01. Cellular and Molecular Pharmacology

01.001 Antineoplastic beta lapachona is not cytotoxic in low concentration in endothelial cells. Alves NM, Cruz VDSC, Graziani DG, Braga KMDSB, Paixão FMDP, Araújo EG UFG

Introduction: The beta lapachone(βLP)is a compound isoleted from sawdust Ipê's wood extraction, that is antibacterial, antifungist and antiretroviral. The ßLP mechanism of action inclued sulfhydryl groups alkylation from enzymes, apoptosis and new oxigen-reactive species development. Previous studies demonstrated that βLP has a cytotoxic activity in neoplasic cells lineage. **Objectives:** Appraising βLP extract issues in cultivated endothelial cells lineage of umbilical cord. **Methods**: TheβLP extract was purchased from Santa Cruz Biotechnology (Dallas, Texas, EUA). The test compounds were dissolved in DMSO (Dimethyl Sulfoxide, Cultilab, Campinas, Brazil), in concentration 1,0 mM and kept at a temperature of -4ºF. The lineage EA. hy926 was bought from "Banco de Células do Rio de Janeiro" (UFRJ – Rio de Janeiro, Brazil) came from ATCC (American Type Culture Collection - Manassas, VA, USA). That cells was cultivated in medium culture Dulbecco modified from Eagle (DMEM) increased by fetal bovine serum 10% (SFB), penicillin and streptomycin(10. 000 U. I. /ml - 10 mg/ml), 1% amphotericin B and 1% L-glutamine (all Cultilab reagents, Campinas, Brazil) and keptin a humidified incubator at 98,6ºF with a atmosphere with 5% of CO2. During culture, the medium was changed every two days until the cells reached 90% minimum confluency. Cells were transferred to 96-well plate and cultured for 24 hours at a concentration of 1x10 4 cells/well, in a humidified incubator at 98,6ºFand 5% CO2 atmosphere. βLP treatments were done for 24 hours at the dosages of 0. 1 µM, 1 µM, 10 µM and negative control group, free of extract. At the end of each treatment period, the medium was discarded and the cells were evaluated by the cell viability assay by the tetrazolium reduction method with addition of 10µl MTT (3-(4,5-dimethyl-2-thiazolyl) 5-diphnyl-2H-tetrazolium) in each well. The plates were incubated for three hours. In order to complete the reaction, 40 µl of sodium dodecyl sulfate (SDS – Vivantis Biochemical) diluted in HCl (0. 01 N) per well was added and the plates were incubated for 24 hours at room temperature. The optical density was quantified in spectrophotometer (Awareness Technology Ine/ Stat Fax 2100, 532nm). The calculation of cytotoxicity was done using the formula: Data were presented in this study as mean and standard error. Statistical comparisons were performed using one-way analysis of variance (ANOVA). Differences were considered statistically significant with $p < 0.05$. Statistical analyzes were performed using GraphPad Prism 5 software (GraphPad Software, Inc. , La Jolla, CA, USA). **Results:** The following absorbance averages were obtained between the three concentrations: 0. 1 µM, 1 µM, 10 µM, respectively: 0. 2155; 0. 0360; 0. 0363. The percentage of cell viability was determined by a calculation [VC%: (absorbance of treatment / absorbance of control group) x 100], resulting: 131. 40 (0. 1 µM), 21. 95 (1 µM), 22, 15 (10 µM). Following the same order of concentrations, the cytotoxicity determination (CT: [100 - treatment absorbance / control group absorbance x 100]) was performed, yielding: (0. 1 µM), 78. 05 (1 µM) and 77. 85 (10 µM). All βLP concentrations tested in this assay presented a statistically significant difference compared to the negative control and between each of the means. The concentration of 0. 1 µM βLP did not show cytotoxicity on endothelial cell lines by the MTT assay. In contrast, the concentration of 0. 3 µM β-lapachone was cytotoxic (64. 81%) for canine osteosarcoma cells, inducing apoptosis and promoting the arrest of CRUZ cell cycle, 2018. By comparing these results, it is possible suggest that β-lapachone presented selective capacity for cytotoxic action at low concentrations, differentiating endothelial cells from umbilical cord and canine osteosarcoma cells. This result is very promising and instills the need for the use of assays that can validate this possibility. **Conclusion:** βLP shows no cytotoxicity at low concentration in umbilical cord endothelial cell line.

01.002 Atorvastatin blocked UTP-induced metastatic prostate cancer cells to endothelial cells. Cardoso TC, Silva CLM UFRJ

Introduction: Prostate cancer is the second most prevalent type of cancer in men in the United States, Europe and Brazil (Bray *et al.* , Lancet Oncol. 13: 790-801, 2012). Tumor metastasis is the main cause of cancer related death. Understanding the molecular mechanisms underlying tumor metastasis is crucial to control this fatal disease (Maishi and Hida, Cancer Sci. 108(10): 1921–1926, 2017). The mutual interaction of prostate cancer cells and endothelial cells (EC) may be associated with higher incidence of metastatic cancer (Wang *et al*. , Mol Cancer Ther; 12: 6, 2013). ATP is released at high levels from malignant cells and can act as potent prometastatic factors (Ferrari *at al*. , Trends Pharmacol Sci. 38(3): 277-290, 2017; Allard *et al*. , Immunol Rev 276: 121–144, 2017) and is known to modulate a variety of processes linked to endothelial cell activation. $P2Y_2$ receptor ($P2Y_2R$) activation by ATP or UTP markedly induced ICAM-1 and VCAM-1 expression in ECs, which may play an important role in cancer cell adhesion to ECs (Jin *et al*. , Breast Cancer Res. 26;16(5): R77, 2014). Numerous non-lipid modifiable effects of statins termed as pleiotropic effects of statins, could be beneficial for the treatment of various devastating disorders, such as improving endothelial dysfunction (Bedi, *et al*. , Naunyn Schmiedebergs Arch Pharmacol. 389(7): 695-712, 2016). Thus, our aim was evaluate the effect of pretreatment with atorvastatin on adhesion of prostate cancer cells DU-145 cells to endothelial cells EA. hy926 induced by $P2Y_2R$ agonist UTP. **Methods:** Endothelial cell lines EA. hy926 and tumor prostate cancer cells DU-145 (androgen independent) were obtained from the Rio de Janeiro cells line bank. EA. hy926 cells were maintained in DMEM supplemented with 10% fetal bovine serum and penicilin (100 U/ml) and streptomycin (100 µg/ml). DU-145 cells were maintained in RPMI supplemented with 10% fetal bovine serum, pyruvate 1%, penicilin (100 U/ml) and streptomycin (100 µg/ml). Both cell lines were incubated at 37 °C and 5% CO_2. EA. hy926 cells were incubated for 24 hour with atorvastatin (1µM), then the $P2Y_2R$ agonist UTP (100 µM) were incubated for 4 hours, for basal condition were used only vehicle. DU-145 cells were treated with calcein 0,2µM for 20 minutes. ECs were washed with DMEM and DU-145 cells (5×10^3 cells/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 DU-145 cells per field was determined by fluorescence microscopy (200X magnification). **Results:** $P2Y_2R$ agonist UTP (100 µM) increased prostate cancer cells adhesion to ECs when compared with basal condition (11. 25 ± 2. 4 and 7. 571 ± 1. 1 cells/field, n = 7, respectively, P < 0. 05). **Conclusion:** Our data indicate the involvement of purinergic signaling on adhesion of metastatic prostate cancer cells to endothelial cells promoted by $P2Y_2R$. **Acknowledgements:** CAPES, CNPq, FAPERJ

01.003 Beta-blockers with moderate intrinsic sympathomimetic activity improve sepsis-induced myocardial dysfunction. Silva KPD[1], Júnior EDS[2], Baker J[3], Cunha FQ[4], Pupo AS
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Introduction: Myocardial dysfunctionis associated with high mortalityin septic patients. Recent findings showed that the cardiac depression in sepsis is related to β-adrenoceptor internalization (Dal Secco, D. , Am J Physiol Heart Circ Physi. , 313: H149, 2017). This study investigates whether β-blockers with intrinsic sympathomimetic activities (efficacies) ranging from "zero" (propranolol) to "moderate"(carvedilol<pindolol<alprenolol<bucindolol=xamoterol) ameliorate the cardiac function and inflammatory response in sepsis by preventing β-adrenoceptor internalization, but still providing some degree of cardiac stimulation. **Methods:** Sepsiswas induced in C57BL/6 male mice (6-8 weeks olds) by cecal ligation and puncture followed by treatment with propranolol, carvedilol, pindolol, alprenolol, bucindolol or xamoterol at doses chosen based on theK_D and half-life of each drug. Drugs were given during the hyperdynamic phase of sepsis. After 24h of sepsis induction, the following experiments were performed: (a) [^{3}H]CGP-12177 binding assay to estimateβ-adrenoceptors density in the heart; (b) isolated heart in Langendorff system to evaluate cardiac function; (c) ELISA to quantify serum and cardiac IL-1β and TNF levels; (d)bacteremia. **Results:** Cell surface β-adrenoceptor density was reduced by ≈50% in the heart of septic mice (Bmaxfmol. mg^{-1} protein=4. 7±0. 7, n=5*vs* 8. 8±1, n=5 in naïve mice). The reduction in β-adrenoceptor density induced by sepsis was prevented by treatments with propranolol (Bmax=13. 8±2. 9, n=5), alprenolol (7. 7±0. 6, n=5) and pindolol (8. 4±0. 8, n=5), but not bycarvedilol (5. 8±1. 7, n=5). The performance of the isoprenaline-stimulated heart from septic mice was severely compromised (Heart rate= 322±59 bpm; dP/dT_{max}=343±61mmHg/s; dP/dT_{min}= -287±51mmHg/s, n=5) when compared to hearts from naïve mice (Heart rate= 556±23; dP/dT_{max}= 894±122; dP/dT_{min}= -1245±210; n=5). Treatment of mice with propranolol and alprenololrestored the heart rate ($Emax_{propranolol}$= 468±15bpm; $Emax_{alprenolol}$= 528±32; n=5), dP/dTmax ($Emax_{propranolol}$= 1162±190 mmHg/s; $Emax_{alprenolol}$= 1090±95; n=5) and dP/dTmin ($Emax_{propranolol}$= -1141±167 mmHg/s; $Emax_{alprenolol}$=-759±211; n=5). However, treatment with carvedilol, pindolol, bucindolol or xamoterol was ineffective in preventing the cardiac depression induced by sepsis. High levels of IL-1β (932±473pg/mL, n=10, undetected in naïve mice) and TNF (785±26pg/mL, n=10, undetected in naïve mice) were found In serum from septic mice. Treatment with β-blockers diminished serum IL-1β(in pg/mL: propranolol= 33±17; carvedilol= 68±16; pindolol= 259±236; alprenolol= 459±292; n=5-7) and TNF levels(in pg/mL: propranolol= 43±18; carvedilol= 86±29; pindolol= 95±76; alprenolol= 72±73pg/mL; n=5-7). β-blockertreatment diminished IL-1βlevels in the heart (in pg. mg^{-1} protein: propranolol= 2±0,4; carvedilol= 2±0. 2; pindolol= 2±0. 3; alprenolol= 2±0. 2; n=5-8 vs untreated septic animals 4. 7±1. 4; n=5);however, only propranolol treatmentreduced bacteremia(Log UFC/ml= 1,2; 95%CI =0. 6-2. 1 vs untreated septic animals Log UFC/ml= 3,5; 95%CI= 2. 8-4. 5; n=7). **Conclusion**: Treatment with β-blockers with moderate intrinsic sympathomimetic activity (e. g. alprenolol) may be a novel approach to protect cardiac function and reduce inflammatory response during sepsis. (CEUA #40/2017).

01.004 Stress-mediated systemic low-grade inflammation and its impact on male reproductive health. Freitas GA[1], Pinna GP[2], Scavone C[1], Avellar MCW[3] [1]ICB-USP, [2]University of Illinois, [3]Unifesp

Introduction: Studies examining stress-related disorders, such as post-traumatic stress disorder (PTSD), have either emphasized a relationship between PTSD and a systemically pro-inflammatory state or identified a link between PTSD and male infertility. The mechanisms by which the neurobiological abnormalities in PTSD impact male reproductive function and fertility still remains largely unknown. Taking advantage of social isolation in mice, that experimentally reproduces the behavioral and neuroinflammatory changes that are found in PTSD, we have recently shown that this protracted stress condition negatively affects immune cellular aspects of the epididymis, an organ of the male reproductive tract with a crucial role on sperm function; a negative effect on qualitative and quantitative sperm parameters was also observed in the stressed mice. Low-grade systemic inflammation is seen as a key mechanism underlying chronic diseases, and may be also present in PTSD. Aim: Here the low-grade systemic inflammation induced by social isolation was correlated with the epididymal inflammatory profile that follows this stress condition. **Methods**: Adult Swiss mice (90 days) were kept in groups (GH, control) or individually (socially isolated, SI) for 4 weeks. Plasma samples were obtained and used to evaluate the profile of systemic cytokine level profile (Milliplex® MAP kit, Millipore). Interleukin-1beta (IL1B)expression level was evaluated by *Western blotting (WB)* analysis. Immunofluorescence studies (IF) were performed in epididymal cryosections (10 µm) with antibodies against innate immune components (IL1B and β-defensin SPAG11C) and epididymal clear cell marker (VATPase). Image analysis were conducted by epifluorescence and confocal microscopy; the relative number of VATPase-positive cells was evaluated from digital fluorescent images acquired from the cauda epididymal region. Proper positive and negative controls were used in the assays. **Results:** The plasma levels of the pro-inflammatory cytokines IL1B (4. 1pg/ml±1. 1 vs. 10. 1pg/ml±1. 2) and IL6 (2. 9pg/ml±1. 3 vs. 26. 9pg/ml±4. 3) increased, while the anti-inflammatory cytokine IL10 (14. 9pg/ml±1. 1 vs. 5. 8pg/ml±3. 1) decreased in SI mice when compared to controls, confirming the systemic inflammatory condition in these animals. In the epididymis from SI mice, WB and IF analysis also revealed higher expression levels and changes in the immunodistribution of the pro-inflammatory mediator IL1B. Confocal analysis further revealed significant changes in the immunodistribution pattern of SPAG11C-positive cells and VATPase-clear cells along cauda epididymis from SI mice. The length occupied by the apical region of VATPase-positive clear cells relative to the total luminal perimeter of the epidydimal tubule increased in tissues from SI mice (28% ± 1. 4 vs. 37% ± 2. 3). **Conclusion:** Collectively, our results support the hypothesis that low-grade systemic inflammation associated with neurobiological abnormalities in PTSD condition negatively impacts epidydimal structure and function, with consequences to male reproductive health.

01.005 Polarization of mesenchymal stem cells isolated from apical papilla human teeth with IFN-y. Dagnino APA, Chagastelles PC, Medeiros RPD, Goldani E, Campos MM, Silva JB PUC-RS

Introduction: MSCs play an important role in the maintenance of tissue homeostasis and regeneration processes. Different strategies increase the therapeutic capacity of MSCs. The activation of cells isolated from the bone marrow of humans with interferon-γ (IFN-γ) induce indoleamine expression, without causing any deleterious effects (Kim DS, EBioMedicine, 28, 261, 2018; Guess AJ, Stem Cells Transl Med. , 6, 1868, 2017). This study analyzed the ability of MSC isolated from the apical papilla of human teeth to polarize into different phenotypes after exposure to IFN-γ, aiming to improve their therapeutic activity, as a step prior to its application in cell therapy. **Methods**: the Institutional Research Ethics Committee approved this project (CAAE: 60389816. 7. 0000. 5336). The cells were cultured with DMEM medium and incubated with IFN-γ (100 and 200 ng/ml), for 24 to 72 h. The levels of kynurenine, the indoleamine activity, the cell proliferation and the lactate dehydrogenase (LDH) were evaluated. The levels of CCL3, CCL4, CCL5 and IL-6 were measured at 24 h and 48 h in the culture supernatant. **Results:** Cells stimulated with IFN-γ (100 or 200 ng/ml) 24 to 72 h produced increased kynurenine levels in the culture supernatant, when compared to the control group ($p < 0.05$). The IFN-γ exposure for 24h induced an increase of indoleamine activity, after 48 and 72 h, even after stimulus withdrawal ($p < 0.05$). There was no difference in proliferation, LDH activity, or cytokine levels. **Conclusion:** Tests performed demonstrate that IFN-γ stimulated cells to produce indoleamine, the major anti-inflammatory mediator of MSCs. In addition, the treatment did not induce cell death, proliferation or inflammatory changes. Additional experiments are in progress to evaluate the immunosuppression capacity of the non-polarized and polarized MSCs on the expression of molecules related to neural regeneration process. **Financial support**: CNPq, CAPES (Finance Code 001) and Fapergs.

01.006 Vitamin D increases dyslipidemia but does not enhance cardiovascular remodeling or oxidative stress on atherosclerotic and osteoporotic mice. [1]Prado AF, [1]Alves GM, [1]Anaissi AKM, Demachki S[1], Nascimento JLM[1], Macchi BDM[1], Ramos J[2], Gerlach RF[2], Issa JPM[2], Azevedo A[2] [1]UFPA, [2]USP, FMRP-USP

Introduction: Atherosclerosis and osteoporosis are very common diseases, with an inflammatory etiology and frequently associated one to another. Osteoporotic patients, often with high cardiovascular risk, receive vitamin D treatment, which could increase cardiovascular diseases according with some studies. Therefore, this work aimed to study morphological and biochemical effects of vitamin D on aorta of atherosclerotic and osteoporotic mice. **Methods**: Wide type (c57) and ApoE (-/-) female mice were submitted to ovarian removal at the 5th week of life and for induction of atherosclerosis these mice received a hypercholesterolemic diet (normal ration plus 1% cholesterol) from the 7th week of life. The animals were treated by gavage with vehicle (olive oil) or vitamin D (0,1µg/kg) from the fifteenth to the eighteenth week of life. Serum cholesterol levels were quantified. Aorta was analyzed with HE to measure layer thickness, Orcein to quantify elastin and Picrosirius red to quantify collagen. Matrix metalloproteinases (MMPs)2 and 9 were analyzed by zymography in situ. Oxidative stress was studied by reactive oxygen species using dihydroethidium probe. **Results:** Female rats with atherosclerosis and osteoporosis that received vitamin D treatment showed higher levels of blood cholesterol than other groups. However, their aorta does not showed differences at medial layer thickness, elastin or collagen density. Otherwise, outer layer (tunica adventitia) showed higher collagen deposits due to atherosclerosis and osteoporosis. Both diseases also enhance oxidative stress and increase MMP levels compared to control group. **Conclusion:** Vitamin D did not induce morphological and biochemical effects on healthy or sick rats. **References**: [1] Azevedo, A. et al. Basic Clin Pharmacol Toxicol. 115: 301-314, 2014. [2] Ceron, C. S. et al. Trends in cell biology. 11: S37-43, 2001. [3] Nagase, H. et al. Cardiovascular research. 69: 562-573, 2006. [4] Rizzi, E. et al. Exp. and Mol. Pathology. 94: 1-9, 2013. Human or Animal Research Ethical Committee: protocol number 2014. 1. 1090. 58. 0, approved by CEUA – FORP USP. **Financial support**: CAPES, CNPq, FAPESP, UFPA.

01.007 Maxadilan, but not PACAP, triggers a disruption in endothelial barrier: Molecular mechanisms associated to changes in endothelial cell phenotype. Barja-Fidalgo TC[1], Nascimento-Silva VN[1], Rodrigues GRDS[1], Svensjö ES[2] [1]UERJ, [2]UFRJ

Introduction: Maxadilan (MAX) is a potent vasodilator isolated from saliva of *Lutzomyialongipalpis*. Its vasoactive and pro-inflammatory effects are mediated through its high selectivity forPAC1 (pituitary receptor type 1), inducing *in vivo*increase in vascular permeability and leukocyte activation. However, the direct effects of MAX and PAC-1 agonists on endothelial cells, and the molecular mechanisms involved in these actions are not well described. In this study,we investigate the effects of MAX on endothelial cells. **Methods:** Human microvascular endothelial cells (HMEC-1), cultured on glass coverslips overnight, were treated with Maxadilan (MAX) or pituitary adenylate cyclase activating polypeptide (PACAP-38),the main endogenous agonist of PAC1 receptor, in the presence or absence of the PAC1 antagonist M65, for different times. Alterations in endothelial permeability was assessed by FITC-dextran. Confocal and epifluorescence microscopy analysis were used to detect VE-cadherin dispersion, *Src* activation, β-catenin, FAK, paxilin subcellular localization and cytoskeleton alterations. Protein expression was detected by Western blot. **Results:** Treatment of HMEC-1 withMAX(7nM) increased endothelial permeability in a PAC1-dependent manner, being inhibited by M65. Interestingly, PACAP-38 (7nM) have no effect in the endothelial permeability. MAX treatment induced phosphorylation (Y^{658}) ,dispersion and internalization of VE-cadherin, and subsequent β-catenin nuclear translocation in the endothelial cells. These effects were accompanied by reorganization of the actin cytoskeleton, redistribution of paxillin and its colocalization with actin in focal adhesions. Furthermore, MAX triggered FAK phosphorylation at residue Tyr397 and its association with actin, and induced Src translocation to membrane and association to VE-cadherin at the adherents junctions. Inhibition of Src reduced the effects of MAX in the endothelial permeability. **Conclusion**: The data indicate that the vascular effects of MAX, contrasting with PACAP, involves the disruption of the endothelial barrier through its interaction with PAC1 receptor. The study can contribute to elucidate the role of PAC-1 receptors in vascular endothelium. Additionally, a better understanding on the effects MAXon endothelium may lead to the development of more specific strategies for the treatment of vascular diseases. **Financial Support:** CAPES, FAPERJ, CNPq.

01.008 Phenotypical and pharmacological differences evoked by *in vitro* aging of LLC-PK1 proximal tubule renal cells. Barros GMO, Silva ACAE, Quintas LEM UFRJ

Introduction: Aging is a progressive, degenerative process that occurs with every cell of the body. It consists in the gradual deterioration of the functional characteristics of the cell, which loses its ability to adapt and repair, and may result in phenotypic transformation and culminates in cell death. In addition, aging modifies the pharmacological response to drugs. In the kidneys, aging is related to changes in renal morphology and decreased renal function, which may be associated with chronic failure. We have shown that bufalin, a cardiotonic steroid that selectively binds to Na/K-ATPase (NKA), induces epithelial-mesenchimal transition (EMT) in LLC-PK1 epithelial renal cells at high passages (P>80). Our objective in this work was to evaluate phenotypical and bufalin-induced response differences in LLC-PK1 cells of high (P>80) and low (P<40) passages. **Methods**: LLC-PK1 cells (porcine proximal renal tubule) of both passages – P<40 and P>80 –were cultured in DMEM with 5% FBS and antibiotics. Cells were cultured in 24-well plates (5000 cells/well) and after 96 h they were trypsinized, centrifuged and Trypan blue viable cells were counted in Neubauer chamber. Also, in 96-well plates 1000 cells/well were cultured for 24, 48 and 72 h and treated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After 4 h, 200 µl DMSO (dimethyl sulfoxide) was added to solubilize formazan crystals and absorbance determined at 570 nm in the Sunrise plate reader (Tecan, Switzerland). Serum-starved cells were treated for 24 h with 20 nM bufalin and morphology was evaluated by phase-contrast microscopy. NKA activity was evaluated by the amount of inorganic phosphate released from ATP hydrolysis according to Fiske and Subbarow method. Protein expression of NKA α1 isoform was evaluated by Western blot. The comparison between groups was performed by Student's t test or two-way ANOVA, followed by Sidak posttest (p<0. 05 was considered statistically significant). The data are expressed as mean ± SEM. **Results:** Both groups are comparable in gross morphological analysis but P>80 grew faster than P<40. Cell number after 96 h was 3. 5x higher for P<80 (14990 ± 3089 P>80 vs. 4238 ± 1086 P<40; p<0. 05, n=6). Also, MTT also corroborated cell counting data, with a statistically significant difference after 72 h (1073 ± 94 P>80 vs 726 ± 165 P<40; p<0. 05, n=5). When both groups were treated with 20 nM bufalin, only P>80 responded by a severe morphological modification compatible to EMT phenomenon. P<40 cells were totally resistant to bufalin. The activity of the molecular target of bufalin, NKA, was similar for both groups (7. 4 ± 1. 2 P<40 vs 6. 7 ± 1. 3 P>80; n=11), but the expression of α1 was 25% lower in P>80 (p<0. 05, n=4). **Conclusion:** Our data show that both at the cellular as well as molecular levels LLC-PK1 feature different characteristics as they age in vitro and may provide the basis for the research of distinct pharmacological and physiological, as cardiotonic steroids are considered a new class of endogenous hormones in man, effects in the aging process. The investigation of the mechanism responsible for this diversity is in progress. **Financial support**: PIBIC/UFRJ, CAPES, FAPERJ e CNPq.

01.009 *In vitro* **Leishmanicidal Activity of New Carbamoyl-N-Aryl-Imine-Ureas against Leishmania Major**. Santos HCN[1], Silva AE[1], Silva JFM[1], Silva JKS[1], Silva KCJ[1], Araújo MV[1], Oliveira GG[1], Avelar JLS[2], Barreiro EJDL[2], Lima LM[2], Moreira MSA[1] [1]UFAL; [2]UFRJ
Leishmaniasis is a group of infectious neglected diseases which affects mainly low-income people. It is caused by parasites of the genus *Leishmania*which have two evolutionary forms; namely, promastigote and amastigote, with the latter being found in tissues of vertebrates. It has two main clinical forms: cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL), with clinical manifestations ranging from mucosal lesions to involvement of viscera, which can lead to death. Despite the great complexity of the disease, the available therapeutic arsenal is still limited, with the use of pentavalent antimonials as the first-choice drugs, and for patient's refractory to them amphotericin B, pentamidine, and miltefosine are used. These drugs present problems related to their cytotoxicity and parasite resistance. Therefore, the present study aimed to evaluate the *in vitro*leishmanicidal activity of new carbamoyl-*N*-aryl-imine-ureas, designed as cysteinyl-proteases inhibitors, against *Leishmania major*. In order to achieve that aim, the MTT (3- [4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium) assay was performed using four different concentrations (100, 30, 10, and 3 μM) of target compounds; the results revealed LASSBio-2077 , 2078, 2079, 2080, 2081, 2082, 2083, 2246, and 2244 and the standard drug pentamidine exhibiting IC_{50}values of 18. 3 ± 5. 8 μM, 32. 5 ± 10. 6 μM, 12. 4 ± 4. 9 μM, 64. 5 ± 6. 4 μM, 47. 5 ± 10. 6 μM, 20. 8 ± 2. 3 μM, 11. 2 ± 2. 6 μM, 18. 8 ± 6. 7 μM, 23. 2 ± 2. 1 μM, and 22. 7 ± 5. 6 μM, respectively. Then,*Leishmania major*amastigote forms were tested using the concentration of1 μM. In this concentration LASSBio-2081 and 2244 had a maximum effect of 73. 0 ± 6. 5% and 83. 5 ± 2. 8%, respectively. Despite their respective IC_{50}values, the derivatives 2081 and 2244 could be considered a promising leishmanicidal lead-candidates, since their effect was higher than 70% with no cytotoxicity to macrophages. Ongoing studies are being performed to establish their IC_{50}values on amastigotes of*L. major*. **Acknowledgements**: LAFI, UFAL, FAPEAL, CNPQ, INCT-INOFAR.
Keywords: Cutaneous leishmaniasis, *Leishmania major*,Carbamoyl-*N*-aryl-imine-ureas.
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01.010 Alpha 7 Nicotinic acetylcholine receptors in macrophages and microglia. Santos VGB, Castro NG, Nazareth AMN, Lima FRSL, Leser FSL UFRJ

Introduction. Inflammatory responses to stimuli are essential body defenses against foreign threats. In the past few years, a number of studies demonstrated the functional expression of nicotinic acetylcholine receptors (nAChRs) of the α7 type in macrophagic immune cells, both in the CNS (microglia) and in the periphery, where they have an essential role in the control of inflammation by the inhibition of pro-inflammatory cytokine release. While the α7 nAChR is well characterized as a calcium-permeable ligand-gated ion channel in neurons, published whole-cell patch clamp experiments suggest that ion channel activity is absent in macrophages and microglia. This divergence may indicate an uniqueness in the receptor's location (possibly intracellular) and function (possibly metabotropic) in these cell types. Therefore, we aimed at elucidating the α7 receptor transduction mechanism, which is critical to understanding the physiological role of these receptors in the immune response. **Methods**. We have used the RAW 264. 7 cell line and primary mouse peritoneal macrophages and microglia (approved animal use protocol A6/19-001-16 CEUA-UFRJ). A fluorescence assay of alpha-bungarotoxin-rhodamine (αBGT-Rh) binding was used to identify α7 nAChRs in these cells. We performed whole-cell patch-clamp recordings and ratiometric (F340/F380) calcium microfluorimetry with fura 2-AM to show agonist-evoked transmembrane currents and variations in intracellular Ca2+ concentration. **Results:** Cell imaging showed that αBGT-Rh (200 nM) bound to the receptors and nicotine inhibited its binding (ANOVA and Tukey's test, P<0. 005), which proved that the labeling was specific and, therefore, the cells were expressing nAChRs-α7. However, only ~20% of the RAW 264. 7 cells showed calcium transients in response to rapid infusion of the agonists 1 mM choline or 1 mM nicotine, with or without the selective α7 potentiator 3 microM PNU-120596. In similar standard conditions, peritoneal macrophages and microglia showed no nicotinic calcium responses, and none of the three cell types showed nicotinic ionic currents. In contrast, >90% of the cells responded to stimulation with 1 mM ATP, showing both ionic currents and calcium transients. Extracellular Ca2+ restriction attenuated the α7 nicotinic calcium response in RAW 264. 7 cells, so the increase of intracellular Ca2+ was partially dependent on Ca2+ influx. It is already known that microglial inflammatory responses are highly temperature-sensitive, so we also performed microfluorimetry assays with temperature control. At 36ºC microglial cultures responded to α7 agonists applied by a heated perfusion system with robust intracellular Ca2+ increases, while a selective α7 antagonist (MLA 10 nM) inhibited these responses. Conclusion. These results suggest that the activation of α7 nAChRs triggers calcium signaling, which at least in part depends on extracellular calcium. This receptor activity is strongly dependent on experimental conditions and is compatible with an ionotropic transduction mechanism.

Support: FAPERJ, CNPq.

01.011 Participation of PI3 kinase and ERK pathways in the differentiation of hematopoietic stem cells by P2 receptors. Souza KF[1], Araújo RT[1], Zaias AB, Torquato HF[1], Paredes-Gamero EJ[2] [1]Unifesp, [2]UFMS

Introduction: Several studies have shown the ability of ATP and their analogs to modulate the cellular activity of mature hematopoietic cells and hematopoietic stem cells (HSCs). The ability of ATP to induce HSCs differentiation has been demonstrated but the receptor subtypes and the intracellular pathways involved in differentiation have not been elucidated. In this study, the activation of intracellular pathways involved in myeloid differentiation by P2 receptors activation was evaluated in HSCs. **Methods:** Differentiation and proliferation state of HSC were evaluated by flow cytometry. HSC was identified using Lineage cocktail (CD3, CD4, CD8, B220, Ter119, Gr-1, Mac-1, FLK-2), c-Kit and Sca-1 antibodies. Bone marrow cells were extracted from femurs. Subsequently, cells were fixed, permeabilized and incubated with primary and secondary antibodies. The follow antibodies were used: phosphor (p)-Pi3K, p-AKTser495 and thr;386 p-protein kinase C pan (PKC); p-Ca^{2+} calmodulin kinase (CaMK) I, II and IV; p-Stats 3 and p-STAT5, pERK1/2. The percentages and the phosphorylated state of proteins were quantified in HSC population. Data analyses were performed usingACCURI C6 flow cytometer andFlowJo software. **Results**: Initially, the differentiation of HSC by ATP was corroborated. It was used the concentration of 1 mM ATP, which did not promote cell death. ATP also decreased the percentage of HSC, increased the expression of Ki67 (a proliferation marker) and reduced Notch-1 receptor expression, showing the loss of quiescence state of HSCs. In addition, the activation of Pi3K/AKT pathway wasobserved. AKT was phosphorylated in both serine 473 and threonine 308, suggesting the participation of PDK-1 enzyme and mTOR protein complex in this signaling process. ThePKC, CaMKI andCaMKII – Ca^{2+}-dependent proteins –were also activated by ATP, but not the CaMKIV protein. Some transcription factors such as Stats 3 and 5, were activated independently of Janus kinases activation. ERK1/2 was activated in 24 h. Corroborating this result, the specific inhibitor for MEK (UO126) was able to reverse the process of ATP-induced differentiation. In addition, the use of the PI3K inhibitor (wortmannin) led to a partial reduction ofthe differentiation process. **Conclusion:** These results suggest that HSCs differentiation caused by P2 receptors activation occurs via several intracellular pathways, such as the ERK1/2 and PI3K pathways. **Funding Suport** Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

01.012 Cytoglobin attenuates neuroinflammation in lipopolysaccharide-activated primary preoptic area cells via NFkB pathway inhibition. Gomes BRB, Sousa GLS, Ott D, Murgott J, Sousa MV, Souza PEN, Roth J, Souza FHV UnB

Introduction: Cytoglobin (Cygb) is a hexacoordinate protein that has been reported to be associated with the transport of oxygen, nitric oxide scavenging, tumor suppression and protection against oxidative stress and inflammation. This protein is expressed in brain areas including the preoptic area of the anterior hypothalamus (POA), the region responsible for the regulation of body temperature. The aim of this studywas to validate the increase in hypothalamic Cygb expression after a pyrogenic dose of LPS, and to investigate its function by examining levels of inflammatory cytokines (TNFα and IL-6) and the activation of transcription factors such as NF-IL6, STAT3, and NFκB, in LPS-activated POA cells co-treated with recombinant rat Cygb. **Methods:** Adults male Wistar rats received intravenous injection of LPS (5µg/kg) or saline (1ml/kg). The animals were euthanized 2. 5or 5h after the administration of LPS, and the hypothalami were collected for the analysis of expression of Cygb by Western blot. We therefore investigated the effect of the treatment with recombinant rat cytoglobin (10 and 20 µg/ml) in POA primary cultures stimulated with LPS (10 µg/ml) for 4h. PBS was used as negative control. After this time, the supernatants were collected for cytokines (TNFα and IL-6) measurements, and the cells were used for immunocytochemistry analysis. The immunoreactivity of the transcriptional factors NF-IL6 and NFκB in microglial cells and STAT3 in astrocyteswere examined by immunocytochemistry. **Results:** We detected the upregulation of Cygb in the rat hypothalamus 2. 5and 5h after intravenous administration of LPS. The levels of TNFα and IL-6 were significantly increased in LPS (10 µg/ml) stimulated POA cells compared with control group. This effect of LPS was attenuated by co-treatment of cells with Cygb (20 µg/ml). We further observed a decrease of the immunoreactivity of the inflammatory transcription factor NFκB, but not NF-IL6 and STAT3 in the nucleus of Cygb-treated POA cells stimulated with LPS. **Conclusion:** These findings suggest that Cygb attenuates the secretion of IL-6 and TNFα in LPS-stimulated POA primary cultures via inhibition of NFκB signaling pathway, revealing that this protein might play important role in the control of neuroinflammation and fever.

01.013 AT1 receptor signaling in cells with high levels of O-GlcNAc-modified intracellular proteins. Silva Neto JA, Abrão EP, Silva J, Costa TJ, Duarte DA, Simões SC, Costa Neto CM, Tostes RC FMRP-USP

Introduction: AT1 receptor is a seven transmembrane protein that, upon binding of angiotensin II, triggers many physiological effects AT1 receptor activation triggers second messengersgeneration and phosphorylation of several intracellular proteinsthat regulate biological functions such as secretion of hormones and neurotransmitters, renal filtration, vasoconstriction and cardiovascular remodeling. *O*-GlcNAcylation is a posttranslational modification of cytosolic and nuclear proteins where a glucosamine derivative is attached to serine and threonine residues, the same theoretical targetsthat are phosphorylated by kinases. So, we have hypothesized that high levels of *O*-GlcNAc-modified intracellular proteins impair the phosphorylation process induced by AT1 receptor activation and, therefore, modify the angiotensin II pharmacological response. **Methods:** HEK 293T cells stably expressing AT1 receptor were cultured in 6 well plates in Dulbecco's Modified Eagle Medium (High glucose) during 16hours (h) in the presence of the O-GlcNAcase enzyme (OGA) inhibitor Thiamet G (1µM, during 16h) or vehicle. Then, a time-effect curve to angiotensin II (0. 1 µM, 1-30 min) was performed and AT1 activation kinetics as well as the proteins related to AT1 receptor pathway were determined. Data were expressed as mean ± SD of arbitrary units (a. u.) and analyzed by Wilcoxon test, considering $p < 0.05$ as statistically different. RESULTS Thiamet G treatment increased the content of *O*-GlcNAcylated proteins [from 1. 4 ± 0. 3 (control) to 8. 5 ± 2 a. u ($p < 0,05$, $n = 5$)] and OGA expression [from 1,6 ± 0,3 to 2,3 ± 0,5 a. u. ($p< 0,05$, $n = 4$)]. The expression of GRK2, ERK 1/2 or beta-arrestin 1 and 2 did not change in *O*-GlcNAcylated cells. The kinetic of ERK phosphorylation was similar in control and Thiamet-G-treated cells but, after 10 min of angiotensin II stimulation, the ratio of phosphorylated/total ERK was decreased in *O*-GlcNAcylated cells [from 71. 2 ± 22 (control) to 35. 8 ± 9 a. u. ($p < 0,05$, $n = 4$)]. **Conclusion:** These preliminary data indicate that high levels of *O*-GlcNAcylated proteins modify the AT1 receptor pharmacological response. **Financial support**: CAPES, FAPESP and University of São Paulo.

01.014 Characterization of extracellular cAMP metabolism in rat airways. Pacini ESA[1], Jackson EK[2], Godinho RO[1] Unifesp[1]; [2]University of Pittsburgh

Introduction: cyclic AMP (cAMP) is a universal intracellular second messenger involved in many biological processes, such as muscle contraction, cell proliferation and gene expression. In the respiratory tract, intracellular cAMP has a crucial role in the smooth muscle relaxation induced by β_2-adrenoceptors (β2-AR)/Gs protein/adenylyl cyclase axis. In addition to its classical intracellular function, in many other tissues, cAMP works as an extracellular third messenger, which depends on its efflux and the sequential extracellular conversion into AMP and adenosine by ecto-phosphodiesterase (ecto-PDE) and ecto-5'-nucleotidase (ecto-5'NT), respectively (Godinho et al. , Front. Pharmacol. 6: 58, 2015). Although we have shown the contracting effects of extracellular cAMP on tracheal smooth muscle (Pacini et al. , J Pharmacol Exp Ther. , 366: 75-83, 2018), theenzymatic metabolism ofextracellular cAMPby airway cells has never been accessed. Thus, the aim of this study was to characterize the extracellular cAMP-adenosine metabolic pathway in the airways. **Methods**: tracheal rings obtained from adult male Wistar rats were mounted in an organ bath containing Krebs-bicarbonate buffer at 37°C, under optimal resting tension. Next, tissues were stabilized for at least 60 minutes and incubated with increased concentrations of cAMP (3, 30 ad 300 µM) for 1, 30 and 60 min. In another protocol, rat tracheas were pretreated with AMPCP (ecto-5'-nucleotidase inhibitor) and incubated with 30 µM cAMP for 60 min. The incubation mediums were collected, centrifuged for 10 min at 10. 000 x g, boiled and the purines (AMP, adenosine and inosine) were quantified from the supernatant using liquid chromatography–tandem mass spectrometry. All values were expressed as mean ± S. E. M. **Results:** incubation of exogenous cAMP to rat trachea induced a time- and concentration-dependent generation of extracellular 5'-AMP, adenosine and inosine in the medium that reached60 ± 5, 126 ± 21 and 89 ± 38 ng/mL, respectively (n=6-8). Conversely, inhibition of the ecto-5'-nucleotidase increased by 6-fold the levels of AMP (16 ± 1. 5versus 94 ± 7. 3 ng/mL) and reduced by 2. 5-fold (38 ± 6. 3 versus 15 ± 1. 2 ng/mL) and 3. 5-fold (46 ± 12 versus 13 ± 4. 8 ng/mL) the levels of adenosine and inosine, respectively (n=6, $p < 0.05$). **Conclusion:** our results show that tracheal tissue expresses an extracellular biochemical cascade (ecto-PDE, ecto-5'NT and adenosine deaminase) that converts cAMP into AMP, adenosine and inosine. The observation that extracellular cAMP is a source of interstitial adenosine supports previous studies from our lab showing that the contracting effects of extracellular cAMP in tracheal smooth muscle depends on A_1-adenosine receptors. **Financial support**: CAPES, CNPq and Fapesp. Animal EthicsCommittee: CEUA #9987150714

01.015 Unveiling the molecular bases of AT1 receptor desensitization and tachyphylaxis. Duarte DA[1], Silva LTPEA[1], Oliveira EB[1], Bouvier M[2], Costa Neto CM[1]USP, [2]Université de Montréal

Introduction: Acute desensitization or tachyphylaxis is a phenomenon occurring in some GPCRs, including the angiotensin II AT1 receptor (AT1R), described as a loss of functional response after continuous administration or repeated treatment with an agonist. Although it has been studied since decades ago, the precise mechanism underlying this event remains unclear. Here, we assessed the molecular bases of thetachyphylaxis of the AT1R, the major player of the renin-angiotensin system. **Methods:** In order toinvestigate the molecular mechanisms behind AT1R tachyphylaxis, wein depth characterized different signaling pathways and properties of AngII and the analogs [Suc[1]]-AngII and [Lys[2]]-AngII, which previously were described to act as non-tachyphylactic agonists. The affinity of the ligands for the AT1R was assessed by radioligand competition binding assays in HEK293T cells expressing the AT1R. We also analyzed kinetic binding of the ligands to calculate association (K_{on}) and dissociation (K_{off}) rate constants. In addition, bioluminescence resonance energy transfer (BRET) assays were performed to evaluatedifferent triggered signaling pathways and receptor translocation afterprolonged orshort ligand stimulation. **Results:** The analogs [Suc[1]]-AngII and [Lys[2]]-AngII presented lower affinity than AngII for the AT1R, but behaved as full agonists for Gq protein activation, intracellular calcium mobilization, as well as concerning to β-arrestin recruitment. Estimation of K_{on} and K_{off}rate constants reveled that both analogs havefaster receptor dissociation kinetics than AngII, meaning that the residence time is lower for bothanalogs than for AngII. We also showed that the activation of Gq protein and the translocation of β-arrestins to the plasma membrane were more transient, and faster reversed in cells stimulated by either [Suc[1]]-AngII or [Lys[2]]-AngII,as compared to AngII. Furthermore, the internalization of the AT1R induced by analogs showed faster recycling. Evaluation of ligands' ability to resensitize the receptor during a tachyphylactic protocol showed that both analogs were able to induce furtherand full Gq protein activation in response to several stimuli, whereas as expected, further stimuli of AngII led to decreased Gq activation. **Conclusion:** Based on our results, we can infer that the molecular bases of AT1Rtachyphylaxis are linked to distinct kinetics of sustained signaling of the agonists, which in turn depends on their dissociation rates. According to our hypothesis, the non-tachyphylactic AngII analogs activate the AT1R in a fast but transient manner due to a low residence time in the receptor, consequently leading the AT1R to be quickly recycled to the plasma membrane, becoming available to subsequent stimuli andresponses. On the other hand, the sustained activation of AT1R by AngII,with low dissociation rate lead to sustained endosomal internalization of the AT1R, delayingrecycling to the plasma membrane. We believe that understanding the molecular mechanisms involved in receptor tachyphylaxis is keyfor better understanding drug/receptor interaction mechanisms and shall shed lightin drug discovery and development.

Funding: FAPESP (2014/09893-0 and 2018/13655-9).

01.016 Inhibitory activity of *Copaifera reticulata* **and** *Piper marginatum* **oleoresin on lipoxygenase enzyme**. Pires TM[1], Silva LAN[1], Oliveira ECP[1], Acho LDR[2], Lima ES[2], Moraes WP[2] UFOPA, [2]UFAM

Introduction: Although there are drugs currently available in the pharmaceutical market to accelerate the healing process of wounds, reducing the inflammatory response, there is a need to search for different compounds from the Amazonian biodiversity that can contribute to the acceleration of the healing process, which are inexpensive. and produce fewer adverse events. Among these products, Copaifera reticulata (OCR) and Piper Marginatum (OPM) oleoresin are prominent alternatives in this scenario, as their medicinal properties have been reported by popular use. Thus, this work aimed to evaluate the in vitro inhibitory activity of OCR and OPM on the Lipoxygenase (LOX) enzyme. **Methods:** This enzymatic activity was determined according to Pinto et al. 2007, with modifications. For the preparation of the enzyme a concentration of 0. 1mg / ml diluted in 50mM TRISMA-HCL buffer pH 8. 5, and for the substrate was diluted: 50µL linoleic acid, 20µL TWEEN 20, 100µL of ethanol and 10mL of 50mM TRIS-HCL buffer pH 8. 5. In the cuvette 850µL of buffer, 50 µL of Lipoxidase enzyme and 50 µL of OCR/OPM or DMSO (Control) were added. It was incubated for 50 min at room temperature, and then 50 µl of the prepared Linoleic Acid (substrate) solution was added. Quercetin was used as the standard drug. The reading was performed in a spectrophotometer, under a wavelength of 234 nm, and was monitored in kinetic mode for 5 min. The IC50 was calculated based on interpolation with the standard curve. All calculations and analysis were done using GraphPad Prism software. **Results:** Few in vitro studies are found that relate oleoresins and LOX. In this study, the OPM showed inhibitory potential on the LOX enzyme, with an IC50 of 65. 3 ± 2. 9 µg / ml. In vivo research with the plant extract of the studied plant, at 5 and 1 g / kg, has proven anti-inflammatory activity by reducing 80 to 90% of mouse-induced paw edema (D'ANGELO et al. 1997). OCR also presented inhibitory activity with IC50 of 92. 5 ± 6. 2 µg / ml, as it can be corroborated with the study by Vargas et al. (2016) who performed tests with OCR diterpenes and this presented IC50 of 157. 8 ± 17. 7 µM, in researches with another species of copaiba a good correlation between copaiba balsam oil and sesquiterpenes was reported, reaching 70%. inhibition of LOX (BAYLAC et al. 2003). **Conclusion:** Oleoresins contain components such as sesquiterpenes and diterpenes, which are responsible for contributing to anti-inflammatory effects and other biological activities. However, the development of pharmaceutical formulations is necessary for clinical trials aiming to demonstrate their application in the treatment of acute skin lesions and / or chronic inflammatory diseases. **Acknowledgments:** Experiments developed with the support of the Laboratory of Biological Activities of the Federal University of Amazonas (UFAM) and Laboratory of Plant Biotechnology of the Federal University of Western Pará. **References:**
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01.017 Protective effect of Aqueous *Coriandrum sativum* **L. extract in the production of reactive species and Aortic wall remodeling in rat neonate offspring after maternal exposure to methylmercury**. [1]Bannwart CM, [1]Ferreira PN, [1]Rodrigues KE, [1]Oliveira FR, [1]Hamoy M, [1]Amarante CB, [1]Nascimento JLM, [1]Macchi BDM, [1]Gerlach RF[2], [1]Silva CAM, [1]Prado AF [1]UFPA, [2]USP

Introduction: Methylmercury (MeHg) intoxication can lead to vascular dysfunctions which pathological mechanism also includes increased Reactive oxygen species production[1,2], and, as *Coriandrumsativum* has known important antioxidant activity and beneficial vascular repercussions[3], this study aims to assess the vascular effects of aqueous *Coriandrumsativum*extract (CSAE) on offspring of rats submitted to methylmercury intoxication during gestational and lactation periods. **Methods:** Animals were randomized into 4 groups, each encompassing 8 animals (n = 8): Control; CSAE; MeHg; MeHg + CSAE. Pregnant rats were intoxicated in the last gestational third (14th gestational day) by adding 40 µg/mL MeHg to drinking water (ad libitum) and CSAE treatment was administered by gavage (360 mg/kg daily) until lactation day 14, which added up to a total of 21 treatment days. The offspring aortas were analyzed with light microscopy after orcein, and HE staining in order to visualize and quantify aortic wall elastin, and thickness respectively; Dihydroethidium (DHE) probe was applied in order to assess in situ reactive oxygen species and hair mercury level was evaluated by atomic fluorescence spectrometry. **Results:** No difference in wall diameter was observed between the four groups. However,there was a significant decrease in elastin levels in the MeHg group compared to the control group. The CSAE treatment prevented total elastin levels decrease in MeHg + CSAE group. An increase in reactive oxygen species was observed in MeHg group compared to control group and a decrease of ROS in the MeHg + CSAE group which presented levels similar to those found in the control group. A total reduction in mercury levels was also observed after comparing the MeHg + CSAE group to the MeHg group. **Conclusions:** We conclude that the CSAE has vasoprotective properties, for the reason that it reduces offspring total MeHg levels, preventing decreased levels of elastin in the aortic wall, as well as promoting a decline in ROS linked to vascular damage. **References:** [1] Genchi, G. et al. Int. J. Environ. Res. Public Health. v. 14 p. 1–13, 2017. [2] Bjørklund, G. et al. Environmental Research, v. 159, p. 545–554, 2017. [3] Patel, D. K. et al. Food and Chemical Toxicology, v. 50, p. 3120–3125, 2012. **License number of ethics committee**: ICB-UFPA 243-14. **Acknowledgments:** CAPES, CNPq, FAPESP. **Keywords:** Mercury; MeHg; Coriandrum sativum; MMPs; Matrix metalloproteinases; Cardiovascular effects

01.018 Action of lysine (K) -deacetylase modulators on cell metabolism in astrocytes exposed to oxidative stress and excitotoxicity: possible neuroprotective role in Amyotrophic Lateral Sclerosis. Torresi JLB, Rosenstock TR, Dutra MB FCMSCSP

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a rare disease with no confirmed pathological mechanism. Yet, many are the mechanisms proposed to subsidize the process of neurodegeneration, among them oxidative stress, excitotoxicity and mitochondrial dysfunction. Therefore, the objective of this project was to evaluate the neuroprotective effect of modulators of lysine deacetylases (KDACs) on mitochondrial metabolism and cell survival in primary astrocyte culture after increased oxidative stress (H2O2) and induction of excitotoxicity (after exposure to the neurotoxin LBMAA). **Methods**: We used primary culture of C57BL mice. For viability assays, we used MTT test; all cells were treated with H2O2 or LBMAA and four different KDACS (TSA, SB, MS-275 and SBHA). The levels of histone H3 and acetylated histone H3 were evaluated by western blot, mitochondrial membrane potential was investigated by TMRE fluorescent assay. Mitochondrial metabolism was also analyzed by the ATP/ADP, pyruvate/lactate and NAD+/NADH ratio, as well as measurement of oxygen uptake and mitochondrial biogenesis. The statistical analysis was done by One-Way anova followed by post-hoc Bonferroni test($p<0.05$ considered statistically significant). **Results:** We observed that in the cellular models used, that mitochondria are depolarized and there is a reduction in the expression of the Pgc1 alpha gene in addition to a decrease in oxygen consumption. These changes are also associated with a decrease in ATP / ADP ratio and decreased cell viability. At the same time, we observe that MS-275 and SB were able to rescue the ATP/ADP ratio, restore mitochondrial membrane potential and restore cell viability. **Conclusion:** We conclude that MS-725 and SB, through epigenetic modifications, have shown neuroprotective effects over oxidative stress and excitotoxicity cellular models, restoring mitochondrial membrane potential, ATP/ADP ratio and cellular viability, therefore these compounds show therapeutical potential for not only ALS, but for any disease where oxidative stress and excitotoxicity are an underlying cause. **Financial Support**: FAPESP, CNPq/PIBIC, FAP Santa Casa de São Paulo