneticin at 37°C and 5% CO_2 in 175 cm^2 flasks until confluence. Doxycycline (2 µg/mL) was then added to the medium and maintained for 48 hours whereas sodium butyrate (5 mM) was added for only 24 hours, in order to increase the density of the D2L receptors. After cell lysis and protein dosage, binding experiments were carried out with [^{35}S]-GTPγS for 40 min at 30°C using a buffer containing either NaCl or NMDG (Koener *et al*., *Prog. Neuropsychopharmacol. Biol Psychiatry* 36:60, 2012). **Results and discussion:** In the NaCl buffer, LASSBio-579 and LQFM 037 produced only a very small increase of the basal [^{35}S]-GTPγS binding: Emax values were 14 ± 3.1% for LASSBio-579 and 10.4 ± 4.9% for LQFM 037. However, when NaCl was substituted by NMDG, both drugs stimulated [^{35}S]-GTPγS binding by 34 ± 2.1% for LASSBio-579 and 19.4 ± 2.1% for LQFM 037. This is in accordance with data showing that the replacement of Na^+ ions by NMDG facilitates the detection of partial agonists with low efficacy (Lin H *et al*., *Br J Pharmacol* 149: 291, 2006). On the other hand, clozapine behaved as an antagonist of the D2L receptors in the two buffers, since no stimulus of [^{35}S]-GTPγS binding was observed. To confirm that LASSBio-579 and LQFM 037 are partial agonists of the D2L receptor, we challenged the full D2 agonist dopamine with increasing concentrations of our compounds. In the NaCl buffer, LASSBio-579 and LQFM 037 antagonized the stimulation of [^{35}S]-GTPγS binding promoted by 1 µM dopamine in a concentration-dependent manner, with IC_{50} of 1.8 and 0.8 µM, respectively. In the NMDG buffer, which favors the stimulatory effect of dopamine, the antagonism exerted by both LASSBio-579 and LQFM 037 was less intense, with a shift to the right of the inhibition curves. In our conditions, aripiprazole acts as a D2L receptor antagonist in the presence of NaCl and as a partial agonist in the NMDG buffer, where it produced a 52% stimulation of [^{35}S]-GTPγS binding, well below the effect of 100 µM dopamine (231% stimulation). As aripiprazole, and unlike clozapine, LASSBio-579 and LQFM 037 can be considered as weak partial agonists of the D2L receptor. **Acknowledgments:** Capes, INCT-INOFAR, UCL

01.003

LDT3 and LDT5: pharmacological evaluation in human alpha-1 adrenoceptors. Nascimento-Viana JB[1], Alcántara-Hernández R[2], García-Sáinz JA[2], Romeiro LAS[3], Noël F[1], Silva CLM[1]
[1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UNAM – Fisiología Celular, [3]UnB – Desenvolvimento de Estratégias Terapêuticas

Introduction: Previously, new *N*-phenylpiperazine derivatives (LDT series) were synthesized and the pharmacological evaluation unveiled a potent α_1-adrenoceptor (AR) antagonism with high affinity for α_{1A}- and α_{1D}-AR (Chagas-Silva *et al.*, *N-S. Arch. Pharmacol.* 387:225; 2014). The lower urinary tract symptoms (LUTS) are the major complaint among aging men. LUTS are usually caused by benign prostatic hyperplasia (BPH), an urological disorder which the usual pharmacological treatment is the use of the selective α_{1A}-AR antagonists to relax prostate. Therefore, the objective of this study was to evaluate *in vitro* the affinity for recombinant human α_1-ARs subtypes expressed in Rat-1 cells the cell antiproliferative effect in BPH cells. **Methods**: All protocols were approved by the Ethics Committee of UFRJ (CAAE-0029.0.197.000-05 and DFBC/ICB011). *Intracellular [Ca^{2+}]*: Cells were established and treated with LDT3, LDT5 or control (tamsulosin, α_{1A}-AR antagonist) (10^{-10}–10^{-5} M), in the absence or presence of phenylephrine (PHE) (100 µM). [Ca^{2+}]i measurement was determined using fura-2. *Cell proliferation and viability assays*: Cell cultures were established and treated with 50 nM of LDTs, or controls, in the absence or presence of PHE (3 µM). We performed MTT and Trypan Blue exclusion assays 48h after treatment. *Western blot assays*: BPH cells were lysed with RIPA buffer, centrifuged at 13,000 g for 15 min and the supernatants were used for evaluation of activation (phosphorylation) of MAP kinases ERK1/2. **Results and discussion:** Previous data of our group showed that LDTs have high affinity for $\alpha_{1A/D}$-AR. Moreover, both compounds inhibited with high affinity (nM) rat prostate contraction mediated by α_{1A}-AR *in vitro* and blocked the phenylephrine-induced increase of intraurethral pressure *in vivo*. In fluorimetric assays, all LDT3 and LDT5 showed an affinity for α_{1D}-AR similar to the ones observed in functional assays (*log* IC_{50} = -8.27 ± 0.33 and -8.32 ± 0.08, LDT3 and LDT5 respectively, n = 6-9) being higher than for α_{1B}-AR (µM range; *log* IC_{50} = -5.97 ± 0.15 and -5.81 ± 0.22, LDT3 and LDT5 respectively, n = 4-5). The low affinity for the later receptors subtype reduces the probability of a putative hypotension as side effect. On the other hand, tamsulosin showed similar affinity for all α_1-AR subtypes. Pre-incubation of BPH cells with BMY7378 or LDTs prevented cell proliferation induced by PHE, corroborating the role of α_{1D}-AR in stromal cell proliferation (Kojima *et al.*, *Prostate*, 69:1521, 2009). The same was not observed for tamsulosin. MAP kinase ERK1/2 signaling is involved in cell proliferation. Preliminary data showed that LDT3 and LDT5 blocked ERK phosphorylation induced by PHE. Such antiproliferative effect could contribute to prevent prostate enlargement reducing LUTS. In conclusion, LDT3 and LDT5 are potential candidates as new prototypes for the treatment of BPH. **Acknowledgements:** Dr. Nasciutti LE and Dr. Souza PAVR for generously provided BPH primary stromal cells. **Financial support:** Capes-PDSE, Capes, CNPq and Faperj.

01.004

Pharmacological evaluation of new N-phenylpiperazine derivatives for the treatment of benign prostatic hyperplasia: intrinsic activity determination for 5-HT1A and muscarinic receptors.

Carvalho AR[1], Nascimento-Viana JB[1], Romeiro LAS[2], Noël F[1], Silva CLM[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]LADETER-UnB – Desenvolvimento de Estratégias Terapêuticas

Introduction: 5-Hydroxytryptamine (5-HT)-$_{1A}$ receptors stimulate prostate cellular proliferation and these receptors have been considered as a new for the treatment of benign prostatic hyperplasia (BPH) (Abdul *et al.*, J. Urol. 154:247, 1995). Previously, *N*-phenylpiperazine derivatives (LDT series) were reported to exhibit high affinity for 5HT$_{1A}$ receptors but we did not know their intrinsic activity. Moreover, muscarinic M_3 receptors have great relevance in the urinary tract being responsible for contraction of bladder smooth muscle and contributing to the clinical conditions of BPH (Cohen *et al.*, JPET. 248:1063, 1988). The objective of this study was to determine the intrinsic activity of LDT3, 5 and 8 at 5HT$_{1A}$ receptors and at muscarinic receptors. **Methods**: All protocols were approved by the Ethics Committee of UFRJ (DFBCICB011). Binding assays: Wistar rats were anesthetized and euthanized. Membrane preparations containing 5HT$_{1A}$ and muscarinic receptors were obtained from hippocampus and prostate, respectively (Hall *et al.*, *J Neurochem*, 44:1685, 1985; Lau *et al.*, *Eur J Pharm* 343:151, 1998). For 5-HT$_{1A}$ receptor assay, the preparation (50 µg) was incubated with [3H]8-OH-DPAT 1 nM (agonist) or [3H]*p*-MPPF 0.5 nM (antagonist), in appropriate conditions, with or without GTP 1 mM, for 15 or 45 min/37°C in the presence and absence of LDT3, LDT5 (10^{-10}M – $3x10^{-7}$M) and LDT8 (10^{-12}M – 10^{-7}M). For muscarinic receptor assay, the preparation (190 µg) was incubated with [3H]QNB (0.1 nM) for 1h/37°C in the presence or absence of LDT3, LDT5 (10^{-7}M – $3x10^{-5}$M). After incubation, the tube content was filtered under vacuum and the radioactivity was quantified. Functional studies: isometric contraction experiments were performed as previously described using rat prostate or bladder (muscarinic receptor) (White el al., JPET. 339:870, 2011). Each segment of tissue was contracted with carbacol (CCh) (10^{-8} x 10^{-3}M) before and after incubation with atropine (10 nM) or LTD3 or LDT5 (100 nM) (1h). The results were analyzed by non-linear regression to calculate the parameters IC_{50}, *Ki* (using Cheng-Prusoff equation), EC_{50}, E_{max} and K_b value (using Schild equation). **Results and discussion:** The affinity of an agonist for G protein-coupled receptors, but not of an antagonist, varies with the receptor conformation due to GTP-binding. The *Ki* ratios for 5-HT$_{1A}$ receptors binding assays [95% CI] of LDT 3, 5 and 8 were, respectively, 1.2 [0.6-2.2]; 2.1 [1.3-3.5] e 35 [3.7-186.2], which were used to predict the intrinsic activity of LDT's (Lahti *et al.*, *Mol Pharmacol*, 42:432, 1992). Therefore, LDT3 and LDT5 can be considered as high affinity antagonists and LDT 8 as a partial agonist of 5-HT$_{1A}$ receptor. At muscarinic binding, the *Ki* for LDT3 and atropine was 11.7 µM and 0.14 nM, respectively. LDT5 showed the lowest affinity. In functional prostatic studies, treatment with LDT3 and LDT5 promoted a shift in the curve of the agonist to the right, typical of competitive antagonism. In the functional bladder, LDT3 and LDT5 did not alter the contraction promoted by CCh. **Acknowledgement:** Faperj and CNPq.

01.005

Immunotherapy against pythiosis: Molecular profile, genetic diversity and evolution of Brazilian isolates of *Pythium insidiosum* based on COX II gene. Ribeiro TC[1], Weiblen C[1], Azevedo MI[2], Monteiro DU[1], Emmanouilidis J[1], Machado VS[1], Pereira DIB[3], Botton SA[1], Santurio JM[1] [1]UFSM, [2]UFRGS, [3]UFPel

Introduction: Pythiosis is a pyogranulomatous disease caused by the oomycete *Pythium insidiosum*. The disease affects humans, domestic and wild animals in tropical and subtropical areas. Failures in antifungal therapies allied to cases not responsive to immunotherapy encourage studies on genetic variations of the Brazilian isolates and research to improve immunotherapy available. Genes encoding metabolic structural proteins such as COX II, have been used as genetic markers in molecular analyzes in eukaryotes. Network analysis studies the minimal evolution and calculates the evolutionary distances for all individuals, also verifies the evolution through a network of haplotypes. This work evaluated the genetic diversity of Brazilian isolates of *P. insidiosum* against Thailand and other American isolates by partial DNA sequencing of the genomic region cytochrome c oxidase II (*Cox II*), as well as studied the evolution of *P. insidiosum* (Azevedo MI, *Vet Microbiol*: 159,141,2012, Kammarnjesadakul P, *Med. Myc* 49;289, 2011). **Methods:** Extraction of DNA of 30 isolates of *P. insidiosum*; PCR for *Cox II* amplification with the primers FM 66 and FM 58. The amplification products were sent to DNA sequencing (MegaBACE500). The chromatograms were visualized using the GAP4 program from the Staden package. Other sequences were used for comparison, like equine isolates from the USA and Costa Rica, human and environmental isolates from Thailand, obtained from GenBank (www.ncbi.nlm.nih.gov/GenBank). The multiple alignments of the data sets were conducted by the Clustal W algorithm in the MEGA 5.3 program. Evolutionary analysis of the isolates was constructed using Network 4.6 (www.fluxus-engineering.com) program using the Median-joining Method: In addition, 5 DNAsp program was also used to measure the levels of nucleotide diversity. **Results:** All isolates were amplified by PCR for *CoxII*. *P. insidiosum* isolates shown to be monophyletic for *Pythium* spp. and composed three major polytomic groups. Americans isolates are inserted into one of these polytomic groups and showed low levels of nucleotide diversity, revealing an evolutionary proximity between all of them. Network analysis of *Cox II* showed recent expansion of *P. insidosum*. **Discussion:** Phylogenetic studies consider relationship and the evolutionary history, under the premise that the most closely related species share a greater number of features in common than the most distant. The isolates of *P. insidiosum* shown to be monophyletic for *Pythium* spp. and composed the polytomic groups. Americans isolates form one of the polytomic groups with low levels of nucleotide diversity, demonstrated the evolutionary proximity between *P. insidiosum*, possibly sharing a common ancestor. Network analysis showed recent expansion of American *P. insidosum* possibly from Asia, since Thailand isolates proved parafiletic toward the American isolates. It was observed a genetic variability among *P. insidiosum* isolates; in addition, it was possible to display the strain used in immunotherapy (Pitium-Vac®) is representative of *P. insidiosum* Brazilian isolates. **Acknowledgments:** CNPq and FAPERGS for the Financial support.

01.006

Pharmacological and molecular evidence on the relevance of kinin receptors in a mouse glioma model. Nicoletti NF[1], Senécal J[2], Pesquero JB[3], Campos MM[1], Couture R[3], Morrone FB[16] [1]INTOX-PUCRS – Biologia Celular e Molecular, [2]INTOX-PUCRS, [2]UdeM, [3]Unifesp – Biofísica

Introduction: Gliomas are among the most deadly and prevalent brain tumors (Wen, PY *et al*; *N Engl J Med* 359: 492, 2008). This study investigated the role of kinins and their receptors (B_1R and B_2R) in malignant brain tumors *in vivo*. **Methods:** GL261 glioma cells were cultured and resuspended at 2 x 10^5 cells/2 µl DMEM. Cells were injected (2 µl/2 min) into the left striatum (coordinates with regard to bregma: 2.0 mm lateral; 3.0 mm at depth) of adult C57/BL6, KOB_1R, KOB_2R or KOB_1B_2R mice (nearly 8-weeks-old, 25–30 g) previously anesthetized with ketamine plus xylazine (80 + 10 mg/kg, i.p.). In some groups, five days after glioma implantation, the animals received the first administration of compounds by intracerebroventricular injection at 5, 10 and 15 days. Tumor size determination, western blot analysis and quantitative autoradiography were carried out. Data is expressed as mean ± standard deviation. All the procedures were approved by the Ethical Committee of the PUCRS (Brazil – CEUA 11/00258). **Results:** Our results demonstrated that both the treatment with the selective B_1R antagonist SSR240612, or B_1R gene ablation increased the tumor size (7.58 ± 1.72 and 13.59 ± 5.45 mm^3, respectively), when compared to C57/BL6 non-treated control group (5.78 ± 1.20 mm^3). The selective B_2R antagonist HOE-140 alone did not produce any alteration of the tumor size in C57/BL6 (4.86 ± 2.00 mm^3) or KOB_2R mice (5.58 ± 4.96 mm^3). However, the treatment with SSR240612 plus HOE-140 significantly decreased the tumor size (2.58 ± 0.78 mm^3) when compared to control (5.78 ± 1.20 mm^3), and a similar reduction was seen in double KOB_1B_2R animals (3.00 ± 1.55 mm^3). The protein expression of B_1R and B_2R was analyzed in the brain tumors of mice subjected to the treatment with kinin antagonists. Protein expression of B_1R was significantly decreased in tumors of mice pretreated with SSR240612 (89.56 ± 2.72%), and a low pattern of B_1R expression was also detected in the HOE-140-treated group (74.83 ± 4.20%), in comparison to C57/BL6 non-treated control group (100% tumor growing). Interestingly, the protein expression analysis of B_1R pointed out that combined treatment with SSR240212 plus HOE-140 brought the levels of B_1R expression to that observed in the brain of sham-operated group. The autoradiography analysis demonstrated that B_1R receptor expression was decreased in brain of animals treated with the B_2R antagonist HOE-140 (23.16 ± 6.78%) and also in the group treated with both antagonists SSR240612 plus HOE-140 (22.87 ± 10.19%). **Discussion:** Taken together our results show that genetic deletion or combined pharmacological inhibition of B_1R and B_2R change the expression and binding sites of kinin receptors and modify the development of the tumor, which might represent an attractive alternative for the treatment of malignant gliomas in the future. **Financial support:** Capes, CNPq, FAPERGS and FINEP/PUCRSINFRA #01.11.0014-00.

01.007

Melatonin inhibits P2Y1 receptor-mediated leukocyte adhesion to endothelial cell. Cardoso TC, Silva CLM ICB-UFRJ

Introduction: Inflammatory process changes endothelial cells phenotype increasing the expression of adhesion molecules that promote the adhesion and migration of immune cells (Carlos and Harlan, Blood 87:2068, 1994). Besides the role of melatonin in the circadian rhythms control, the hormone (1 – 100 nM) reduces leukocyte adhesion to rat microcirculation *in vivo* stimulated by leukotriene B_4 (Lotufo *et al.*, *Eur J Pharmacol* 430: 351, 2001). Similar results were observed *in vitro* (Lotufo *et al.*, *Eur J Pharmacol* 534: 258, 2006). It was also shown that melatonin reduces endothelial adhesion molecule expression (Marçola *et al.*, *J. Pineal Res* 54: 162, 2013) and mRNA levels of adhesion molecules (Hung *et al.*, *J. Pineal Res* 55: 247, 2013). The activation of purinergic receptor $P2Y_1$ ($P2Y_1R$) present on endothelial cells by ADP increases leukocyte rolling to the endothelium (Zerr *et al.*, Circulation 123:2404, 2011) having an important role in inflammation (Burnstock and Ralevic, *Pharmacol Rev* 66: 102, 2014). Thus, our aim was to evaluate the melatonin effect on $P2Y_1R$-induced leukocyte adhesion to mesenteric endothelial cells (MEC). **Methods:** The protocol was approved by the institutional ethics committee (DFBCICB011). MEC were obtained from male Wistar rats and maintained in DMEM supplemented with 20% fetal bovine serum, gentamicin, and incubated at 37°C and 5% CO_2. MEC were incubated for 4 hours with vehicle (basal), the $P2Y_1R$ agonist 2MeSATP (60 μM) in the absence or presence of melatonin 30 nM. Alternatively, the $P2Y_1R$ antagonist MRS2179 (0.3 μM) or the melatonin MT receptor antagonist luzindole 0.3 μM were used. Rat mononuclear cells (MNC) were isolated from blood and purified by Ficoll-Paque Plus gradient (Oliveira *et al.*, Plos One 6:e23547, 2011). At the end of the fourth hour, 1E4 MNC/well were added and incubated for 30 minutes. After this period the non-adherent cells were removed and four fields per well were randomly chosen and analyzed. The number of adherent MNC per field was determined by microcopy (400X magnification). **Results:** 2MeSATP (60 μM) increased MNC adhesion to MECs when compared with basal condition (20.3 ± 2.4 and 10.8 ± 1.1 cells/field, n = 12, respectively, $p<0.05$). MRS2179 blocked the effect induced by 2MeSATP (7.5 ± 0.8 cells/field, n = 12, $p<0.05$). Melatonin did not alter basal MNC adhesion but it inhibited the effect induced by 2MeSATP (from 33.12 ± 2.6 cells/field, n = 33, to 18.36 ± 1.4 cells/fields, n = 33, $p<0.001$). Additionally, melatonińs action was mediated by MT receptors since the antagonist luzindole (10 μM) blocked its effect (from 26.83 ± 3.6 cells/field (n = 12) to 13.42 ± 1.1 cells/field (n = 12), in the absence or presence of luzindole, respectively, $p<0.05$). **Conclusion:** Our data indicate that melatonin has an inhibitory effect on the stimulation of endothelial-leukocyte adhesion promoted by $P2Y_1R$, having therefore a wider range of anti-inflammatory action. **Acknowledgements:** Capes, CNPq, Faperj

01.008

Protective effect of 4,4'-bischloro-diphenyl diselenide against inhibition of thioredoxin reductase by methylmercury. Leite GO[1], Lugokenski TH[2], Rocha JBT[3], Wagner C[2,1] [1]UFSM – Farmacologia, [2]Unipampa, [3]UFSM – Bioquímica Toxicológica

Introduction: The thioredoxin (Trx) system, involving redox active Trxs and thioredoxin reductases (TrxRs), sustain a number of important Trx-dependent pathways. These redox active proteins support several processes crucial for cell function, cell proliferation, antioxidant defense, and redox-regulated signaling cascades. Methylmercury (MeHg) is an important environmental toxicant that has a high affinity for thiol groups and can cause oxidative stress. The Trx system is the major system responsible for maintaining the redox state of cells and this function involves thiol reduction mediated by selenol groups in TrxRs. Selenium compounds are known to present a protective effect against MeHg toxicity. MeHg has a great affinity to thiols and selenols, thus the potential toxic effects of MeHg on TrxR inhibition, as well as, protective effect of 4,4'-bischloro-diphenyl diselenide and selenol compound form were determined in the current study. **Methods:** TrxR activity was measured by the method described by Holmgren and Bjornstedt (1995). The purified TrxR was added to a cuvet containing the reaction mixture in presence or absence of MeHg (final concentrations were 0.05, 0.1, 0.2 μM). Fresh compound (did not previously reduced) 4,4'-bistrifluoromethyl-diphenyl diselenide (0.1, 0.4, 0.8, 1.6, 3.2, 6.4 μM) or selenol form (previously reduced 4,4'-bistrifluoromethyl-diphenyl diselenide in their selenol form) at same concentrations as added in TrxR activity inhibited by different concentrations of MeHg. **Results and discussion:** The results demonstrated that MeHg can inhibit TRxR activity in a concentrations of 0.05 μM, 0.1 μM and 0.2 μM. Fresh (did not converted in selenol form) 4,4'-bischloro-diphenyl diselenide (concentration of 3.2 and 6.4 μM) presented a protective effect only when the TrxR was exposed to minor concentration of MeHg (0.05 μM). Selenol form (previously reduced) of 4,4'-bischloro-diphenyl diselenide at a concentration of 6.4 μM can protect against enzyme inhibition induced by MeHg in both, high and low concentrations (0.05 and 0.2 μM) of mercury. This results suggested that the selenol form of selenium compound was more effective in protect the enzyme TRxR against MeHg inhibition, in addition, selenol form of 4,4'-bischloro-diphenyl diselenide can be an important molecule to prevent mercury intoxication. Freitas, A.S., Rocha, J.B.T. *Neuroscience Letters*. 503: 1-5, 2011. Holmgren, A *Annu Rev Biochem* 54: 237–271, 1985. Zhao R, Masayasu H, Holmgren A. *Proc Natl Acad Sci USA* 99:8579–8584, 2002. Wagner C, Sudati JH, Nogueira CW, Rocha JBT *BioMetals* (Oxford), 23:1171-1177, 2010. **Financial Agencies and acknowledgments**: Capes, FAPERGS, CNPq

01.009

Novel digoxin derivatives not mimic the cellular effects of digoxin. Silva NP[1], Noël F[1], Barbosa LAO[2], Quintas LEM[1] [1]ICB-UFRJ, [2]CCS-UFSJ

Introduction: Digitalis glycosides are clinically used for decades in the treatment of heart failure. Their classic mechanism of action consists of the inhibition of Na/K-ATPase (NKA) activity. Recently, novel functions of NKA have reestablished the interest of digitalis drug development for innovative therapeutic uses. Many studies have investigated their antitumor effect, but life-threatening cardiotoxicity limits the utilization of standard digitalis compounds. Interestingly, whereas killing tumor cells, some cardiotonic steroids are known to induce proliferation in normal cells. We have shown that original digoxin derivatives (DGB1 to DGB7) present different patterns of NKA inhibition. Here we evaluated their effect on cell proliferation. **Methods:** DGBs were obtained through organic synthesis with addition of functional groups to the lactone ring of digoxin. LLC-PK1 cells (normal porcine proximal renal tubule) were treated for 24, 48 or 72 h with 1, 10, 100 or 1000 nM DGB1-DGB7 and the cell quantity was evaluated by counting the number of Trypan blue-viable cells. Digoxin was used as a standard. **Results:** Digoxin resulted in a hormetic effect, stimulating cell proliferation at lower (1-100 nM) concentrations whereas producing cell death at 1 µM. Interestingly, while DGBs have distinct inhibition patterns (i.e., NKA activity is not inhibited by DGB1 and DGB7, is inhibited by DGB2 and DGB4 with a similar potency to digoxin, and is 7-35 times less potently inhibited by DGB3, DGB5 and DGB6), as we previously showed, they had no effect in cell proliferation at any concentration studied (1 nM-1 µM), except for DGB3 which had an effect similar to digoxin at 1 µM. **Conclusion:** The results showed that DGBs had no proliferative profile in low concentration in normal cells which could be of therapeutic interest. Moreover, the inhibition of NKA activity is not essential for LLC-PK1 cell proliferation, so other mechanisms may be involved. Differences of activation of intracellular proliferative pathways between digoxin and DGBs are under investigation. **Financial Support:** Faperj, CNPq, Capes.

01.010

Evaluation of cytotoxicity and expression of genes after human melanoma cells treatment with biflorin. Ralph ACL[1], Souza LGSS[2], Lemos TLG[2], Montenegro RC[3], Smith MC[4], Calcagno DQ[5], Vasconcellos MC[1] [1]UFAM – Pharmaceutical Sciences, [2]UFC – Organic and Inorganic Chemistry, [3]ICB-UFPA – [4]Unifesp – Morphology and Genetic, [5]UFPA – Oncology

Introduction: Biflorin is an *o*-naftoquinone [6,9-dimethyl-3-(4-methyl-3-pentenyl)naphtha[1,8-*bc*]-pyran-7,8-dione] prenilated from *Capraria biflora* L. (Schrophulariaceae) roots. It has been reported its antitumoral activity in around 13 tumor cell lines (murine melanoma, prostate, colon, gastric) and inhibition of tumor development in mice Erlich and Sarcoma 180 model. Nowadays is known its direct interaction with DNA, although it mechanism of action has not been elucidated yet. In this study, we analyzed the cytotoxicity and expression of genes involved on cell cycle progression, replication, DNA repair and methylation at SK-Mel 103 human melanoma cell line, considering knowing characteristic of mutated *NRAS*. **Material and Methods:** The tests were developed using human melanoma cell lines SK-Mel-103 (DMEM high glucoses, FBS 10%). For cytotoxicity evaluation were performed trypan blue exclusion test using 1.0, 2.5 and 5.0 µM of biflorin during 24, 48 and 72 hours of treatment. The IC_{50} were determined using Alamar Blue assay, starting with 20.0 µM of biflorin during 24 hours of treatment. Doxorubicin (5.0 µM) was used as positive control. All real-time RT-qPCR reactions were performed in triplicate using *TaqMan®*, FAM™ MGB at *7500 Fast* for both the target gene *BRAF* (Hs00269944_ml), *MELK* (Hs01106440_ml) *MGMT* (Hs00172470_ml), *RAD54L* (Hs00269177_ml), *TYMS* (Hs00426586_ml), *DNMT1* (Hs00154749_ml), *DNMT3B* (Hs00171879_ml), *MBD1* (Hs00242770_ml), *MBD2* (Hs00187506_ml), *MBD3* (Hs00172710_ml), *MBD4* (Hs00187498_ml), *MeCP2* (Hs00172845_ml) and the internal control *B2M* (Hs00984230_ml). The $1x10^6$ cells/per well were treated with 1.0, 2.5 and 5.0 µM of biflorin during 24 hours. Were used GaphPad Prism 5.0 for statistical analysis. **Results and discussion:** Biflorin caused intense growth inhibition of SK-Mel 103 cells at 2.5 and 5.0 µM after 48 and 72 hours. The IC_{50} after 24 hours treatment with biflorin was 1.85 µM (CI_{95} 1.62-2.10) for SK-Mel 103 and with doxorubicin was 4.16 µM (CI_{95} 3.48-4.98). Analyzing the genes expression, biflorin caused overexpression of *BRAF*, *RAD*, *TYMS* and *MGMT*. However, the methylation genes had not been changed after 24 hour treatment. We suggest that the overexpression of *BRAF* after biflorin treatment and *NRAS* mutation in SK-Mel 103 leads to activation of RAS/BRAF pathway using a new epigenetic programming in response a DNA damage caused by biflorin. **Conclusion:** Complementing the direct interaction of biflorin with the DNA, it has the capacity to change genes expression involved at cell cycle progression, replication and repair which is influencing the melanoma cells proliferation, besides that, biflorin can change epigenetics interactions. These results contribute for the determination mechanism of action of biflorin and raises knew research about epigenetic mechanisms involved at biflorin cytotoxicity. **Financial Agencies**: CNPq/Fapeam.

01.011

Analysis of message-address concept of adenosine receptor system by molecular modeling studies. Tesch R[1], Sant'Anna CMR[2], Fraga CAM[1] [1]ICB-UFRJ – Farmacologia e Química Medicinal, [2]ICE-UFRRJ – Química

Introduction: The message-address concept was postulated by Schwyzer in 1977 and describes compounds that have attached groups related to the message (activation or inactivation of their target) and groups related to the address (*e.g.* receptor subtype affinity). This concept was used by Philip Portoghese to design the selective δ opioid receptor antagonist naltrildol through modifications of the non-selective opioid receptor antagonist naltrexone. The same concept can be applied to other G-protein-coupled receptor (GPCRs) systems, such as the agonists and antagonists of adenosine receptors. Adenosine receptors are divided in four subtypes as known, A_1, A_{2A}, A_{2B} and A_3. The extensive involvement in inflammatory, cardiac, pulmonary and neurodegenerative diseases has turned the adenosinergic system as a promising therapeutic target in the past few years. The knowledge of the message-address concept can be explained by molecular modeling studies. Only the A_{2A} subtype has been characterized by x-ray crystallography thus being necessary for docking studies the creation of three dimensional models of A_1, A_{2A} and A_3 adenosine receptor. **Aim:** The aim of this work was the construction of three dimensional models of other subtypes and use of the docking approach to analyze the interaction of different selective adenosine receptor agonists and antagonists to explain their message-address properties. **Methods:** The models were generated from X-ray structures of A_{2A} receptor co-crystallized with the endogenous agonist adenosine (PDB 2YDO) and with the antagonist ZM241385 (PDB 3EML). The docking of selective agonists and antagonists of each subtype was performed with GOLD 5.1 software (CCDC) and different score functions were analyzed for each subtype in order to validate the ability of the model to identify their selective agonists and antagonists. **Results:** The models were validated by analysis of Ramachandran plots and presented appropriate percentage of amino acid residues in the most favorable regions. The percentages for A_1, A_{2B} and A_3 were respectively 90.3%, 89,1% e 90,2% for models created from PDB 3EML and 91,0%, 90,7% e 92,5% respectively for models created from PDB 2YDO. The adenosine receptor active site can be divided into intracellular, membrane and extracellular regions. Through the interaction pattern of adenosine receptor agonists it can be observed that all adenosine analogues have the sugar moiety interacting in the intracellular region of the active site, which is related to the interaction of the endogenous agonist adenosine in the x-ray structure of A_{2A} adenosine subtype. This result indicated that groups attached to the adenine moiety are responsible for the selectivity of each subtype and interact with amino acid residues of the extracellular region which is non-conserved. The same pattern is observed in selective antagonists. Although almost all adenosine agonist had a sugar moiety, the A_{2B} agonist LUF5835 which does not have this moiety have made interactions through *m*-hydroxiphenyl group exemplifying that agonism can be achieve without the presence of the sugar moiety. The agonist message seems to be related to the intracellular region of adenosine receptor especially regarding interactions with residues serine 277 and histidine 278 while an antagonist message is sent by the absence of these interactions. The address, *i.e.* selectivity seems to be related to the interactions in the extracellular region of the adenosine receptors. **Conclusion:** The molecular modeling studies such as docking could be applied to identify the message-concept in the adenosinergic system. Through the visual analysis it was observed that not only the ligands

are related to the message-address concept but also the adenosine receptor regions.
Financial support: INCT-INOFAR; Capes; CNPq; Faperj

01.012

Red propolis from State of Alagoas induced anti-hypertensive effect in SHR. Herculano EA[1], Costa CDF[2], Silva JCG[2], Beiriz RV[2], Tenório EP[2], Moura MT[2], Nascimento TG[2], Ribeiro EAN[2], Araújo-Júnior JX[1] [1]UFAL – Chemistry Biotechnology, [2]UFAL – Pharmacy and Nursing

Introduction: Propolis is a resinous substance collected by honeybees from various plant sources (Maruyama, Biol Pharm Bull 32, 1244, 2009). Some compounds contained in propolis might have a potential anti-hypertensive effect (Aoi, *Biochem Biophys Res Commun*, 432, 650, 2013). The red propolis variety is obtained in the northeast of Brazil this unique propolis composition has not been found elsewhere out from this country. Has been reported to have various biological activities beside differences in chemical composition (Frozza, *Food Chem Toxicol*, 52, 137, 2013). In this sense, we evaluate the Red Propolis from Alagoas' about effects on blood pressure of the spontaneously hypertensive rats (SHR). **Methods:** Male SHR were used for all experiments. For measurement hypotension and bradycardia catheters were inserted and after 24 hours the experiments were performed. In other experimental series the animals received Red Propolis Dry for 28 days. Systolic blood pressure (SBP) was measured weekly in unanaesthetized animals by an indirect tail-cuff Method: For *ex vitro* vessel tone analysis the superior mesenteric arteries were isolated and maintained in organ bath solution until use. All analysis was performed using GraphPad™ Prism software, version 5.0®. Protocol approved by the ethics committee for animal experimentation nº 09/2014. **Results and discussion:** In conscious SHR, the crude extract (CE) of red propolis (0.1-10 mg/kg, i.v, randomly), elicited immediate and dose-dependent decreases in mean arterial pressure (-6.3 ± 0.5%; -12.1 ± 1.6%; -12.2 ± 0%; -25.8 ± 0.4% and -16.5 ± 9.7%, respectively). Similarly, the chloroformic fraction (CF) of red propolis (0.1-10 mg/kg, i.v, randomly) induced a hypotensive effect (-5.5 ± 3%; -8.4 ± 1.8%; -16.3 ± 4.3%; -20.8 ± 5.5% and -22.7 ± 6.4%, respectively). The red propolis dry extract (DE) (0.1-10 mg/kg, i.v., randomly) presented hypotensive (-9.8 ± 2.9%; -14.4 ± 3.1%; -11.3 ± 3%; -19.4 ± 1.6% and -22.5 ± 3.8%, respectively) effect without statistical difference between groups (CE,CF and DE) and as well as without heart rate effects. The same hypotensive effect was observed after administration of the RPF (0.1, 0.5, 1, 10 and 10 mg/kg, i.v,) in unconscious rats (-10.7 ± 2.2%; -14.2 ± 3%; -18.5 ± 4.6%; -18.7 ± 4% and -20.3 ± 3.5%, respectively). These data suggest that RPF exerts its hypotensive effect through the periphery and not the central nervous system. Diastolic blood pressure was also lower in SHR treated with DE (150 mg /kg/day, p.o. per gavage) after 14th treatment days. These results suggest that propolis produce an antihypertensive effect. We therefore sought to investigate whether DE induces its hypotensive effect through the vasculature. Mesenteric artery rings were pre-contracted with Phe (10^{-6}M). DE (1–500 µg/mL) induced vasorelaxation in a concentration-dependent manner in endothelium-intact mesenteric arteries (E_{max} = 100% and pD_2 = 1.06 ± 0.05). In contrast, the vasodilatory effect of DE was significantly attenuated in endothelium-denuded rings (E_{max} = 56.7 ± 4.01% and pD_2 = 1.46 ± 0.1). In conclusion, we found that red propolis produce an antihypertensive effect and hypotension that may be mediated by vasodilatation. **Acknowledgement:** UFAL, Fapeal, CNPq, Capes

01.013

Pharmacological evaluation of marinobufagin derivatives: Search for functional selectivity among cardiotonic steroids. Rendeiro MM, Noël F, Cunha-Filho GSA, Touza NA, Quintas LEM
ICB-UFRJ

Introduction: Na/K-ATPase (NKA) is a plasma membrane protein well-known as an energy-transducing ion pump. This action is inhibited by a class of compounds called cardiotonic steroids (CTSs). In addition, by the interaction with NKA, these substances are able to trigger signal transduction pathways that underlie newly recognized cellular effects like proliferation and differentiation. The possibility to discriminate between the two mechanisms (i.e., to have functional selectivity) is a challenge for the development of CTS with unique therapeutic applications. Previous studies in our laboratory showed that telocinobufagin induced cell death, while the structurally similar CTS marinobufagin (MBG) caused proliferation in renal cells. MBG was also the best CTS that could distinguish ATPase inhibition from signaling activation, turning out to be a promising candidate. Here we are evaluating these properties with original MBG derivatives. **Methods:** MBG and 20S,21R-epoxymarinobufagin (EMBG) were obtained from toad *Rhinella schneideri* parotoid gland secretion, while 3β-acetoxy-marinobufagin (AMBG), 3β-acetoxy-20S,21R-epoxymarinobufagin (AEMBG) e 14H,15-oxo-marinobufagin (OMBG) were obtained from chemical modifications of MBG. For the enzymatic experiments, the colorimetric determination of Pi released by ATPasic reaction was carried out after incubation of NKA-enriched human kidney preparations with increasing concentrations of each MBG derivative. The gross cellular effect was evaluated by counting the number of Trypan blue-viable porcine proximal renal tubule LLC-PK1 cells treated with 30nM, 300nM and 3uM MBG MBG derivatives for 24, 48 and 72 h. **Results:** We observed that all MBG derivatives had similar or lower potencies for NKA inhibition in comparison to MBG (MBG IC50 = 1.3 ± 0.3 μM, AMBG IC50 = 0.79 ± 0.18 μM, EMBG IC50 = 9.0 ± 1.8 μM and AEMBG IC50 = 1.96 ± 0.25 μM), except for OMBG that had no effect. Ongoing cell proliferation studies revealed a significant reduction of cell number with 3 uM EMBG (48 h, 54 ± 2%, $p<0.05$; 72 h, 44 ± 5%, $p<0.05$) but no effect at lower concentrations. **Conclusion:** The addition of a second epoxy group on MBG (i.e., EMBG) had a detrimental effect on the NKA inhibition potency and changed the proliferative profile of MBG to an antiproliferative one at the highest concentration used. The next steps are to determine the cellular effects on with the other derivatives and investigate the putative mechanisms. **Financial Support:** Faperj, CNPq, Capes.

01.014

Expression and purification of an acidic phospholipase A2 from *Bothrops pauloensis* snake venom. Borges IP, Isabel TF, Gimenes SNC, Homsi-Brandeburgo MI, Rodrigues RS, Silva FH, Rodrigues VM UFU

Introduction: Phospholipases A_2 (PLA_2) are enzymes that catalyze the hydrolysis of phospholipids releasing free fatty acids and lysophospholipids (HIGUCHI *et al.*, 2007). Snake venom phospholipases are widely studied due to variety of their pharmacological effects as neurotoxicity, miotoxicity, cardiotoxicity, activation or inhibition of platelet aggregation, anti-coagulation, edema, convulsion and hypotension (GUTIÉREZ and LOMONTE, 1997). In the present study, we report the expression and purification of a recombinant acidic phospholipase A_2 from the venom gland of the *Bothrops pauloensis*. **Material and Methods:** The phospholipase A_2 was expressed using *Pichia pastoris* system. The gene was designed based on the sequence of BPr-TXI, an acidic PLA_2 from *Bothrops pauloensis*. This gene was cloned into pTZ57R/T and after sequencing for confirmation; the insert was subcloned into the expression vector pPICZαA and transformed into *Pichia pastoris*. After selection of positive clones, expression was induced by addition of 0.5% methanol by every 24h up to 144h. Purification was performed by affinity chromatography on nickel and the protein eluted with increasing concentrations of imidazole. **Discussion and Results**: Subsequently, the acidic recombinant phospholipase A_2 *B. pauloensis* (BPr-TXI) was tested for its catalytic activity, however this protein did not show satisfactory activity when compared to BpPLA2-TXI, a native acidic phospholipase A_2 purified from the venom of *B. pauloensis*. **Conclusion:** Future studies will allow the biochemical, functional and structural characterization of acidic recombinant phospholipase A_2 from *B. pauloensis* and may provide important information for clarifying the precise biological role of the recombinant protein as well as take advantage of future and possible therapeutic use of this compound or derivatives. **Reference:** Higuchi, D. A.; *Biochemie*, v. 89, p. 319-328, 2007. Gutiérrez JM, Lomonte B; Wiley, Chichester, p. 321-352. 1997. **Financial support:** FAPEMIG, CNPq, Capes, INCT Nano-biofar, UFU, INGEB

01.015

Purified polysaccharides of *Genipa americana* leaves: Anticoagulant, antiplatelet and antithrombotic activities. Madeira JC[1], Nogueira FC[2], Souza ROS[1], Pereira LP[1], Assreuy AMS[1], Pereira MG[2] [1]ISCB-UECE, [2]FECLESC-UECE

Introduction: Heparin, a highly sulfated polysaccharide, is the most employed anticoagulant and antithrombotic drug in clinical practice, despite the disadvantages of bleeding, thrombocytopenia and allergies (HIRSH, *New England J Medicine*, v.324, p.1565, 1991). Non-sulfated plant polysaccharides, different from sulfated polysaccharides isolated from marine alga, have been little explored in coagulation and thrombosis. Aimed to isolate, characterize and evaluate the anticoagulant, antiplatelet and antithrombotic effects of polysaccharides from *Genipa americana* leaves. **Methods**: Leaves of *G. americana* were collected in Quixadá-CE, Brazil (exsiccate nº 46796). Dry powder (5 g) of the leaves was depigmented with methanol and extracted in 0.1 M NaOH to obtain the Total Polysaccharides-TPL (YOON, *Thromb Research*, v.106, p.51, 2002). TPL were purified using anion exchange chromatography (DEAE-cellulose) and the acidic polysaccharide fractions (PL-f) eluted by step wise with NaCl (0.1-1.0 M). TPL and PL-f were characterized by chemical analysis of carbohydrates, uronic acid and proteins and tested *in vitro* for anticoagulant (activated partial thromboplastin time-aPTT and prothrombin time-PT) (BIOS Diagnostic) and antiplatelet (platelet rich plasma) (BORN & CROSS, *The J Physiology*, v.168, p.178, 1963). To obtain the human plasma the research obeyed the Principles of Resolution 196/96 of the National Health Council. The participants were advised about the study and signed the Informed Consent of the Hematology Center of Ceara. Antithrombotic activity was performed using the model of venous thrombosis (Vogel, G. M. T., *Thromb Research*, v.54, p.399, 1989). Wistar rats were anesthetized by intramuscular injection of 5% ketamine-90 mg/kg and xylazine-10 mg/kg. The animals were manipulated in compliance with the Guide for the Care and Use of Laboratory Animals (NIH publication n° 85-23, revised 1985), being approved by ethics committee of UECE with nº 0920046320. Results were expressed as mean ± S.E.M. and analyzed by ANOVA and Bonferroni test ($p<0.05$). **Results**: TPL contains low levels of protein (0.56%) and high levels of carbohydrate (54.6%, with 21.1% of uronic acid). Fractionation of TPL resulted in two main fractions eluted with 0.1 (FI) and 0.25 M (FII). FI and FII are composed, respectively, of 36% and 23% of carbohydrate (including 9% and 30% of uronic acid) and 4% and 5% of proteins. TPL and FI, at 0.1 mg/mL, prolonged the aPTT test in 3.6 times (140.7 ± 3.7 s) and 1.7 times (69.0 ± 3.1 s), respectively, compared with saline (38.7 ± 0.9 s). These polysaccharides had no activity in the PT test. TPL and FI (100 µg/µL) inhibited the platelet aggregation induced by ADP (3 µM) in 23% and 48%, respectively. In the model of venous thrombosis, TPL exhibited potent antithrombotic effect at 1.0 mg/mL: 45% (2.58 ± 0.25 mg), FI 54% (2.14 ± 0.28 mg) and FII 37% (2.96 ± 0.36 mg) compared with saline group (4.7 ± 0.10 mg). **Conclusion**: Polysaccharides isolated from *G. americana* leaves present anticoagulant, antiplatelet and antithrombotic effects. **Financial agencies**: Capes, FUNCAP, CNPq

01.016

Cardiovascular effect of LQM 001 a derivative aminoguanidinic. Costa CDF[1], Herculano EA[1], Lima RR[1], Bernadino AC[1], Beiriz RV[1], Silva JCG[1], Araújo-Júnior JX[2], Ribeiro EAN[1] [1]UFAL – Pharmacy and Nursing, [2]UFAL – Chemistry Biotechnology Institute

Introduction: The high blood pressure is the most important risk factor for cardiovascular diseases. This diseases remains the leading cause of death worldwide and one of the world's greatest public health problems (Hobbs, Heart, 90, 1217, 2004). Thereupon, attention has recently been focused on Several Medicinal Chemistry strategies in the molecular design of new candidate therapeutic agents or new drugs prototypes acting on cardiovascular system (Barreiro, Rev. Virtual Quim., 1, 26, 2009). The aminoguanidinics derivatives such as clonidine, guanabenz and guanfacine are example of important blood-pressure-lowering drugs. The synthetic aminoguanidine derivatives have been used in the treatment of a variety of diseases, but the major success has been their action as antihypertensive drugs (Huang, *Eur J Pharmacol*, 699, 233, 2013). Therefore, the present study was designed to evaluate the cardiovascular effects of LQM001, a new aminoguanidinic derivative through *in vivo* and *in vitro* approach. **Methods**: Male spontaneously hypertensive rats were used for all experiments. For measurement hypotension and bradycardia catheters were inserted and after 24 hours the experiments were performed. For *in vitro* vessel tone analysis, the superior mesenteric arteries rings were maintained in organ bath solution until use. The changes in systolic blood pressure and heart rate of rats and vascular tone of mesenteric rings *in vitro* were measured using pressure transducer and force transducer, respectively, connected to a multichannel recording system. All analysis was performed using GraphPad™ Prism software, version 5.0®. Protocol approved by the ethics committee for animal experimentation: 009481/2011-21. **Results and Discussion**: In conscious SHR LQM 001 (0.1; 0.5; 1; 5 and 10 mg/kg, i.v, randomly), elicited immediate and dose-dependent decreases in mean arterial pressure (MAP) (-23,5 ± 3,6%; -32,9 ± 3,9%; -38,2 ± 3,8%; -49,0 ± 2,4 % and -50,3 ± 1,3%, respectively). LQM 001 presented hypotensive effect and no presented heart rate effects. The vascular effect was investigated, LQM001 ($3x10^{-8}$ - 10^{-4}M) induced vasorelaxation of a concentration-dependent manner in rings with intact endothelium, pre-contracted with Phe (10^{-6}M) (Emax = 114.19 ± 0,04% and pD2 = 6,00 ± 0.04). The relaxant effect induced was not changed in endothelium-denuded rings (Emax = 107.97 ± 0.03 % and pD2 = 5,71 ± 0.03), suggesting that the presence of the endothelium not important for vasodilator effect. To check the participation of Ca^+ channels in the mechanism of relaxation of this substance, the desnuded mesenteric rings were contracted with KCl 80 mM (Emax = 39,31 ± 1,3%). These results suggest the partial involvement of Ca^+ channels in this effect. The present study demonstrates that this substance induces vasorelaxation endothelium-independent with partial involvement of Ca^+ channels. In conclusion, we found that LQM 001 produce a hypotension effect that may be mediated in part by vasodilatation. **Acknowledgement**: UFAL, Fapeal, CNPq, Capes

01.017

Report of a novel GPCR signaling pathway: Evidences from site-directed mutagenesis at the angiotensin II AT1 receptor. Parreiras-e-Silva LT[1], Reis RI[1], Duarte DA[1], Prando EC[1], Maria AG[1], Santos GA[1], Beautrait A[2], Oliveira EB[1], Schertler GF[3], Deupi X[3], Bouvier M[2], Costa-Neto CM[1]
[1]FMRP-USP – Biochemistry and Immunology, [2]UdeM – Immunology and Cancer, [3]PSI – Biomolecular Research

Introduction: G protein-coupled receptors (GPCRs) constitute the largest family of membrane receptors and are currently known to have signaling mechanisms of higher complexity than initially thought, including G protein-dependent and independent pathways. Concerning to G protein-independent pathways it has been reported activation of signal transduction pathways by β-arrestins and transactivation of the epidermal growth factor receptor (EGFR). Findings from the last years have also reported the so-called "biased agonists", which preferentially trigger the activation of one of the pathways over the others. The angiotensin II AT1 receptor is known to trigger ERK1/2 activation by G protein-dependent and independent pathways, and is one of the prototype GPCRs in the study of biased agonists. **Methods:** In this study we engineered by site-directed mutagenesis a mutant AT1 receptor, in which a highly conserved proline residue at transmembrane 5 (Pro^{207}) was replaced by an alanine residue. Analysis of the functional profile of this mutant (P207A) was performed by intracellular calcium mobilization, direct analyses of G protein, β-arrestin 1 and β-arrestin 2 coupling by Bioluminescence Resonance Energy Transfer (BRET), pharmacological blockage of the EGRF phosphorylation, and ERK1/2 phosphorylation levels by western blotting. **Results and Conclusion:** Our data revealed that the endogenous ligand angiotensin II (AngII) behaved as a partial agonist in G protein-dependent and independent signaling pathways. More interestingly, activation of the P207A mutant by the arrestin-biased agonist Sar^{1}-Ile^{4}-Ile^{8}-AngII (SII) revealed that it was not able to trigger none of the known signaling pathways but nevertheless was still able to lead to ERK1/2 phosphorylation. We believe that this data unveils the existence of a novel GPCR signaling pathway, which still needs to be identified. **Financial support**: Fapesp, Capes, CNPq and FAEPA.

01.018

Study of monastrol-kinesin interactions by quantum biochemistry calculations. Bezerra EM[1], Diniz-Filho J[2], Pessoa CO[2], Meira AS[2], Freire VN[3], Costa RF[4] [1]UFC – Análise Clinica e Toxicologia, [2]UFC – Fisiologia e Farmacologia, [3]UFC – Física, [4]UFERSA – Física

Mitosis is a well-established, validated intervention point for cancer therapy. Diverse antimitotic drugs acting on cytoskeleton proteins are currently being used in the clinic. Despite successful reports, the coexistence of undesirable effects toward important targets, like the nervous system, poses a challenge to those therapies. On the other hand, it has been demonstrated that human Kinesin-5 (Eg5) is sensitive to small-molecule inhibitors that allosterically block its activity. Eg5 is poorly expressed in normal non-proliferating cells, while in contrast it is over-expressed in actively proliferating cells, as observed in cancer. In this sense, molecules that specifically and solely inhibit Eg5 are potential alternatives to be explored for drug development. Monastrol was uncovered as a mitotic inhibitor, thus leading to the classical monoastral form of the mitotic spindle. Although being active against Eg5, monastrol does not produce significant alterations of microtubules. Through this line of development, newly derived drugs could potentially decrease the above mentioned undesirable effects. Therefore, monastrol and other small-molecule Eg5 inhibitors, provide a useful toolbox for studies involving Eg5 and cancer. In the present work, we investigated the electronic properties of monastrol, using Gaussian09. In particular, Infra-Red and Raman spectra were explored. The corresponding assignment for molecular segments was also done using VEDA. We further used the crystallographic structure by X-ray of Eg5 co-crystallized with monastrol (from Protein Data Bank), in order to study the interactions in the ligand site. For each amino acid detected in the ligand site, we computed the energy of interaction with monastrol, using the program DMOL3 via a variant of the MFCC method (Multiple Fragmentation Conjugate Caps). The calculations were made according to the Density Functional Theory (DFT), using the local density approximation and gradient generalized (LDA). We also used the functional Perdew-Wang (PWC) with dispersion term OBS and Double Numerical plus Polarization (DNP) basis set. According to the respective residue and interaction energy value (kcal/mol), we found the following sequence: LEU214(-21.62); PRO137(-15.52); ARG119(-15.18); TYR211(-15.09); GLU116(-12.75); GLU215(-12.50). This sequence presents accurate results that could help in the rational design of more effective Eg5 inhibitors and in the combat of cancer. We thank CNPq, UFC and CENAPAD-UFC

01.019

SRC-MAP kinase pathway activation by bufalin promotes epithelial to mesenchymal transition in LLC-PK1 cells. Martins-Ferreira J, Cunha-Filho G, Quintas LEM, Noël F ICB-UFRJ

Introduction: Cardiotonic steroids (CS) are now known to bind to Na^+/K^+-ATPase located in caveolae and transduce the extracellular signal to intracellular compartments via activation of different protein kinases, including Src tyrosine kinase-Ras-ERK1/2 pathway. Consequently, they regulate cell cycle and gene expression, thus playing an important role in the control of renal and cardiac functions. Fibrosis is characteristic of the advanced stages of chronic renal and cardiac disease. Epithelial to Mesenchymal Transition (EMT) is a biological process involved in the establishment of fibrosis. One of the first signs of EMT is the endocitosis of membrane E-cadherin and impairment of cell-cell adhesion followed by gene activation of SNAIL which in turn inhibits E-cadherin and enhances mesenchymal proteins, as vimentin gene transcription, resulting in a phenotype transformation. Src kinase has been shown to contribute to EMT in cancer. Recent studies have identified both ouabain and marinobufagenin as endogenous steroids whose production and secretion are regulated by multiple physiological and pathological stimuli including angiotensin II and epinephrine in human. This study aims to characterize the mechanism involved in CS causing EMT. For this purpose, LLC-PK1 epithelial cell cultures were exposed to bufalin, a CS purified from toad venom, and EMT characteristics along with Src-Ras-ERK1/2 signaling pathway were evaluated. **Methods:** EMT characterization and signaling pathway were evaluated by phase contrast and fluorescent microscopy, high content screening microscopy, surface protein biotinylation and immunoblotting assays at 4 or 24 hours after incubation with 20 nM bufalin. **Results:** Bufalin 20 nM treatment for 4 or 24 hours increased endocytosis of E-cadherin as shown by immunofluorescence staining and a decrease of almost 45% of surface/total E-cadherin ratio in 20 nM bufalin-treated cells ($n=3$, $p<0.05$) evidenced by surface biotinylation. In 24 hours we detected a change from epithelial to fibroblast-like morphology in bufalin-treated cells, and those cells expressed a considerably higher content of stress fibers, whose formation was prevented by inhibition of Src (2 μM PP2) and MAP kinases (10 μM U0126) activation as well as changes in cell morphology. ERK1/2 activation increases around 120% in cells treated for 24 hours. **Discussion:** The Na^+/K^+-ATPase bufadienolide ligand bufalin activates Src and MAP kinase signaling pathway and causes EMT in cultured renal cells. Loss of surface E-cadherin expression is in between the first steps of EMT phenomenon and is induced by bufalin. These results indicate that CS may be a key player in cell differentiation and fibrosis common in later stages of kidney chronic disease. Defining the intracellular pathways involved in this process contribute to the development of novel pharmacological therapies. **Financial Support:** Capes; CNPq; Faperj.

01.020

Role of prostaglandin D_2 and their receptors in immunomodulatory functions of eosinophils.

Luna-Gomes T[1], Bozza PT[2], Bandeira-Melo C[1] [1]IBCCF-UFRJ, [2]IOC-Fiocruz

Introduction and objectives: Eosinophils are effector cells classically involved with allergic inflammation. However, immunomodulatory roles have been described to eosinophils. This immunomodulatory role is based in fact of eosinophils contain cytokines and chemokines pre-formed within granules. This pre-formed cytokines, with diverse biologic activities are selectively and rapidly released by vesicular transport. Prostaglandin (PG) D_2 is a key mediator of allergic inflammatory diseases, such as asthma. This eicosanoid is a cyclooxigenase product synthesized mainly by mast cells, T cells and eosinophils. The functions of PGD_2 are mediated by high-affinity interaction with two receptors, the prostanoid DP_1 receptor and the chemoattractant receptor-homologous molecule expressed on Th2 cells receptor, the DP_2 receptor. Here, we investigated the role of PGD_2 and their receptors in induce cytokines secretion and control immunomodulatory functions of eosinophils. **Methods:** *in vitro:* Human eosinophils isolated from peripheral blood of health volunteers with a negative selection kit (052/09 CEP UFRJ/HUCFF) and mouse eosinophils differentiated from mouse bone marrow cells were used *in vitro* assays. Cells were pre incubated with 25nM PGD_2, DP_1 agonist or DP_2 agonist for 1 hour 37^0C. Cytokines release of supernatants was evaluated by multiplex analyses. Values are expressed as means ± SEM of at least three distinct donors. + *P* £ 0.05 compared with control. *in vivo*: Sensitized BALB/c mice treated with PGD_2 24 hour before analyses and mouse model of eosinophilic allergy (allergic pleurisy model) pre-treated with HQL-79 (HPGDS inhibitor 1 mg/kg) were used *in vivo* assays (CEUA Fiocruz LY-32/12).The pleural fluid was centrifuged and cell free supernatants were used for the quantification of cytokines by multiplex analyses. Values are expressed as means ± SEM of at least six animals. + *P* £ 0.05 compared with control. **Results and discussion:** Using mouse model of allergy we observed that after 24 hours of challenge, the allergic stimulation induced significant increase of pleural levels of cytokines IL-6 and TNF-a, RANTES, MCP-1 and KC. The pre-treatment with HQL-79, decreased PGD_2 production, as expected, reduced production of IL-6, RANTES, MCP-1, KC and increased levels of IL-10. The administration of PGD_2 in BALB/c sensitized mice induced pleural eosinophilia, as previously described. Here, the PGD_2 treatment increased pleural levels of RANTES, MCP-1 and KC, same whose secretion is controlled by the endogenous activity of PGD_2 synthesized during eosinophilic allergic reaction. To detect if eosinophils are able to secrete cytokines in response to PGD_2, we used mouse and human eosinophils *in vitro*. We showed that mouse eosinophils stockpile IL-4 and RANTES and that the stimulation with PGD_2 induced RANTES secretion but not IL-4 secretion. To study the role of PGD_2 receptors on cytokine secretion we used specifics agonists. In human eosinophils, the DP_2 agonist ($DK\text{-}PGD_2$) induced secretion of IL-4, IFN-γ, IL-6 and TNF-α. But, lost the effect on the mobilization of MCP-1 and MIP-1α observed when both receptors are activated. Differently, use of DP_1 agonist (BW245c) induced IL-6 and TNF-α secretion and appears to be responsible for the mobilization of chemokines MCP-1 and MIP-1α. Surprisingly, unlike PGD_2 stimulation itself, selective DP_1 receptor activation was able to select IL-10 secretion of pool preformed cytokine. Our results suggest that PGD_2, are able to control eosinophilic effector functions and displays immune-modulatory functions, as showed by its ability in mediate the cytokine profile produced during allergic reactions, mechanism controlled differently by PGD_2 receptors. **Financial Support:** CNPq, Faperj, Capes, Fiocruz.

01.021

Trypanocidal effect *Dinoponera quadriceps* venom. Lima DB[1]; Mello CP[1], Tessarolo LD[1], Menezes RRPPB[2], Torres AFC[1], Pereira TP[1], Bandeira ICJ[1,] Sampaio TL.[1], Costa MFB[2], Fernandes LC[1], Quinet YP[3], Martins AMC[1] [1]UFC – Clinical and Toxicological Analysis, College of Pharmacy, [2]UFC – Physiology and Pharmacology, [3]ICB-UECE

Introduction: The investigation of the therapeutic potential of the toxins comes, increasingly, arousing interest from the scientific community as a source of molecular models for the design of new drugs. In this work, we evaluated the trypanocidal potential of *Dinoponera quadriceps* venom (DqV) aiming at discovering a pharmacological tool and / or therapeutic substance value in the treatment of Chagas disease. **Methods:** Different concentrations of DqV were incubated with cultures of epimastigotes of the Y strain of *Trypanosoma cruzi* (grown in LIT medium at 28 º C) for 24 and 48 hours [1] and different concentrations of DqV were incubated with cultures of trypomastigotes of the Y strain of *Trypanosoma cruzi* (obtained by infection of LLCMK2 cells and cultured in MEM at 37 ˚C and 5% CO2) for 24 hours [2], and then cell viability was determined by counting in a Neubauer chamber. Flow cytometry markers, Annexin V-FITC and propidium iodide (PI) was used to evaluate the type of cell death of epimastigotes forms in IC50 concentration (28.32 µg / ml) [3]. **Results:** DqV showed a dose-dependent cytotoxic effect after 24 hours (IC50 = 28.32 µg / ml) and 48 hours (IC50 = 20.67 µg / mL) of incubation to epimastigote forms. DqV also decreased the viability of tripomastigote forms, the infective form in humans (IC50 of 1.978 µg / mL). The flow cytometric analysis showed a significant increase of percentage double labeled cells with PI and Annexin V- FITC. These results suggests the presence of necrotic and apoptotic involvement in the trypanocidal effect of DqV. **Discussion:** Studies with antiparasitic activity of invertebrates venoms are scarce in the literature, but a previous work with the honeybee *Apis mellifera* venom also observed trypanocidal effect, affecting the growth, viability and structure of different forms of *T. cruzi*. Different types of cell death have been suggested in epimastigotes form and apoptotic structures in trypomastigotes. These results demonstrate the heterogeneous cell death involved with the antiparasitic effects of invertebrates' venom [4]. In a later study, the same author attributed all the effects reported above to a component exists in the *Apis mellifera* venom, the Melitin, corresponding 40-50% of venom dry weight. It follows that poisons invertebrates, although little explored, show great pharmacological potential [5]. **References:** [1] CAMARGO EP *Rev Inst Med Trop São Paulo*, v6, p93, 1964. [2] GONÇALVES AR *Parasitol Res*, v88, p598, 2002. [3] DEOLINDO, P. *Mem Inst Oswaldo Cruz*, v 100, p. 33, 2005. [4] ADADE, CM. *Parasitology*, v11, p1444, 2012. [5] ADADE, CM. *Toxicon*, v69, p227, 2013.

01.022

The absence of 5-lipoxygenase impairs alveolar bone loss in an experimental periodontal disease by *Aggregatibacter actinomycetemcomitans*. Madeira MFM[1], Queiroz-Junior CM, Correa JD[2], Machado FS[3], Soriani FM[4], Cunha TM[5], Garlet GP[6], Teixeira MM[7], Silva TA[2], Souza DG[8] [1]UFMG – Patologia Oral/Microbiologia, [2]UFMG – Patologia oral, [3]UFMG – Bioquímica, [4]UFMG – Genética/Imunofarmacologia, [5]USP – Farmacologia, [6]USP – Ciências Biológicas, [7]UFMG – Bioquímica/Imunofarmacologia, [8]UFMG – Microbiologia

Introduction: Periodontal disease (PD) is a chronic inflammatory infectious disease that affects the attachment structures of the teeth. The oral subgingival biofilm, in close association with periodontal tissues, is the etiologic factor of PD. *Aggregatibacter actinomycetemcomitans* (*Aa*) is a Gram-negative periodontophatogen with a range of potential virulence factors, including its LPS (*Aa*LPS). Inflammatory mediators are involved in the pathogenesis of PD, among them arachidonic acid metabolites, such as leukotrienes. The enzyme 5-lipoxygenase (5-LO) catalyzes the conversion of arachidonic acid in leukotriene B4 (LTB4), a potent lipid mediator with various biological activities mediated by specific cell surface receptors (BLTs). **Objective:** In the present work, our aim was to evaluate the role of 5-LO in the experimental model of PD induced by *Aa*. **Methods:** C57BL6 wild type (WT) or 5-LO deficient ($5LO^{-/-}$) mice received a direct injection of 1 x 10^9 CFU/mL of *Aa* strain FDC Y4, diluted in PBS, into palatal gingival tissue. Immediately after, was performed an oral administration of the same inoculum with 1.5% of carboxymethylcellulose. The protocol was repeated 48 and 96 hours later. Negative controls received only PBS (NI). After 30 or 60 days of infection, animals were euthanized and tissues removed, processed and analyzed in order to evaluate the levels of cytokines and chemokines, myeloperoxidase activity, histology, neutrophil influx and alveolar bone loss. *in vitro*, RAW 246.7 cells activated by *Aa*LPS were used to evaluate osteoclast differentiation and activity in the presence of CP 105696 (BLT1 antagonist), Montelukast, LTB4 or PAF. **Results:** WT mice presented increased myeloperoxidase activity ($p<0.01$) and IL-6 production ($p<0.001$). Furthermore, WT mice presented significant alveolar bone loss after 30 ($p<0.01$) and 60 days ($p<0.001$) of infection. $5LO^{-/-}$ mice presented no significant increase of myeloperoxidase activity, when compared to WT mice. Interestingly, although a significant production of IL-6 ($p<0.05$), no significant alveolar bone loss or TRAP-positive cells were observed in $5LO^{-/-}$ mice. Furthermore, the differentiation and activity of osteoclasts stimulated with *Aa*LPS were diminished in the presence of CP105696 but not in the presence of Montelukast, assessed by the number of TRAP-positive cells, even in the presence of PAF. Moreover, levels of LTB4 were increased when osteoclast-like cells were cultured with PAF. **Conclusion:** 5-LO appears to develop an important effect on the pathogenesis of PD and this effect seems to be related to the pro-inflammatory product from 5-LO pathway, the LTB4. This study shows that LTB4 is important to the *Aa*-induced alveolar bone loss by impairment in neutrophil recruitment and directly affecting osteoclast differentiation and activity and that the production of LTB4 in osteoclasts is induced by PAF. This study was approved by CETEA/UFMG nº 256/2008 and supported by Capes, CNPq and FAPEMIG.

01.023

Evaluation of the effects of lectins in pancreatic acinar cells stimulated by bile salts and alcohol: involvement of cell death. Pantoja PS[1], Damasceno SRB[2], Franco AX[2], Morais CM[2], Girao DKFB[2], Oliveira TB[2], Mendes TS[2], Lima FRF[2], Mendonça VA[1], Silva KES[1], Lima MAS[1], Souza MHLP[2], Soares PMG[2] [1]UFC – Biomedical Sciences, [2]UFC – Physiology and Pharmacology

Introduction: Acute pancreatitis features pancreatic necrosis and multiple organ failure requiring intensive care. Lectins are molecules of great use in experimental models. The aim of this work was evaluate the effects of lectins in pancreatic acinar cells stimulated by bile salts and alcohol as well as the involvement of cell death in this process. **Methods:** Protocol was approved in the local ethics committee (nº 99/2013). Pancreatic acinar cells were isolated with collagenase from pancreata of Swiss mice (8–12 week old). Experiments were performed at room temperature and cells used within 4 hours of isolation. The extracellular solution contained (in mmol/L): 140 NaCl, 4.7 KCl, 1.13 $MgCl_2$, 1 $CaCl_2$, 10 D-glucose, and 10 HEPES (adjusted to pH 7.35). Cells were pretreated with Concanavalin A (Con A, 200 µg/ml) and Concanavalin Br (Con Br, 200 µg/ml) with 1 hour of incubation, and then were incubated for a half hour with bile acid (taurolithocholic acid sulphate; TLC-S, 500 µM) or alcohol (850 mM). To assess the participation of its specific binding sugars, lectins were incubated for 1h at 37 ºC with the binder sugar (mannose) or a non-binding sugar (galactose). The cell death pathways were detected with propidium iodide (PI); after, cells were washed and resuspended in Ca2+ free buffer with the nuclear stain Hoechst 33342 and observed in the Confocal microscope Olympus®. To evaluate the interaction of lectins with pancreatic cellular structures, cells were incubated with Con A and Con Br linked to fluorophore fluorescein isothiocyanate (FITC). **Results and discussion:** The group of cells treated only with TLC-S or alcohol showed a high level of necrosis (75.5 ± 3.2% and 64.6 ± 4.6%, respectively). Pretreatment of the cells with lectins significantly decreased the percentage of necrosis (Con A: 38.5 ± 2.4 % versus TLC-S group and 19.68 ± 3.1% versus alcohol group; Con Br: 38.5 ± 1.5 % versus TLC-S group and 24.1 ± 2.9% versus alcohol group). When lectins were preincubated with the binder sugar mannose, its effect of preventing necrosis caused by TLC-S (72.5 ± 3.2%) was abolished (Con A 80.0 ± 3.6% and Con Br 66.6 ± 4.0%) when compared with the not linked to the mannose lectin group (Con A 38.5 ± 2.4% and Con Br 38.5 ± 1.5%), showing that the cell-lectin interaction is possibly dependent binding to sugar. To confirm this effect, another experiment was performed with galactose, a non-binding sugar of Con A and Con Br. The results showed that the binding of lectins with galactose did not alter the anti-necrotic effect of Con A and Con Br. The images obtained by confocal microscopy of cells labeled lectin/FITC showed binding of concanavalin Br in pancreatic cell membrane and binding of concanavalin A to intracellular structures. These results suggest that Con A and Con Br protect pancreatic acinar cells from necrosis caused by harmful agents, possibly due to its interaction with sugars present in the cell. **Financial Support:** CNPq.

01.024

Zymosan promotes NLRP3/ASC/Capsase-1 inflammasome activation by a phagocytosis-independent mechanism. Silva RL[1], Lopes AHP[1], Zamboni DS[2], Cunha FQ[1], Cunha TM[1] [1]FMRP-USP – Pharmacology, [2]FMRP-USP – Cell Biology

Introduction: Zymosan (ZY) is an insoluble preparation of cell wall from *Saccharomyces cerevisiae*. It is widely used to study the role of innate immune system in the recognition of microbes and to induce inflammatory response in pre-clinical studies. The initial recognition of ZY by immune cells is mediated by pattern recognition receptors (PRR), e.g. TLR-2 and Dectin-1, which induce the release of several cytokines, including IL-1β. The production of mature IL-1β depends on two stages: 1) the production of pro-IL-1β, which is triggered by PRRs activation, 2) and IL-1β maturation, which is dependent on inflamassome activation (an intracellular multi-protein complex). However, which inflammasome component is involved in the maturation of IL-1β triggered by ZY is still unclear. Thus, the aim of the present study was to investigate the molecular mechanisms by which ZY promote IL-1β maturation in macrophages focusing in the role of inflammasome. **Methods:** Peritoneal naive macrophages (MΦ) were harvested from C57BL/6 wide type (WT), NLRP3$^{-/-}$, NLRC4$^{-/-}$, ASC$^{-/-}$, Caspase-1$^{-/-}$ mice and cultured at 5% CO_2, 37 °C. They were incubated with ZY (10-100 µg/mL) from 6 to 12 hours. Culture cells supernatants were used to measure the levels of IL-1β and TNF-α by ELISA. ZY-FITC complex was used to verify the inhibition of phagocytosis using fluorescence microscopy. This study was approved by Local Ethical Commission in Animal Research: Protocol n° 077/2012. **Results:** Initially, we observed that ZY (10-100 µg/mL) promotes the release of active IL-1β from WT MΦ in a concentration-dependent manner. The maximum release of IL-1β was observed in the concentration of 30 µg/mL, without difference between 6 and 12 h after incubation. In contrast, IL-1β release was severe impaired when MΦ obtained from NLRP3$^{-/-}$, ASC$^{-/-}$ or caspase-1$^{-/-}$ mice. On the other hand, TNF-α release by MΦ from NLRP3$^{-/-}$, ASC$^{-/-}$, caspase-1$^{-/-}$ or NLCR4$^{-/-}$ mice stimulated with ZY was similar to WT MΦ. ZY-induced IL-1β release in WT MΦ, but not TNF-α, was completely inhibited by KCl (50 mM, which blocks K^+ efflux), and glyburide (100 µM, a potassium channel inhibitor). However, IL-1 β release was not affected by BAPTA (1 mM, an extracellular calcium chelator). Interestingly, ZY induced-IL-1β release was not inhibited by apyrase (2-20 U/mL; a ATPase), carbenoxolone (50 µM, a pannexin-1 channel inhibitor) or by cytochalasin D (CytD, 5 µM; a phagocytosis inhibitor). Importantly, this concentration of CytD was able to reduce the phagocytosis of ZY. **Conclusions:** The present results indicate that ZY induced the production and maturation of IL-1β though a mechanism dependent on NLRP3/ASC/caspase-1 inflammasome assembling, which seems to be independent on phagocytosis. Moreover, NLRP3 activation by ZY seems to be independent on extracellular ATP and pannexin-1 channels, different from many other NLRP3 activators. Based on our data, we suggest that ZY induced NLRP3 activation could be triggered by an intracellular signal, since ZY phagocytosis is not essential to IL-1β release. Supported by CNPq, Fapesp.

01.025

Cardiovascular effects *in vitro* of a selective and potent agonist for the BK potassium channel. França PL[1], Barenco TS[1], Maciel L[2], Nascimento JH[2], Bendhack LM[3], Suarez-Kurtz G[4], Ponte CG[1] [1]NCB-IFRJ, [2]IBCCF-UFRJ – Eletrofisiologia Cardíaca, [3]FCFRP-USP – Farmacologia, [4]InCA – Farmacologia

Introduction: Arterial Systemic Hypertension (ASH) and Pulmonary Hypertension (APH) remain undertreated in many individuals and may involve vascular smooth muscle hyperactivity. Considering the pivotal role of the high conductance BK potassium channels in controlling the vascular smooth muscle tonus, the modulation of this channel by new agonists is referred to in the literature as having a great potential as a therapeutic strategy, and has attracted the interest of the pharmaceutical industry worldwide. Our group has characterized a family of tetrahydroquinolines which are able to activate the BK channel and, among the various analogues tested, a compound named "Z" has been found to be the most potent and selective. For future experiments to evaluate the therapeutic potential of compound Z for the treatment of HSA and APH in animal models initially employ some experimental models *in vitro* to assess the effects of the compound Z on the cardiovascular system (CS). **Methods:** Modulation by compound Z of the CS muscle tissue (aorta, mesenteric arteries, portal vein and isolated heart) was tested using tissue samples from male Wistar rats (200 – 250 g). The various tissues were dissected and assembled on several different incubation chambers filled with Krebs–Henseleit solution at 37°C, aerated with a carbogenic mixture (95% O_2 and 5% CO_2) for recording muscular activities and cardiac function. For the records of contractile force of the aorta, portal vein and the second branch of the mesenteric artery, we used the Mulvany-Halpern myograph. With the perfusion of the mesenteric arterial bed isolated evaluate peripheral vascular resistance and the Langendorff system to evaluate left ventricular function and electrocardiographic parameters (CEUA UFRJ – Protocol IBCCF156). **Results:** In the artery models, the aortic tension, the tension of the second branch of the mesenteric and the resistance to the flow in the mesenteric arterial bed was reduced by compound Z (IC_{50} = 3.4, 1.1 and 6.8 μM, respectively). In the portal vein model system, relaxation was observed as well (IC_{50} = 1.6 μM). Regarding the cardiac function, compound Z did not significantly alter neither the electrocardiographic parameters nor the left ventricle function in concentrations up to 10 μM. In the tissues in which compound Z caused relaxation, the addition of the selective BK channel blocker (IbTX) promoted complete reversion of the relaxant effects. **Discussion:** The results obtained *in vitro* are encouraging since they suggest that compound Z could potentially reduce the systemic arterial pressure by reducing the peripheral vascular resistance, in addition to a reduction of the cardiac preload without causing any significant cardiotoxicity. In summary, our results indicate that compound Z is a promising drug candidate to be tested in the regulation of the arterial pressure and should, therefore, be tested *in vivo* in rat models of ASH and APH. **Support:** Faperj, INCA-FAF and IFRJ.

01.026

Antioxidant activity of novel derivative of ferulic acid on *Saccharomyces cerevisiae* model.

Rêgo SC[1], Taimo MRD[2], Gomes Junior AL[2], Mata AMOF[1], Alencar MVOB[2], Araruna Junior AA[3], Santos JSB[1], Freitas RM[2], Cavalcante AACM[2] [1]UNINOVAFAPI, [2]UFPI, [3]FSA

Introduction: There is a great interest in studies involving free radicals, which are produced by distinct environmental chemicals as well as by endogenous metabolism (FARIAS, K.S., *Rev Bras Plant Med*, v. 15, p. 520, 2013). Excess of radicals in the body induces oxidative stress and damage lipids, proteins, DNA and carbohydrates, findings related to the emergence of chronic diseases such as cancer, atherosclerosis, cataracts, Alzheimer and diabetes. However, these radicals can be avoided by consumption of foods or substances with antioxidant properties, which has the role to remove these chemical species (GUARIENTI, C., Rev. Inst. Adolfo Lutz, v. 69, p. 107, 2010). Among the methods for determining the antioxidant property, tests with eukaryotic microorganisms such as *Saccharomyces cerevisiae* are frequently used, since its metabolism may be associated with that of higher eukaryotes. This property is determined by survival of yeasts treated with an analyte and oxidizing substance, such as hydrogen peroxide (H_2O_2) (GUARIENTI, C., RBCEH, v. 7, p. 144-150, 2010). This study evaluated the antioxidant activity of a derivative of ferulic acid on proficient and deficient *S. cerevisiae* strains in enzymatic defenses. **Methodology**: Petri dishes (pretreatment, co-treatment and post-treatment), which were seeded with proficient (Superoxide dismutase wild) and deficient *S. cerevisiae* strains (Superoxide dismutase cytoplasmic, mitochondrial superoxide dismutase, superoxide dismutase and cytoplasmic was conducted mitochondrial cytoplasmic superoxide dismutase and catalase, and only the catalase) were exposed to concentrations of 83.3, 125, 166.6 and 250 mg/mL of a novel ferulic acid and H_2O_2. Zones of inhibition were measured (mm). Negative control was exposure to NaCl 0.9%. Positive control was represented yeast cells treated by H_2O_2. Results were statistically significant if $p<0.05$. **Results and discussion:** The derivative revealed antioxidant activity at all concentrations tested against several strains of *S. cerevisiae*.

01.027

Extracellular cAMP induces contraction of airway smooth muscle via adenosine formation and activation of adenosine receptors. Pacini ESA, Godinho RO Unifesp-EPM – Farmacologia

Introduction In the airway smooth muscle, activation of receptors coupled to stimulatory G protein (Gs) triggers relaxation signaling by activating adenylyl cyclase/cyclic AMP (AC/cAMP) axis. In many other cells, including skeletal muscle, cardiac fibroblasts and glomerular mesangial cells, intracellular accumulation of cAMP is followed by its efflux via a probenecid-sensitive transporter. At the extracellular compartment, cAMP is sequentially converted to AMP and adenosine by ecto-phosphodiesterases and ecto-5'-nucleotidases, respectively. In those cells, this signaling cascade known as "extracellular cAMP-adenosine pathway" modulates the primary GsPCR signaling. Taking into account the importance of GsPCR such as β_2-adrenoceptors, for the regulation of airway smooth muscle contraction, in the present study we analyzed the existence of a functional "extracellular cAMP-adenosine pathway" in the rat tracheal smooth muscle. **Methods** Tracheal rings obtained from 3-4 month-old male Wistar rats were mounted in an organ bath tissue containing Krebs-bicarbonate solution at 37°C, 95% O_2/5% CO_2 under optimal resting tension (1 g). After 1 h stabilization, tracheal rings were challenged with 1 µM carbachol, rinsed and after 30 min incubated with the following drugs: a) 100-300 µM adenosine, b) 100-1000 µM cAMP alone or c) in the presence of inhibitors of adenosine uptake (uridine) and adenosine deaminase (EHNA) or d) in the presence of ecto-5'-nucleotidases inhibitor AMP-CP (n = 3-12). The isometric contraction force was collected and analyzed using PowerlabChart 8 software and data were expressed as mean ± S.E.M. *Number of the Animal Ethics Committee*: CEUA N° 9987150714 **Results** 100 to 1000 µM cAMP induced a phasic contraction of rat tracheal rings that reached up to 13.6 ± 2.9 mg of tension. The contraction induced by 1 µM carbachol reached 482 ± 20 mg of tension. The contracting effect of cAMP was mimicked by adenosine. While EHNA and uridine alone were not able to induce contraction, pre-incubation of tracheal rings with this cocktail inhibitors increased by up to 373% the cAMP-induced contractile response. Conversely, pre-incubation of tracheal rings with AMP-CP, an inhibitor of ecto-5-'nucleotidase, reduced by 80% the cAMP-induced contraction, indicating that cAMP effect depends on extracellular adenosine availability. **Discussion** The present results support the existence of "extracellular cAMP-adenosine pathway" in rat trachea. The inhibition of cAMP-induced contraction by AMP-CP revealed a physiological role for ecto-5'-nucleotidases in regulating extracellular adenosine nucleotide content and, as consequence, the extracellular adenosine levels. Furthermore, inhibition of adenosine uptake and adenosine deaminase increased the cAMP-induced contractile responses supporting the idea of cAMP as an extracellular source of adenosine. Thus, considering that cAMP is not able to cross plasma membrane, the present study indicates that tracheal smooth muscle contraction induced by cAMP depends on extracellular generation of adenosine and perhaps on the activation of adenosine receptors. **Financial Support:** Capes, CNPq and Fapesp.

01.028

Cyclic AMP – adenosine pathway modulates skeletal muscle contraction induced by electrical stimulation of peripheral nerve. Duarte T, Godinho RO Unifesp-EPM – Farmacologia

Introduction: Adenosine is an endogenous purine nucleoside that acts as neuromodulator of the central and peripheral synapses. Interestingly, in the skeletal neuromuscular system, cAMP represents an important source of extracellular adenosine. We have shown that direct or receptor-dependent activation of skeletal muscle adenylyl cyclase (AC) increases the generation of intracellular cAMP which is followed by the cyclic nucleotide efflux (Godinho and Costa-Jr, *Br J Pharmacol* 138: 995-1003, 2003). Outside the muscle cell, cAMP is sequentially metabolized by ecto-phosphodiesterases and ecto-nucleotidases into AMP and adenosine (Chiavegatti T, *et al*., *Br J Pharmacol* 153: 1331-40, 2008), which in turn is able to stimulate postsynaptic A_1 adenosine receptors (A_1R) associated to negative inotropic effects (Duarte T, *et al*., *J Pharmacol Exp Ther*. 34: 820-8, 2012). Although, adenosine-dependent activation of pre-synaptic A_1 and A_{2A} receptors subtypes has been associated with reduced ACh release from motor neurons and as a result with attenuation of muscle contraction (Garcia N, *et al*., *Eur J Nerurosci*. 38: 2229-41, 2013), it is not clear whether *the extracellular cAMP-adenosine pathway* is able to modulate skeletal muscle contraction triggered by pre-synaptic mechanisms. **Methods:** In order to investigate the influence of *the extracellular cAMP-adenosine pathway* on the nerve-evoked skeletal muscle contraction, we analyzed the effects of cAMP (100 μM), adenosine (100 μM, adenosine), clenbuterol (100 nM; β_2-AR agonist), CGS 15943 (1 μM, non-selective AR antagonist) and d-tubocurarine (1 μM, acetylcholine receptor inhibitor) on the isometric contractility of adult mouse diaphragm muscle induced by electrical stimulation (0.1 Hz, 2 ms duration) of phrenic nerve, (n=3-4). *Number of the Animal Ethics Committee* (CEP n. 0022/12). **Results and discussion:** Incubation of phrenic-diaphragm preparation with 100 μM cAMP resulted in a 10% negative inotropic response. A negative inotropic effect was also observed in phrenic-diaphragm preparation incubated with 100 μM adenosine. Preincubation of phrenic-diaphragm with CGS-15943 (1 μM) prevented the cAMP-induced negative inotropic response, indicating that extracellular cAMP modulates nerve-evoked muscle contraction via activation of adenosine receptors. In order to evaluate whether cAMP efflux/*extracellular cAMP-adenosine pathway* could be triggered by receptor-dependent activation of adenylyl cyclase, nerve-muscle preparation was incubated with 100 nM clenbuterol. The β_2-AR agonist increased by 18% the diaphragm contraction force induced by electric stimulation of phrenic nerve. The maximal positive inotropic effect was followed by a descending phase, which was inhibited by CGS-15943. At the end of all experiments, incubation of 10 μM d-tubocurarine abolished diaphragm contraction which ensured that it was mediated by ACh-dependent activation of postsynaptic nicotinic receptors. In summary, our results demonstrate that *the extracellular cAMP-adenosine pathway* modulates nerve-evoked skeletal muscle contraction through activation of adenosine receptors. This pathway is also elicited by activation of postsynaptic β_2-AR, indicating that it may function as a paracrine signaling that creates a regulatory feedback loop between muscle fibers and motor neurons. **Financial Support:** Capes, CNPq and Fapesp.

01.029

Chronic treatment with fluoxetine modulates vascular sympathetic responses by mechanisms that involve inhibition of norepinephrine synthesis/reuptake and increased nitric oxide generation. Pereira CA[1], Ruginsk SG[2], Mestriner FLAC[1], Antunes-Rodrigues J[2], Resstel LB[1], Tostes RC[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Fisiologia

Introduction: Fluoxetine, a selective serotonin reuptake inhibitor, has properties that go beyond its antidepressant effects, such as inhibition of receptors and ion channels involved in the regulation of vasomotor tone (Ni *et al*., *Proc Natl Acad Sci USA*, 94: 2036, 1997; Pancrazio *et al*., *J Pharmacol Exp Ther* 284: 208, 1998) in different types of arteries (Pacher *et al*., *Br J Pharmacol* 127: 740, 1999; Ribback *et al*., *J Physiol Pharmacol* 63: 119, 2012). Fluoxetine has been shown to increase blood pressure (Crestani *et al*., *Eur J Pharmacol* 670: 527, 2011) and sympathetic tone (Lazartigues *et al*., *Fundam Clin Pharmacol* 14:443, 2000). Whereas many studies focused on the acute effects of fluoxetine in the vasculature, chronic effects of this SSRI are still limited. **Objective**: The aim of this study was to evaluate vascular responses to sympathetic stimulation after chronic treatment with fluoxetine. **Methods**: *Wistar* rats, 230-270 g, were treated with: (i) vehicle (Veh, water for 21 days) or (ii) fluoxetine (Fluox, 10 mg/kg/day for 21 days in the drinking water). Vascular reactivity was determined in mesenteric arterial bed (MAB) according to McGregor (McGregor, *J Physiol* 177: 21, 1965). Dose-response curves to phenylephrine (PhE) in the absence and presence of L-NAME, to potassium chloride (KCl) in the absence and presence of Prasozin and frequency-response curves to periarterial nerve stimulation (PNS) in the absence and presence of desipramine were performed. Nitric oxide (NO) levels, NO synthase (NOS) activity, tyrosine hydroxylase (TH) and endothelial NOS (eNOS) expression were determined by chemiluminescence, immunohistochemistry and western blot analyses. Data are presented as (mean ± standard error of mean, Veh *vs*. Fluox, N). **Results**: Chronic treatment with fluoxetine decreased vasoconstrictor response to PhE (pD_2, 8.01 ± 0,15 *vs*. 6,94 ± 0,13; n=4-5, $p<0.05$), but not to PNS. KCl-induced vasoconstriction was augmented in MAB of fluoxetine group when compared to vehicle (Emax, 30.2 ± 2.4 *vs*. 44.9 ± 6.4; n=7; $p<0.05$), but prasozin abolished the differences in contractile responses. Fluoxetine treatment significantly decreased TH expression (7269 ± 499 *vs*. 3921 ± 981; n=3-5, $p<0.05$). Desipramine increased PNS-induced contractile responses in the vehicle-treated group, but not in the fluoxetine group (F_{50}, 14,5 ± 4,0 *vs*. 12,8 ± 4,2; n=5, $p<0.05$). L-NAME significantly increased vasoconstrictor responses to PhE in both groups (pD_2, 8,4 ± 0,12 *vs*. 7,5 ± 0,9; n=5-7, $p<0.05$). Fluoxetine increased vascular nitrate levels (1.2 ± 0.13 *vs* 2.0 ± 0.13 µM/µg, $p<0.05$), NOS activity (Fluox= 76%) and eNOS phosphorylation [arbitrary units, 0.36 ± 0.10 *vs*. 1.2 ± 0.08, $p<0.05$]. **Conclusion**: Chronic treatment with fluoxetine negatively modulates sympathetic responses possibly by mechanisms that involve inhibition of norepinephrine synthesis/reuptake and increased NO generation. **Financial support**: Capes, CNPq. Approved by the Ethics Committee on Animal Experimentation of Ribeirao Preto Medical School (013/2013).

01.030

The role of TLRs and NLRs in carrageenan-induced TNFα and IL-1β production by macrophage. Lopes AHP[1], Silva RL[1], Talbot J[1], Cunha FQ[1], Zamboni DS[2], Couillin I[3], Ryffel R[3], Cunha TM[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Biocel, [3]CNRS – Immunologie et Neurogénétique Expérimentales et Moléculaires

Introduction: Carrageenan (Cg) is an algae carbohydrate that is widely used as food additive. Interestingly, Cg can induce local inflammation and have been used in animal models of acute inflammation. However, the mechanisms involved in Cg-induced inflammation are not clear. In the last decades, pattern recognition receptors (PRRs) have become the most important molecules of the innate immune system. Among these receptors, Toll-like receptors (TLRs) and NOD-like receptors (NLRs) are the well characterized. Whereas TLRs seems to be important in the production of pro-inflammatory cytokines (TNFα, IL-1β), NLRs seems to be involved in the formation of intracellular protein complexes called inflammasomes, which in last instance promote caspase-1 activation and the maturation of pro-IL-1β into active IL-1β. Thus, in the present study, the role of PRRs and NLR in Cg-induced activation of innate immune system was investigated in an *in vitro* model of macrophage (MΦ) cell culture. **Methods**: Naïve peritoneal MF were isolated from male C57BL/6 mice (WT) and from mice genetically deficient to $TLR4^{-/-}$, $MyD88^{-/-}$, $TLR2^{-/-}$, $TRIF^{-/-}$ $P2X7^{-/-}$, $NLRP3^{-/-}$, $Casp1^{-/-}$ and adaptor molecule $ASC^{-/-}$ (Ethical committee process CETEA-FMRP n. 149/2011). The MF were stimulated with Cg (100-300 µg/ml) and the supernatant was removed in attempt to measure IL-1β and TNF using ELISA. Cells were pretreated with carbenoxolone (CBX-pannexin inhibitor), A740003 (P2X7 antagonist) and cytochalasin (CytD; phagocytosis inhibitor). **Results:** Firstly, the incubation of macrophage with Cg induced the production of IL-1β and TNFα in a concentration and time (6 – 12h) dependent manner. Cg-induced IL-1 β and TNFα production was not observed in macrophages derived from $TLR4^{-/-}$ mice, but was enhanced in $TLR2^{-/-}$ derived macrophages. Interestingly, the production of IL-1β was not reduced in macrophage from $MyD88^{-/-}$ mice, but it was in $TRIF^{-/-}$ derived macrophages. The production of mature IL-1β, but not of TNFα, triggered by Cg was also reduced in macrophages form $Caspase\text{-}1^{-/-}$, $NLRP3^{-/-}$ and $ASC^{-/-}$. The treatment with A740003 and CBX, but not with CytD also reduced the production of IL-1β. Corroboration, the production of IL-1β was also reduced in macrophages from $P2X7^{-/-}$ mice compared with WT macrophages. **Conclusion**: The present results demonstrate that carrageenan-induced macrophage production of TNFα and IL-1β in a TLR4-dependent manner. Whereas TNFα is dependent on MyD88-dependent signaling, IL-1β production is triggered by $TRIF^{-/-}$ signaling pathway. The maturation of IL-1β is dependent on NLRP3/ASC/caspase-1 inflammasome assembling which seems to be activated by P2X7/pannexin pathway. On the other hand, phagocytosis of carrageenan is not necessary for IL-1β production. In conclusion, these results elucidate the molecular mechanisms involved in the activation of innate immune system by one of the most used inflammatory stimulus. **Financial support**: Capes, Fapesp.

01.031

Investigation of antitumoral effect of new pyrimidobenzimidazoles in human melanoma cell lines. Azevedo JG[1], Amaral SS[1], Maria-Engler SS[2], Lenz G[3], Wink MR[1] [1]UFCSPA, [2]USP, [3]UFRGS

Melanoma treatment options are surgery for early stages, chemotherapy, immunotherapy and radiotherapy for late stages. Even for the more advanced treatments, such as immunotherapy, resistance is almost certain. Thus, the aim of this study was to investigate a possible antitumor action of a series of seven different pyrimidobenzimidazoles (PBZ 01-07) in human melanoma cell lines, focusing on long term effects and combinations with current used drugs. Treatment for 120 hours and analysis of cell viability with MTT lead to IC_{50} of 3.2 µM and 4.0 µM, for the two more potent compounds, PBZ01 and PBZ04, respectively, for SK-Mel-103. For the less aggressive cell line SK-Mel-28, an IC_{50} of 9.7 µM and 9.6 µM was obtained for PBZ01 and PBZ04, respectively. Treatment of the cells for 5 days, followed by cumulative population doubling analysis for the next 28 days showed that PBZ01 and PBZ04 showed similar potency, as given by the number of cells after 28 days, when compared to the standard drug used to treat melanomas, dacarbazine (DTIC). Importantly, PBZ04 significantly potentiated the effect of DTIC in SK-Mel-28 after 28 days. The results demonstrate that molecules derived from PBZ are able to reduce long term survival of melanoma cells as a single treatment, but more importantly, act together with DTIC to reduce the number of cells and therefore is a good candidate to reduce the probability of melanoma resistance. Human Ethics Committees: project 865/11, 1507/1. **Financial support**: CNPq, FAPERGS, Capes.