ABSTRACTS

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01 Cellular and Molecular Pharmacology

01.001 TGF-Beta1 Modulates The binding of prazosin to human Alpha1A Adrenergic receptors in HEK-293 transfected cells. Quaresma BMCS[1], Waghabi MC[2], Silva CLM[1], Noël F[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]IOC-Fiocruz – Genômica Funcional e Bioinformática

Introduction: Benign prostatic hyperplasia (BPH) is a multifactorial clinical condition characterized by an increased number of stromal and epithelial cells in the periurethral region, which may be related to increased cell proliferation and/or error in programmed cell death (Bushman, Am. J. Physiol. Renal Physiol. 311:817, 2016). Many studies point to the inflammatory process as an important factor in the development and progression of prostatic diseases (Gomes, Inflamm. Res. 67:43, 2018). In the human prostate, fibroblast growth factor (FGF) and transforming growth factor β1 (TGF-β1) are produced by stromal smooth muscle cells and secreted by glandular epithelial cells and are involved in the regulation of prostate growth and proliferation of stromal cells (Story, World J. Urol, 13:297, 1995). As TGF-β1 has been shown to modulate β-adrenergic receptor expression (Mak, Arch. Pharmacol. 362:520, 2000), we raised the hypothesis that TGF-β1 could increase the response of endogenous α1A adrenoceptor (AR-α1A) agonists and thus partially explain the tonic component of BPH. Our objective was to evaluate whether TGF-β1 (one of the main factors of the inflammatory process) could alter the expression or responsiveness of the AR-α1A. **Methods:** Human embryonic epithelial cells (HEK-293) transfected with the human AR-α1A were pretreated with 2.5 ng/ml TGF-β1 for 24 h or 48 h before starting the binding assay with 0.1 nM [^{3}H]-prazosin at 30° C for 1 h. The non-specific binding was determined in the presence of 1 μM prazosin. After the incubation period, the cells were resuspended with the incubation medium and an aliquot was withdrawn for protein dosing. The remainder suspension was filtered under vacuum on glass fiber filters and the radioactivity (Counts per min-CPM) counted in a liquid scintillation counter. For the proliferation assays, the cells were counted by the trypan blue method. **Results:** The specific binding of [^{3}H]-prazosin was significantly lower ($p=0.048$, $n = 6$) in the cells treated with TGF-β1 for 24 hours (4.97 CPM/μg protein) than in control cells (9.59 CPM/μg protein). After TGF-β1 treatment for 48 h, the decrease of [^{3}H]-prazosin binding in the treated group was lower (from 5.29 to 3.42 CPM/μg protein) and not statistically significant ($p=0.12$, $n = 6$). Treatment with TGF-β1 for 24 h did not change the number of cells but there was a small increase (18%) ($p=0.04$, $n = 6$) in the number of cells after 48 hours treatment. **Conclusion:** Our data indicate that TGF-β1 is able to decrease the specific binding of [^{3}H]-prazosin to alpha1A adrenoceptors, at least in HEK-293 transfected cells *in vitro*. As this preliminary experiment was performed using only a non-saturating concentration of the radioligand, we conclude that TGF-β1 may have decreased either the number of AR-α1A or the affinity of prazosin for this receptor. **Financial support**: CNPq

01.003 Luteolin attenuates the inflammatory process and oxidative stress on intestinal mucositis induced by irinotecan in a PPARg dependent way Boeing T[1], De Souza P[1], Speca S[2], Somensi LB[1], Cury BJ[1], Mariano LNB[1], Mena AM[2], Dubuquoy L[2], Bertin B[2], Desreumaux P[2], Da Silva LM[1], Andrade SF[1] [1]Univali – Pharmaceutical Sciences, [2]Université de Lille - Médecine

Introduction: Intestinal mucositis refers to mucosal damage caused by cancer treatment, and irinotecan is one of the agents most associated with this condition when used alone or in combination with other medicines to treat several types of cancer. Focusing on the development of alternatives to deal with this adverse effect, we evaluated the effect of luteolin, a flavonoid widely diffused in medicinal plants, vegetables and fruits, known for its innumerable biological properties. **Methods:** Female mice were treated for 14 days by oral or intraperitoneal route with vehicle (water plus 1% tween) or luteolin (3, 10 and 30 mg/kg) while naïve did not receive any treatment. From the 7th to 10th day, irinotecan was given intraperitoneally (75 mg/kg) to induce mucositis. The mice body weight and diarrhea score were analyzed. After euthanasia, the weight, dehydration and vascular permeability of small intestine and colon were measured. Moreover, histological, oxidative and inflammatory parameters were analyzed, as well as, the tight junctions ZO-1 and occludin by immunofluorescence. The possible interference of luteolin in the antitumor activity of irinotecan was evaluated in mice with melanoma. To determine the mechanism of action of luteolin *in vitro* studies were performed. Fistly, the potential of luteolin in reducing inflammation and oxidative stress induced by irinotecan in CACO-2 cells were evaluated by PCR through mRNA expression of inflammatory and oxidative genes and by ELISA at the protein level. Then, to assess whether the ability of luteolin to control irinotecan-induced damage occurs in a PPARγ dependent manner, experiments were done on cells downregulated for PPARγ (shCACO-2). **Results:** Luteolin attenuated irinotecan-induced intestinal damage by reducing weight loss, diarrhea score of mice, and others macroscopic signs of damage. The histological analyses showed that luteolin prevented villous shortening, vacuolization and apoptosis of cells and preserved mucin production. Moreover, the oxidative balance (LPO, GSH, CAT) was restored and inflammation was reduced (MPO, NO, TNF, IL-6) as well as the disruption of the tight junctions ZO-1 and ocluddin, even though luteolin did not reduce the antitumor activity of irinotecan. In the *in vitro* studies using CACO-2 cells, luteolin significantly increased mRNA expression of PPARγ decreasing mRNA expression of pro-inflammatory cytokines, while, irinotecan decreased mRNA expression of PPARγ and increased cytokines. When the cells were co-stimulated with luteolin and irinotecan, the flavonoid reverted the inflammation and oxidative stress evoked by the chemotherapeutic. However, when these experiments were performed in cells downregulated for PPARγ, luteolin lost the capacity to increase PPARγ expression and decrease mRNA expression of the oxidative genes NRF-2, NQO-1 and pro-inflammatory cytokines. **Conclusion:** In conclusion, the results obtained in the present study evidence for the first time the effect of luteolin in attenuating intestinal mucositis induced by irinotecan, correlating this effect with antioxidant ability and anti-inflammatory capacity of luteolin in a PPARγ dependent way. **License number of ethics committee**: 020/13p. **Financial support**: CAPES, CNPQ

01.004 Polymorphisms of PIK3CA are not associated with the risk for erectile dysfunction. Ferezin LP[1], Azevedo AMM[2], Miyazaki AHL[3], Anselmi GB[3], Molina CAF[4], Tucci JrS[4], Tanus-Santos JE[2], Lacchini R[3] [1]FMRP-USP – Genética, [2]FMRP-USP – Farmacologia, [3]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas, [4]FMRP-USP – Cirurgia e Anatomia

Introduction: Erectile dysfunction (ED) is defined as a constant inability to reach and maintain erection sufficient for a satisfying sexual intercourse. Several factors are involved in the risk and progression of ED, such as increased oxidative stress and reduced production of nitric oxide (NO). The PI3K-AKT axis can affect several signaling pathways. We highlight the phosphorylation of eNOS-nNOS, activating these two enzymes and thereby increasing NO production[1]. Studies have shown PI3K involvement in cardiovascular diseases[2-4]. Since the role of nitric oxide in these diseases is clear, it makes us think that the PI3K pathway may have great cellular and physiological relevance in the context of ED. Therefore, the objective of this study was to evaluate whether PIK3CA (PI3K α isoform) polymorphisms (rs6443624, rs9838411 and rs2699887) are associated with the risk of erectile dysfunction and the severity of symptoms. This is useful to predict patients at higher risk that could benefit from more incisive treatments. **Methods:** were enrolled 136 ED patients from the urology clinic at the Faculty of Medicine of Ribeirao Preto and 98 healthy controls from general population. All subjects had blood collected for genetic tests and were interviewed. Erectile function was assessed by the International Index of Erectile Function (IIEF) Questionnaire. Genotyping was performed by Real Time Polymerase Chain Reaction. Statistical analysis was performed using Chi-squared tests or Kruskall-Wallis test followed by Dunns post-hoc test. **Results:** we found no associations between rs6443624, rs9838411 and rs2699887 and erectile dysfunction (P = 0.661, P = 0.341, P = 0.877, respectively). In addition, polymorphisms were not associated with changes in the IIEF score, either in patients (rs6443624 p = 0.437; rs9838411 p = 0.371; rs2699887 p = 0.772) or in healthy controls (rs6443624 p = 0.878; rs9838411 p = 0.959; rs2699887 p = 0.321). Our future objectives in this study are to implement more sophisticate analyses (such as multivariate linear and logistic regression models), and the evaluation of the association of these genetic markers with Sildenafil responsiveness (data still being generated). **Conclusion:** PIK3CA polymorphisms rs2699887, rs6443624 and rs9838411 are not associated with ED risk, nor with the intensity of disability. [1] HURT, K. J. et al. Proc Natl Acad Sci U S A, v. 109, p. 16624, 2012.; [2] CONTRERAS, C. et al. Br J Pharmacol, v. 161, p. 350, 2010; [3] GHIGO, A. et al. Curr Heart Fail Rep, v. 8, p. 168, 2011; [4] GUO, D. et al. Circ Res, v. 107, p. 1275, 2010. **License number of ethics committee**: 51408515.9.0000.5393 **Financial support**: FAPESP (grant 2016/04449-0)
Key-words: erectile dysfunction, polymorphisms, PIK3CA

01.006 Telmisartam inhibits osteoblastic differentiation of mesenchymal stem cells from hypertensive rats Patrocinio MS[1], Brito VBG[1], Barreto AEA[1], Sousa MCL[1], Frasnelli ST[1], Balassoni BB[1], Lara VS[2], Santos CF[3], Oliveira SHP[1] [1]FOA-Unesp – Ciências Básicas, [2]USP – Patologia, [3]USP – Farmacologia

Introduction: Local renin-angiotensin system is been related to osteometabolic disorders, and therefore, antihypertensive drugs have been related to bone homeostasis, such as telmisartan (Telm), an angiotensin II type 1 receptor (AT1R) antagonist, which also exhibit partial PPAR-γ agonist effect. Then, we aimed to investigate the effects of Telm during *in vitro* osteoblastic differentiation of bone marrow-derived mesenchymal stromal cells (BM-MSC) from Spontaneously Hypertensive Rats (SHR). **Methods:** Male SHR (70 day-old) were euthanized and femurs were dissected for BM-MSC harvest, cells were expanded in culture flasks and after reaching confluence, were seeded into 24-well plates, where they received the osteogenic medium (OM; DMEM plus ascorbic acid 50 µg/mL, dexamethasone 10^{-8} M and β-glycerophosphate 10 mM) and Telm treatment (0.005, 0.05, and 0.5 µM). We analyzed cell viability (MTT assay) and alkaline phosphatase activity (ALP) at day 0, 7, 14 and 21 (enzymatic colorimetric assays), and mineralization at day 14 and 21 (Alizarin Red S staining). Institutional Animal Care and Use Committees approval, nº 00430-2017. **Results:** Control medium group showed increased proliferation rate during the analyzed periods (OD value; d0: 0.46±0.02, d7: 1.55±0.16, d10: 1.78±0.05, d14: 1.87±0.11, d21: 2.51±0.25), while OM group showed lower proliferation rate (d0: 0.46±0.02, d7: 0.62±0.04, d10: 1.08±0.07, d14: 1.08±0.04, d21: 0.60±0.14), and decreased viability at d21, compared to control medium. Higher concentrations of Telm were cytotoxic at d7 (0.05 µM: 0.54±0.03 and 0.5 µM: 0.50±0.04), d10 (0.94±0.08 and 0.97±0.06) and d14 (0.94±0.05 and 1.00±0.02), compared to OM group (non-treated). The OM group showed a characteristic ALP pick at d10 (U/mg of protein; 0.36±0.01), compared to control medium group (0.27±0.05), with a posterior ALP decrease at d14 (0.21±0.04) and d21 (0.14±0.06). Telm reduced ALP at d10, in a dose-dependent manner (0.005 µM: 0.26±0.3; 0.05 µM: 0.25±0.02, 0.5 µM: 0.18±0.02), compared OM group. The mineralization was observed in OM group at d14 (% stained area; 21.90.36±6.68), which was intensified at d21 (58.87±5.70), compared to control medium group (d14: 0.42±0.16 and d21: 0.70±0.14), as expected. Telm 0.005 µM did not altered mineralization (d14: 27.64±4.80 and d21: 55.47±5.57), however, higher doses had an inhibitory effect at d14 (0.05 µM:2.26±1.52 and 0.5 µM: 6.80±1.10) and d21 (0.05 µM:5.76±0.73 and 0.5 µM: 13.46±0.35), compared to MO group. **Conclusion:** Our data demonstrated an inhibitory effect of Telm on *in vitro* osteoblastic differentiation of BM-MSC from SHR, saw by ALP inhibition and decreased mineralization, possibly by AT1R antagonism and/or partial PPAR-γ activation. Further studies are current been conducted to clarify this hypothesis. **Financial support:** FAPESP 2017/02271-2 e 2015/03965-2. **License number of ethics committee**: 00430-2017 **Financial support**: FAPESP 2017/02271-2 e 2015/03965-2.

01.007 Supernatant from osteoblasts of normotensive rats increases mineral deposition of mesenchymal stem cells from Spontaneously Hypertensive Rats (**SHR**). Ballassoni BB[1], Barreto A[1], Brito VB[1], Jordão JVR[1], Patrocínio MS[1], Frasnelli ST[1], Oliveira SHP[1] [1]Unesp – Imunofarmacologia

Introduction: The proliferation and differentiation of osteoblasts is controlled by several local and systemic factors that contribute to bone homeostasis, however hypertension is a systemic pathology that interfere negatively in this balance. Spontaneously Hypertensive Rats (SHR) are a good animal model of human essential hypertension and it is known that the osteoblastic differentiation of these animals is compromised in relation to a normotensive related model (Wistar). In this study, we propose to evaluate if the bone damage of SHR is due to local or systemic factors and if this cell is responsive to exogenous differentiation factors from osteoblasts of normotensive rats. **Methods:** 70-days-old SHR and Wistar males were euthanized for dissection of the femurs and bone marrow collection, which was placed in cell culture flasks and after in to 24-well plates, where they received osteogenic medium (OM: MEM, plus 50 µg/mL ascorbic acid, 10 mM β glycerophosphate, and 10^{-8} M dexamethasone) for 7 days. Then, SHR cells were treated with different dilutions (1:1, 1:2, 1:3) of 24 hours supernatant medium (SM) from Wistar osteoblasts. Cell viability (MTT assay), alkaline phosphatase (ALP) and total proteins were evaluated at days 7, 10 and 14 and mineralization was accessed at day 14 by Alizarin Red and quantified by pixel densitometry using Image J software. The protocol was approved by Institutional Animal Care and Use Committees (School of Dentistry of Araçatuba; Process 00686-2016). **Results:** The OM groups had a reduction in the proliferation rate of cell on day 7 (0,36 ± 0,06), day 10 (0,48 ± 0,09) and day 14 (0,46 ± 0,04). Treatment with SM 1:1 reduced cell viability at day 10 (0,30 ± 0,02) and day 14 (0,28 ± 0,03). However, SM 1:2 at day 10 (0,45 ± 0,01) and day 14 (0,42 ± 0,08) did not reduce cell proliferation at both periods, as well as SM 1:3 (day 10: 0,46 ± 0,01; day 14: 0,42 ± 0,02). The total protein content did not change in the initial periods in all groups, only at day 14, in which the group treated with SM 1:1 had a reduction in the protein content (0,20 ± 0,04), while SM 1:2 (0,25 ± 0,01) and 1:3 (0,23 ± 0,05) did not promote significant modifications. ALP activity increased over time in the OM group (day 7: 2,29 ± 0,10; day 10: 19,6 ± 1,63), while the SM 1:1 promoted a reduction in this enzyme activity (day 10: 15,8 ± 1,06; day 14: 6,12 ± 0,21). Mineralization showed that the group treated with SM 1:1 demonstrated a higher mineral density (10.237 ± 3.550) compared to the group treated with OM (58.368 ± 4.741). The SM dilutions gradually reduced this parameter according to the dilution used. **Conclusion:** The supernatant of osteoblastic cells from Wistar rats was able to improve the osteoblastic differentiation of mesenchymal stem cells from SHR. It suggests that factors that impair bone formation in hypertensive rats are derived from bone itself microenvironment and such cells are responsive to exogenous factors from control animals. Acknowledgments: FAPESP (2015/03965-2). **License number of ethics committee**: 00686-2016 **Financial support**: FAPESP (2015/03965-2).

01.008 Conditioned medium derived from obese adipose tissue induces 3T3-L1 adipogenesis via Toll-like receptor 4. Moreira Nunes CV[1], Ramos LR[1], Lemos LC[1], Barja-Fidalgo TC[2], Renovato Martins M[3], Moraes JA[1] [1]UFRJ – Farmacologia, [2]UERJ – Biologia Celular e Molecular, [3]UFF – Biologia Celular e Molecular

Obesity is characterized by a chronic low-grade inflammation and is a major problem of public health, especially because of its association with several diseases. Thus, understanding the molecular mechanisms underlying this condition is critical to find therapeutic targets. Adipogenesis is the differentiation of preadipocytes into adipocytes, leading to an increase of adipocytes cell. Once in a proinflammatory state the mature adipocytes secrete adipokines, such as TNFα, IL-6 and IL-1β. Toll Like receptor 4 (TLR4) is expressed in immune cells and other cell types, including preadipocytes and its main function is to launch an inflammatory response in the presence of LPS from Gram-negative bacteria. However, fatty acids can also bind and activate TLR4 mediated pathways. In this scenario, we aimed to evaluate if adipose tissue conditioned medium from lean and obese mice regulate adipogenesis in 3T3-L1 preadipocytes as well as the role of TLR4 on these effects. 3T3-L1 viability was assayed by MTT method. Western blotting was performed to PPARɣ e CEBPα. ROS production was analyzed by DCF probe. ELISA was performed to IL-1β, IL6 and TNF-α. To adipogenesis assay cells were stimulated with differentiation medium, 20% conditioned medium (CM) derived from adipose tissue (AT) explants obtained from lean mice or 20% CM from obese (Ethic committee number: 042/16). Lipid accumulation was quantified using Oil Red O (ORO) stain. Initially, we evaluated that CM-derived from obese did not affect 3T3-L1 viability. Additionally, we showed that 3T3-L1 preadipocytes stimulated with CM- derived from obese AT showed higher lipid accumulation, compared to cells treated with CM- derived from lean AT. PPARɣ and CEBPα are two master regulators of adipogenesis and the protein expression levels of these was upregulated by the obese CM- after 48h and 72h. We also observed that 3T3-L1 activation was accompanied by an increase of ROS production. Furthermore, we observed that CM-derived from obese AT induced release of IL1- β, TNF- α and IL6. Interestingly, when an antagonist of TLR4 (TAK) receptor was added the effects of the obese CM- were inhibited. Our results suggest that the fatty acids from the obese CM, but not from lean, activates TLR4 receptor that in turn stimulated PPARɣ and CEBPα expression leading to an increment in lipid accumulation. Our data indicate that the effects mediated by obese CM is suppressed when 3T3-L1 preadipocytes were treated with a TLR4 antagonist. In this regard, this suggests that TLR4 can be a therapeutic target to attenuate the proinflammatory profile observed in obesity attributable to a decrease in inflammation and generation of new adipocytes. **License number of ethics committee**: 042/16 **Financial support**: CAPES, CNPq, FAPERJ

01.009 Oxidative stress in mesenteric endothelium cells: **Relevance on schistosomiasis endothelial dysfunction** Monteiro MMLV[1], Posso SMV[2], Valença SS[2], Silva CLM[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UFRJ – Farmacologia e Inflamação

Introduction: Schistosomiasis is a neglected tropical disease caused by *Schistosoma mansoni*. The parasite lives inside mesenteric vessels establishing a chronic infection sustained by host immune responses and leading to portal-mesenteric inflammation. Under homeostatic conditions endothelium cells inhibit leucocyte adhesion, however, during schistosomiasis occurs an endothelial dysfunction inducing an inflammatory phenotype (Oliveira et al., 2011, Plos One, 6:e23547). Endothelial dysfunction could be secondary to cell stress caused by an increased reactive oxygen species (ROS) production such as superoxide anion. The aim of the present work was to evaluate ROS formation and antioxidant enzymes expression in mesenteric endothelial cells (MEC) from control and *S. mansoni*-infected mice. **Methods:** CEUA 048/16. Male Swiss mice were divided in two groups (control and *S. mansoni*-infected mice). Seventy-five-day-old mice were anesthetized and euthanized. Mesenteric vessels were removed, minced and plated with DMEM enriched with 20% fetal bovine serum and streptomycin/penicillin, and maintained at 37ºC, 5% CO_2 until confluence. ROS quantification assay adapted from Choi *et al.*, 2006, J. Immunoassay Immunochem, 27:31 used blue nitrotetrazolium (NBT). Confluent MEC (1st passage) were incubated with NBT at 37ºC for 1h, the formazan crystals were solubilized (DMSO/KOH 2M) and read at 630 ŋm. Results were expressed in arbitrary units (a.u.) of ROS/10^3cells. Total protein extracts were harvested from CEM, lysed in RIPA buffer, supplemented with protease inhibitors and analyzed by Western blotting for superoxide dismutase (SOD1), glutathione peroxidase (GPx1) and the oxidative stress marker nitrotyrosine (PNK) expressions. Statistical analysis was performed by using Student's t-test expressed as means ± SEM and $P < 0.05$ was considered significant. **Results:** MEC from infected group (0.06 ± 0.005 a.u.) produced twice ROS as compared to control (0.028 ± 0.005 a.u., n= 5 ***P<0.001). Peroxynitrite ($ONOO^-$) is formed by the reaction of superoxide anion ($O_{2^{\cdot-}}$) and nitric oxide (NO), and it causes nitrosylation of tyrosine residues (PNK). Infected group (INF) had less PNK levels than control (CO) (CO = 1,84 ± 0,05 u.a., INF = 1,44 ± 0,13, n=3, *P<0,05). The smallest production of $ONOO^-$ in *S. mansoni*-infected mice could result from the reduced NOS expression and NO production in this group (Oliveira et al., 2011, Plos One, 6(8):e23547). Preliminary WB data showed an enhanced expression of SOD1 in *S. mansoni*-infected mice (0.07 a.u.) than control (0.05), which could be a compensatory response. There was no alteration of endothelial GPx1 expression (CO = 1.2 ± 0.04 a.u.; INF = 1.3 ± 0.04 a.u., n=3, *P<0.05). **Conclusion:** The phenotype of mesenteric endothelial cells during schistosomiasis is altered by an increased superoxide anion production, a hallmark of endothelial dysfunction. **License number of ethics committee**: CEUA 048/16 **Financial support**: CAPES FAPERJ CNPQ

01.010 A pharmacological and non-pharmacological method of inducing cellular rhythm to visualize Dicer expression pattern in adipocytes. Ruiz GP[1], Rodrigues JQD[1], Castrucci AML[2], Mori MAS[1] [1]Unicamp – Bioquímica e Biologia Tecidual, [2]IB-ICB-USP

Introduction: Adipose tissue plays an important role in metabolism regulation. It is well known that the desynchronization of the circadian clock triggers the development of obesity and metabolic diseases. DICER is a key enzyme in microRNAs processing, and it has been described to exhibit a circadian pattern of expression in several tissues, and this is lost in metabolic diseases (Yan et al., 2013). Yet, there is no evidence that supports a rhythmic expression of DICER in adipose tissue. **Aims:** To investigate the daily rhythm of Dicer mRNA and clock genes in a murine model of beige adipocytes (9W cells) subjected to synchronization by fetal bovine serum (FBS) and dexamethasone (Dex). **Methodology:** 9W pre-adipocytes were mantained and differentiated into beige adipocytes using a protocol previously described by our group. After differentiation, two different treatments were applied to synchronize circadian rhythm, *i.e.* FBS shock and Dex stimulation (Balsalobre et al., 1998; Balsalobre et al., 2000). At circadian time 0 (CT 0), cell culture medium was replaced either with DMEM 50 (50% FBS) for 2 hours (FBS shock), or DMEM 0 (0% FBS) 100nM dexamethasone for 15 min (Dex stimulation). The medium was then changed to DMEM 0, and samples were collected every 4 hours for a period of 24 hours starting 12 hours after the first medium exchange (CT12). Quantitative qRT-PCR was performed using specific primers for *Dicer*, *Per1*, *Bmal1* and *18S* or *36-B4* genes (for housekeeping normalization). The repeated-measures ANOVA statistical test was used followed by the Bonferroni post-test. Gene expression circadian rhythms were analyzed using the Cosinor equation. **Results:** *Dicer* expression displayed a circadian pattern in 9W beige cells synchronized with FBS shock or Dex. Importantly, the control group did not exhibit statistically significant circadian oscillations, demonstrating that both treatments were effective in synchronizing the beige adipocyte culture. In the FBS protocol, acrophase was observed 24 hours post-shock, and the amplitude registered was 0,51. In the Dex protocol, acrophase was observed 28 hours post stimulation, and the amplitude was 0,85. The amplitude is calculated as the difference between the maximum value (acrophase) and the mesor. Expression of *Bmal1* and *Per1* clock genes after the FBS shock protocol did not present statistically significant differences when compared to the control group, but they did display a circadian oscillation with the Dex stimulation, the amplitude registered of *Bmal1* expression was 0,43. Moreover, the different synchronizing agents induced non-simultaneous periods, as expected, which can be visualized through the distinct profiles of expression for *Dicer.* **Conclusion:** *Dicer* shows a circadian pattern of expression in beige adipocytes treated with FBS and dexamethasone. Dexamethasone treatment is also able to synchronize the clock genes, besides inducing a greater amplitude in *Dicer* expression, showing to be a more potent synchronizer than the FBS shock. **Financial support**: FAPESP (2017/01184-9; 2016/17075-1; 2012/50214-4) and CNPq (303070/2015-3).

01.011 *In vitro* **evaluation of the vasodilating effect of neolignan conocarpan in different rat arteries**. Souza PDN[1], Barenco TS[2], Marques AM[3], Nascimento JHM[2], Ponte CG[1] - [1]IFRJ – Fisiologia e Farmacologia, [2]UFRJ – Biofísica e Fisiologia, [3]Fiocruz – Química de Produtos Naturais

Introduction: Systemic arterial hypertension (SAH) and pulmonary arterial hypertension (PAH) are diseases that affect vascular function, affecting a large part of the population. Although there are medications for SAH, there are still patients pharmacologically subtreated and for PAH there is still no effective class of drugs for treatment. In order to find new therapeutic substances for treatment of these diseases, our research group developed a study with neolignan conocaropan, a compound extracted from the *Piper rivinoides*, in vascular reactivity in different types of rat arteries. **Objective:** To evaluate the effect of neolignan conocarpan *in vitro* in the ring of aorta and pulmonary arteries and perfusion of rat mesenteric arterial bed. **Methods:** Were used male Wistar rats, with 250 - 350 g (CEUA / CCS / UFRJ protocol nº 087/15). The vasodilator effects of conocarpan was evaluated using isometric tension of aorta and pulmonary isolated rings (n = 5) in a modified Krebs solution. The basal tension was 1 g of force and the maximum tension was obtained with 1 µM of phenylephrine. The perfusion of mesenteric arterial bed (n = 7) *in vitro*, after bowel remove, is made by the mesenteric artery with constant flow of modified Krebs solution. At the constant flow rate of 5 ml per minutes of solution the arterial resistance was maintained throughout the experiment in the presence of 10 µM of phenylephrine. All experiments were maintained at 37 ° C and aerated with the mixture of 95 % O_2 e 5 % CO_2 and were submitted to cumulative concentrations of conocarpan between 0,1 µM and 30 µM. Statistical analysis with Shapiro-Wilk normality test followed by Anova with Tukey post test at $p < 0.05$ considered significant. **Results:** The conocarpan has a significant vasodilator activity only at a concentration of 30 µM in aorta and pulmonary artery rings. The mesenteric arterial bed was more sensitive to the effect with an IC50 of 1.8 (± 0,0069) µM. **Conclusions:** For being a natural products with relaxing properties of resistance vessels in an arterial bed *in vitro*, conocarpan is a candidate for *in vivo* studies with models of PAH and SAH. **License number of ethics committee**: CEUA / CCS / UFRJ protocol nº 087/15 **Financial support**: CNPq, Faperj e IFRJ

01.012 Conditioned medium derived from human obese adipose tissue induces neutrophil activation via Toll-like receptor 4. Ramos LR[1], Lemos LC[1], Moreira Nunes CV[1], Guimarães-Bastos D[2], Andrade IR[2], Barja-Fidalgo TC[2], Renovato-Martins M[3], Moraes JA[1] [1]UFRJ – Farmacologia, [2]UERJ – Biologia celular, [3]UFF – Biologia Celular e Molecular

Introduction: Obesity is a disease characterized by excessive accumulation of body fat, which causes deleterious effects on health. This abnormal development of adipose tissue functions leads to an inflammatory site, reflecting in the increase of the number of immune system cell infiltrates, such as neutrophils. These cells can be classified as N1 (proinflammatory neutrophils) and N2 (anti-inflammatory neutrophils). The neutrophil polarized to the N1 profile produces high amounts of proinflammatory cytokines (TNF-gamma, IL-6 and IL-1 beta), whereas N2 cells exhibit low microbicidal activity. Obese adipose tissue is able to release fatty acids, which in turn are capable of binding and activating Toll-Like receptor 4 (TLR4) mediated pathways, which are expressed in immune cells, such as neutrophils. In this study we investigated the effect of molecules secreted by adipose tissue from obese and lean individuals in neutrophil activation, focusing on the possible involvement of TLR4. **Methods and materials**: Human neutrophils (PMN), isolated by Percoll gradient, were incubated in the presence of conditioned medium (CM) derived from adipose tissue (AT) explants obtained from obese patients or lean individuals or LPS (10 µg / mL). Chemotaxis was assessed in modified Boyden's chamber after 1 h of migration. Apoptosis was evaluated by morphological analysis after 20 hours of treatment. ROS production was analyzed by the DCF probe until 90 minutes of treatment. Analysis of IL-6, IL-1β and TNF-gamma were performed by ELISA assay. **Results:** We showed that CM obtained from the AT of obese patients reduced the number of apoptotic neutrophils, whereas this effect was not observed with the CM obtained from AT of lean individuals. In addition, we observed that CM from AT of obese patients induced increased production of reactive oxygen species and increased IL1-beta and TNF-alpha. In addition, when we pretreated the cells with the TLR4 receptor antagonist (TAK), we showed that the effects observed by treatment with CM from AT of obese patients were inhibited. Finally, we observed that CM from AT of obese patients was able to induce neutrophils migration, an effect that was shown to be independent of TLR4 activation and dependent on AKT pathway. **Conclusion:** In this study we observed that the CM obtained from AT of obese patients was able to activate and induce different effects on neutrophils, most of these effects being dependent on TLR4 pathway. Thus, we point TLR4 as a possible pharmacological target for the combat against inflammation during obesity, which could reduce the appearance of several comorbidities. **License number of ethics committee**: 36880914.0.0000.5259
Financial support: CAPES, CNPq, FAPERJ

01.013 Blockade of β1 adrenergic receptor impairs osteogenic differentiation in mesenchymal stem cells from normotensive rats. Barreto AEA[1], Brito VGB[1], Patrocínio MS[1], Sousa MCL[1], Frasnelli SCT[1], Balassoni BB[1], Santos CF[2], Lara VS[3], Oliveira SHP[1] [1]FOA-Unesp – Ciências Básicas, [2]FOB – Farmacologia, [3]FOB – Estomatologia

Introduction: The sympathetic nervous system (SNS) is pointed as important regulator of bone metabolism through adrenergic receptors on the surface of the osteoblasts. The hypertension is an example of high activity of SNS, which can lead to bone loss. In this way, the blockade of adrenergic receptors using anti-hypertensive drugs are showed to be protective against this effect. However, the role of β-adrenergic receptors is not clear in the osteogenic differentiation process. Thus, in this study we aimed to evaluate the effect of β-adrenergic receptor on osteogenic differentiation of mesenchymal stem cells from normotensive rats and spontaneously hypertensive rats (SHR). **Methods:** 70-days-old male Wistar and SHR rats were used for bone marrow collection from femurs, which was placed in cell culture flasks and after in to 24-well plates, where they received osteogenic medium (OM: MEM, plus 50 µg/mL ascorbic acid, 10 mM β glycerophosphate, and 10^{-8} M dexamethasone) and the treatment with Carvedilol (1µM), non-selective adrenergic receptor antagonist, or Nebivolol (0,1 µM), β1-adrenergic receptor antagonist. Cell proliferation (MTT assay) and alkaline phosphatase specific activity (Alp) were analyzed at day 7, 10 and 14. Mineralization was accessed at day 14, by Alizarin Red S. Gene expression of osteogenic markers were evaluated at day 7 and β1 receptor (Adrb1) expression was evaluated at day 7 and 10, by real time-RT-PCR. The protocol was approved by Institutional Animal Care and Use Committees (School of Dentistry of Araçatuba; Process 00686-2016). **Results:** Alp activity increased in OM group of Wistar rats at day 7 (50,6 ± 6,9) and the Nebivolol group reduced its activity at day 7 (26,4 ± 2,8) and day 10 (33,7 ± 1,4). SHR OM group did not present Alp increase (13,3 ± 1,3), and the treatment with both drugs did not change it (CV: 12,5 ± 0,6; NB: 19,9 ± 0,6). Nebivolol treatment decreased Runx2 (1,4 ± 0,1), Osterix (0,7 ± 0,1) and β-catenin (0,3 ± 0,1) in Wistar compared to OM (Runx2: 1,9 ± 0,5; Osx: 2,5 ± 0,1; Catnb: 1,3 ± 0,1). In SHR, only Osterix was reduced (0,7 ± 0,1). in Wistar cells treated with Nebivolol, Alp (0,3 ± 0,1), Osteocalcin (597,3 ± 30,6) and Bone sialoprotein (117,3 ± 4,8) have significative decrease compared to OM (Alp: 1,1 ± 0,1; OC: 3058,8 ± 86,6; BSP: 162,4 ± 14,1). Nebivolol reduced Adrb1 expression at day 7 in Wistar group (4,1 ± 0,1) compared to OM (6,2 ± 0,5). Mineralization showed that Nebivolol reduced mineral deposition in Wistar (16,6 ± 1,1) compared to OM (72,1 ± 4,8). **Conclusion:** β1 adrenergic receptor seems to be involved in the osteogenic differentiation of cells from Wistar rats but not from SHR at day 14. Financial support and acknowledgments: FAPESP (Grant: #2015/03965-2) and CAPES. **License number of ethics committee**: 00686-2016 **Financial support**: FAPESP (2015/03965-2) and CAPES

01.014 Cinnamic acid and their derivatives promotes fibroblast migration *in vitro* **by activating the PKA signaling pathway**. Aquino FLT[1], Dias AO[1], Ferro JNS[2], Conserva LM[3], Barreto EO[1] [1]UFAL – Biologia Celular, [2]UFPE – Terras Raras, [3]UFAL – Química de Produtos Naturais

Introduction: Cinnamic acid and its derivatives occur naturally in high levels of plant-based foods. Among various biological activities, these compounds are associated with wound healing activity. It is well established that fibroblast proliferation and migration play important roles in healing. However, the effect of cinnamic acid and its derivatives on fibroblast proliferation and migration are still relatively little known. Here, we aimed to investigate the effects of cinnamic acid and two of its derivatives, the methyl cinnamate and p-coumaric acid, on migration and proliferation processes *in vitro* wound-healing scratch assay. **Methods:** NIH 3T3 fibroblast were cultured in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 0.02% penicillin/streptomycin, and maintained at 37°C with 5% CO2 in a humidified atmosphere. Cells were exposed at 3, 10, and 30 µM cinnamic acid (CA), methyl cinnamate (MC), or p-coumaric acid (p-CA) during 30 hours. Next, cell viability was determined using MTT assay, while fibroblast migration was investigated using the scratch assay using mitomycin C. Fibroblasts were cultured until confluent. Then a linear scratch wound was created and treated with cinnamic acid and its derivatives. Three representative areas of the scratch in each culture were photographed and the scratch area was quantitated using image analysis software (ImageJ). Inhibitors of p38MAPK, JNK, PKA and CREB were used to investigate whether these signaling pathways are involved in migratory events. Additionally, the effect of cinnamic acid and its derivatives on the G2/M phase from cell cycle of fibroblasts was assessed by flow cytometry. Statistical significance between groups was determined by ANOVA followed by Bonferroni's test ($p<0.05$). **Results:** We noted through the MTT assay that cinnamic acid and its derivatives (3-30 µM) had no cytotoxic effects. Compared to untreated cells, treatment with 30 µM CA, MC, and p-CA significantly increased fibroblast migration in 38%, 37%, and 40%, respectively. The increase in migratory activity induced by CA and MC, but not by p-CA, were inhibited by treatment with PKA inhibitor, PKI-(6-22)-amide, in 22% and 78%, respectively. In contrast, only p-CA-induced migration was inhibited (46%) by SB203580, a specific inhibitor of p38-MAPK pathway. On the other hand, the selective JNK inhibitor, JNK-IN-8, was able to reduce in 65% the fibroblasts migratory response induced by MC but not by the treatments with CA or p-CA. By flow cytometry, treatment with CA, MC, and p-CA increased the percentage of fibroblasts in G2/M stage of the cell cycle in 30%, 22% and 29%, respectively. **Conclusion:** Therefore, the results indicate that cinnamic acid and its derivatives possessed a positive effect on the migration of fibroblasts through distinct mechanisms. In addition, these compounds seem to favor the cell proliferation by increasing the number of cells at G2/M phase. Application of these compounds may be beneficial as a wound-healing stimulating agent.
Financial support: CNPq

01.015 Evaluation of the neolignan conocarpane effect in vascular reactivity and monocrotaline-induced arterial hypertension in rats. Barenco TS[1], Souza PDN[2], Marques AM[3], Nascimento JHM[1], Ponte CG[2] [1]UFRJ – Biofísica e Fisiologia, [2]IFRJ – Fisiologia e Farmacologia, [3]Fiocruz – Química de Produtos Naturais

Introduction: Systemic arterial hypertension (SAH) and pulmonary arterial hypertension (PAH) are diseases that affect vascular function, causing high rates of morbidity and mortality. Given the need to discover new therapeutic tools, there is interest in researching new biologically active substances from plant species, such as lignans, secondary metabolites with a wide biological activities' variety. Among them, the neolignan conocarpan, although it has some known biological effects, little is known about its activity on cardiovascular system. **Objective:** To evaluate the neolignan conocarpan's effect on resistance vessels, characterizing its mechanism of action and identifying a possible therapeutic effect on an experimental model of PAH. **Methods:** Male Wistar rats weighing 200-300 g were used in all experiments. Conocarpan's effect on vascular reactivity, and in presence or absent of inhibitors/blockers, was evaluated using Mulvany's Miograph, in rings of 2nd mesenteric branch arterioles. The rings were maintained in Krebs-Henseleit solution at 37ºC, aerated with carbogenic mixture, and contracted by phenylephrine. The conocarpan's therapeutic effect on a monocrotalin-induced (MCT) PAH model in rats, distributed in 3 groups (n=5 for each group): control, monocrotalin-induced PAH and conocarpan-treated PAH was evaluated. The animals received an injection of MCT (60 mg/kg i.p.) and after 14 days the 2-week treatment with conocarpan started. The parameters were evaluated by electrocardiography, echocardiography, blood pressure and biometric data. (CEUA/CCS/UFRJ protocol No. 087/15). **Results:** In vascular reactivity, conocarpan had vasodilator activity on tension generated by mesenteric arterioles' rings with intact endothelium (IC_{50}=1.08 µM); this effect was significantly reduced in rings without endothelium (IC_{50}=11.9 µM). In presence of inhibitors/blockers, we have seen that conocarpan's vasodilatory capacity was partially inhibited by iberiotoxin, glibenclamide, 4-AP and loratadine, totally inhibited by ODQ, minimally reduced by indomethacin and atropine had no effect on it. In PAH model, hypertensive animals showed increased right ventricular systolic pressure (RVSP) and right ventricular hypertrophy (RV), as well as decreased systemic arterial pressure (SAP) and increased QT interval, pulmonary vascular resistance and relative weights of heart and lung. The treatment with conocarpan increased the animals' survival, but despite having improved the RV hypertrophy index, it was not able to significantly decrease its area and thickness. In QT interval, the observed decrease was also not significant. The treatment was not able to improve neither pulmonary vascular resistance, as observed by echocardiography and PSVD, nor SAP. Despite this, cardiac hypertrophy and pulmonary congestion rates were reduced. **Conclusion:** We conclude that conocarpan has a vasodilatory effect on resistance arteries, with great influence of the endothelium. New studies are needed about its effects on PAH, as its therapeutic potential should be evaluated on other experimental models involving changes in resistance vessels, such as SAH. **License number of ethics committee**: CEUA / CCS / UFRJ protocol No. 087/15 **Financial support**: CAPES, CNPq, IFRJ

01.016 Uvaol Improves angiogenic functions of endothelial cells and accelerates fibroblast migration through JNK- and PKA-mediated mechanisms. Carmo JOS[1], Cavalcante RWC[1], Ferro JNS[2], Correia ACC[3], Lagente V[4], Barreto E[1] [1]UFAL – Biologia Celular, [2]UFPE – Química Fundamental, [3]UPE-Garanhuns, [4]University of Rennes – NUMECAN

Introduction: Uvaol is a natural pentacyclic terpenoid exhibiting a wide range of pharmacological actions, including wound healing properties. However, the effects of uvaol in both fibroblasts and endothelial cells functions are still poorly understood. Thus, we aimed to investigate *in vitro* the effect of uvaol on cellular events associated with wound healing, such as fibroblast activation and the angiogenesis. **Methods:** The mouse cell lines tEnd.1 and NIH 3T3 fibroblasts were maintained respectively in RPMI and DMEM medium, both supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 0.02% penicillin/streptomycin and maintained at 37°C with CO2 (5%) in a humidified atmosphere. Endothelial cells (tEnd.1) were exposed at 10, 50, and 100 µM uvaol during 24 hours. Next, cell viability, cellular migration, and angiogenic potential were evaluated by using MTT test, scratch migration assay, and matrigel-based tube formation assay, respectively. In another set of experiments, the production of fibronectin, laminin and type-I collagen in the uvaol-treated fibroblasts at 50 µM for 24 h were evaluated by immunofluorescence. In addition, the scratch assay was used to evaluate the fibroblasts migration after uvaol treatment. In that same experiment were used inhibitors of p38MAPK, JNK, and PKA to investigate whether these signaling pathways are involved in uvaol-induced migratory events. All assays were performed in three independent controlled experiments. Statistical significance between groups was determined by ANOVA followed by Tukey's test ($p<0.05$).

Results: Compared to untreated cells, the migration of tEnd.1 cells was enhanced by treatment with uvaol at 10 or 50 µM in 26% and 28%, respectively. In addition, uvaol (10 µM) markedly improved in 59% and 73% the tube formation of capillaries by t.End.1 cells at 3h and 6h upon incubation on matrigel, respectively. Using immunofluorescence assay we observed that uvaol, only at 50 µM, increased expression of laminin in 16% and fibronectin in 23% compared to untreated cells. Uvaol, in all concentration tested, did not affect the production of collagen type I in fibroblasts. It was also found that in scratch assay, the uvaol (50 µM) enhanced the migration of fibroblasts in 22% compared to untreated cells. The uvaol-induced fibroblasts migration evaluated in the scratch assay was abolished by the JNK inhibitor (JNK-IN-8) and PKA inhibitor (PKI-(6-22)-amide), but not by p38MAPK inhibitor (SB203580). No cytotoxicity of uvaol was found in our experiments. **Conclusion:** Our data provide evidence for uvaol to induce angiogenic effects by stimulates endothelial cell migration and formation of tube-like structures *in vitro*. Furthermore, we also have shown that uvaol stimulated the expression of laminin and fibronectin by fibroblasts, beyond increasing migratory response by JNK and PKA signaling pathway. **Financial support**: CNPq and FAPEAL.

01.017 Study on the participation of endogenous hydrogen sulfide (H2S) on the cholinergic induction of saliva secretion in mice. Alves MCO, Teixeira SA, Costa SKP, Muscará MN ICB-USP – Farmacologia

Introduction: Saliva is of utmost importance for the maintenance of oral and general homeostasis. Hydrogen sulfide (H_2S) exerts multiple effects on most of the organic systems, participating of the regulation of cellular metabolism, vascular and inflammatory responses. However, the role of H2S on the salivation process has never been addressed to date. In this way, we aimed to study the participation of endogenous H_2S on the ex vivo carbachol (CCh)-induced salivation in mice. **Methodology:** The experimental protocol was approved by the local Ethics Committee for Animal Experimentation (CEUA-ICB/USP 81/2017). Female Swiss mice (6-8 wk-old) were anesthetized with tribromoethanol 200 mg/kg and the submandibular and sublingual salivary glands were collected and immediately placed in 5 ml of Krebs solution at 37°C under continuous bubbling with O2/CO2 (95/5 %v/v). After 30 min stabilization, a sample of the bath solution was collected to measure the secreted (basal) amylase activity and cumulative CCh concentrations (10-8 - 10-6 M) were immediately added after the sample collections every 10 min. The procedure was repeated in the presence of propargylglycine (PAG; 10 mM) and aminooxyacetic acid (AOAA; 20 mM), respective inhibitors of the H2S producing enzymes cystathione gamma lyase (CSE) and cystathionine beta synthase (CBS). Amylase activity was measured spectrophotometrically using 2-chloro-4-nitrophenyl maltotrioside (CNP-G3) as substrate, and the secretion responses were expressed as percentage of the basal values. Salivary gland homogenates were analyzed for CBS, CSE and 3-mercaptopyruvate sulfurtransferase (3MST) expression (Western blotting) and H2S production in the presence of PAG, AOAA and β-cyano-L-alanine (BCA; another CSE inhibitor). In the latter, H2S production was quantified by optical densitometry following the lead sulfide precipitation method. Differences among the groups were analyzed by ANOVA followed by the Dunnett test. Values of $P<0.05$ were considered statistically significant. **Results:** CCh induced a concentration-dependent amylase secretion (up to 189±10% of the basal response with 10-6 M CCh) which was unaffected by PAG (216±38%) but significantly potentiated by AOAA (1,139±374%; $P<0.05$). H2S generation by salivary gland homogenates (average rate 0.83 nmol H2S/min/mg protein) was completely abolished by 30 mM AOAA and inhibited by 17% in the presence of 30 mM PAG or 6% by 30 mM BCA. Protein expression of CSE and 3-mercaptopyruvate sulfurtransferase (3-MST), but not CBS, were detected in the glands. **Conclusions:** Mouse submandibular and sublingual salivary glands produce H2S, mainly mediated by CBS and/or 3MST enzymes. Endogenously produced H2S attenuates amylase secretion secondary to cholinergic salivation. The effects of endogenous H2S on other salivation pathways are under current investigation. **License number of ethics committee**: CEUA-ICB/USP 81/2017 **Financial support**: CAPES, FAPESP, CNPq

01.018 Molecular docking of usnic acid in human acetylcholinesterase Cazarin CA, Correa R, Souza MM Univali – Ciências Farmacêuticas

Introduction: Molecular docking is a tool that can predict a better orientation of a ligand in a protein, where it is possible to characterize the behavior of some molecules in the binding site of target proteins and elucidate molecular interactions. Usnic acid [2, 6-diacetyl-7, 9-dihydroxy-8,9b-dimethyl-1,3- (2H, 9H) -diabenzofuran] is one of the most common and abundant lichen metabolites that has performed anti-inflammatory activity, antinociceptive and neuroprotective. Thus, it has a particular pharmacological interest, especially in Alzheimer's disease, where neuroinflammation, oxidative stress and loss of cholinergic neurons are involved in the pathogenesis of the disease and current therapy is totally palliative. **Methods:** The docking was perform using ArgusLab 4.0.1., ArgusDock algorithm, in which the binder behaves as flexible. By means of the RSCB Protein Data Bank, the structure of the enzyme Acetylcholinesterase complexed with a selective inhibitor Donezepila was obtain. All the binders were developed by the ArgusLab 4.0.1 workspace, which were submitted to later geometric optimization by Hamiltonian approximation packages such as MNDO (Modified Neglect of Diatomic Differential Overlap) and PM3 (Parametric Method), observed by quantum mechanics. After separation of the acetylcholinesterase structure into chains, the amino acids characterizing the active site of the enzyme were select. By determining these spaces as a coupling site, successive dockings were performed in the volume of the bounding box of active site. After the docking procedure was start, grids were generated, with data of the intermolecular interactions of the molecules present in the enzyme, in a resolution of 0.4 Ângtron. ArgusDock mechanism was used to obtain the docking energy, with regular precision, the binder was admitted as flexible. In the Maestro platform, the enzyme Acetylcholinesterase was import through the PDB being prepare by the Protein Preparation Wizard tool, where corrections were made for hydrogens and disulfide bonds were made. Modifications and corrections were made at the receiver. The preparation of the enzyme was complete with the removal of water and minimization of its structure. For the ligants, preparation with LigPrep was perform, in which using Force Field OPLS package (Optimized Potentials for Liquid Simulations), the most stable conformation was obtain. Using the complex selective inhibitor, active site grids were produce using the Grid Generation tool. **Results:** Acetylcholinesterase obtained from PDB (1EVE) had its active site complexed with E20 (Aricept) known as Donepezil, with presentation of the ribosomes. The protonated docking of the binder showed Donezepil Hbonds and Van der Waals (VDW) interactions and their interactions with the active site, with docking energy of -10.9 kcal / mol. In the interactions of Tacrina with the amino acids of the active site it was possible to observe the stabilization of the drug by pi-pi stacking and ionic interactions. In relation to the usnic acid it was possible to observe interactions of VDW with amino acids of the active site, where the stabilization is observed through pi-pi stacking type interactions and ellectrostatic forces. **Conclusion:** The docking energy of the usnic acid (-10.4 kcal / mol) was similar to the binding energy of Donepezil itself (-10.9 kcal / mol) by placing the single acid molecule in a promising position to follow with studies of pathologies involving the loss of cholinergic neurons, such as Alzheimer's disease. **Financial support**: UNIVALI, CAPES

01.019 Protective communication between HUVECs and vascular smooth muscle cells from hypertensive rats. Paulo M[1], Vercesi JA[1], Grando MD[1], Bendhack LM[11]FCFRP-USP – Física e Química

Introduction: Paracrine interactions between endothelial cells (EC) and vascular smooth muscle cells (VSMC) act as critical regulators of vessel wall assembly, vessel integrity and proliferation. Communications between ECs and VSMCs occur through synthesis and release of mediators or through direct cell-to-cell contact. Vascular relaxation molecules, such as nitric oxide (NO) and prostacyclin produced by ECs, are known to have a growth-inhibitory action on cultured VSMCs. The abnormal proliferation and migration of VSMCs are key components of various vascular diseases, including hypertension. Unfortunately, the biology of isolated cells in culture may be quite different from cells *in vivo*. To better simulate the vascular wall in the *in vitro* environment, we used *transwell*® techniques in which ECs and VSMCs are cultured together (co-culture). This study aimed to evaluate the modulation between the ECs co-cultured with VSMCs and monocultured VSMCs in reactive oxygen species (ROS) production and cells proliferation. **Methods:** We tested the NO and ROS production measured by fluorimetric assay and VSMCs proliferation measured by the proliferating cell nuclear antigen (PCNA) expression by Western Blot, in VSMC, extracted from renal hypertensive (2K-1C), normotensive (2K) rats and SHR in monoculture and co-culture with ECs. **Results:** NO production was lower in the VSMCs from 2K-1C (936.0 ± 11.0 FI, $P<0.05$) than in 2K VSMCs (1,234.1 ± 20.0 FI) whereas ROS production was higher in 2K-1C VSMC (1,072.6 ± 17.0 FI, $P<0.05$) than in 2K VSMC (958.02 ± 27.1 FI). In the same way, NO production was lower in the VSMCs from SHR (3,700.3 ± 12.0 FI, $P<0.05$) than in normotensive VSMCs (4,633.3± 22.0 FI) whereas ROS production was higher in SHR VSMC (4,460 ± 19.0 FI, $P<0.05$) than in normotensive VSMC (3,317 ± 23.1 FI). In co-culture for 72 h of VSMCs and ECs from 2K-1C aortas, generated ROS (938.0 ± 12.0 FI, $P<0.05$) was lower than in VSMCs in monoculture 2K-1C (1,072.7 ± 17.3 FI) and SHR (1,312.0± 10.1,FI). Otherwise, the NO levels were greater in VSMCs co-cultured (1,096.0 ± 29.0 FI, $P<0.05$) with ECs than in monoculture (929.66 ± 13 FI). ROS production was higher in 2K-1C and SHR than in 2K VSMCs. On the other hand, PCNA expression was similar in VSMCs grown in monoculture (0.344 ± 19.0) and in co-culture (0.337 ± 17.0), but PCNA expression was greater in VSMCs from 2K-1C and SHR grown in monoculture (2K-1C: 0.77± 15.0; SHR: 0.013 ± 11.0) than in co-culture (2K-1Ccc: 0.53 ± 22.0; SHRcc: 0.007 ± 11.0, $P<0.05$). **Conclusion:** Our data suggest that ECs protects VSMCs from hypertensive rats because ECs negatively modulate the proliferation and ROS production in VSMC from hypertensive rat aortas. **License number of ethics committee**: 15.1.1293.60.1 **Financial support**: FAPESP and CNPq

01.020 Pharmacological approach to synchronize clock genes in beige adipocytes

Rodrigues JQD[1], Ruiz GP[1], Mori MAS[1] [1]Unicamp – Bioquímica e Biologia Tecidual

Introduction: Adipose tissue plays an important role in metabolism regulation. It is well known that the desynchronization of the circadian clock triggers the development of obesity and metabolic diseases. DICER is a key enzyme in microRNAs processing, and it has been described to exhibit a circadian pattern of expression in several tissues, but there is no evidence that supports a rhythmic expression of DICER in adipose tissue. **Aims:** To investigate the daily rhythm of Dicer mRNA and clock genes in a murine model of beige adipocytes (9W cells) and a pharmacological approach to possible synchronized clock genes. **Methodology:** 9W pre-adipocytes were maintained and differentiated into beige adipocytes using a protocol previously described by our group (Mori et al., 2012). After differentiation, we used dexamethasone to synchronize circadian rhythm (Balsalobre et al., 2000). At circadian time 0 (CT 0), cell culture medium was replaced with 100nM dexamethasone for 15 min. The medium was then changed to fresh medium, and samples were collected every 4 hours for a period of 24 hours starting 12 hours after the first medium exchange (CT12). Quantitative RT-PCR was performed using specific primers for *Dicer*, *Per1*, *Bmal1* and *18S* or *36-B4* genes (for housekeeping normalization). Then, we have screened the best concentration as well the best exposure time to 4-hidroxytamoxifen (10-20µM; 24h to 72h), in order to knock-out Dicer, through the inducible Cre-Lox recombination system. The repeated-measures ANOVA statistical test was used followed by the Bonferroni post-test. Gene expression circadian rhythms were analyzed using the Cosinor equation. **Results:** *Dicer* expression displayed a circadian pattern in 9B beige cells synchronized with dexamethasone. Importantly, the control group did not exhibit statistically significant circadian oscillations, demonstrating that dexamethasone treatment was effective in synchronizing the brown adipocyte culture. The acrophase was observed 28 hours post stimulation, and the amplitude was 0,85. The amplitude is calculated as the difference between the maximum value (acrophase) and the mesor. Expression of *Bmal1* and *Per1* clock genes did display a circadian oscillation with the dexamethasone stimulation and the amplitude registered of *Bmal1* expression was 0,43. We also have successfully established the optimum parameters to knockout dicer, through Cre-Lox recombination system, using 4-hidroxytamoxifen 20 µM for 48 hours. Now, using the optimized protocol, we will knockout Dicer for the purpose to investigate whether clock genes rhythmicity is related to dicer expression. **Conclusion:** *Dicer* shows a circadian pattern of expression in beige adipocytes after dexamethasone synchronization. Also, dexamethasone was able to synchronize the analyzed clock genes. **Financial support:** FAPESP (2017/01184-9; 2016/17075-1). **Financial support**: FAPESP (2017/01184-9; 2016/17075-1)

01.022 Methyl Gallate effect over osteoclast differentiation and activity Alabarse PVG[1], Correa LB[2], Henriques MG[2], Rosas EC[2], Fukada SY[1] [1]FCFRP-USP – Física e Química, [2]Fiocruz – Farmanguinhos

Introduction: Methyl Gallate (MG) has been recently studied as a promising drug to improve bone-related diseases. Bone remodeling imbalance is observed in several diseases such as in osteoarthritis, osteoporosis, etc. Two main cell types are responsible for bone remodeling: osteoblast and osteoclast. Osteoclast differentiation and/or activity inhibitors had been investigated as prospective target for bone-related diseases. In this context, the aim of this research was to evaluate the effect of MG over osteoclast differentiation and activity. **Methods:** Bone marrow cells obtained from C57/BL6 mice were cultured in alpha-MEM medium supplemented with 10% FBS, and stimulated with M-CSF (30 ng/mL) for 72h at 37ºC/5% CO_2 in petri dishes (approximately $1.3x10^7$ cells). Afterwards, the adherent cells were harvested, seeded in culture plates and stimulated with RANKL (10 ng/mL) with MG at concentration of 3, 10, 30 and 100 µM. Several analyses were performed at different time points: osteoclast differentiation by counting the number and area of tartrate-resistant acid phosphatase (TRAP) positive cells were evaluated at 96h of culture. Osteoclasts marker gene expression were evaluated at 72h by real-time PCR using specific primers for Nuclear factor of activated T-cells, cytoplasmic 1 (Nfatc1), beta-integrin 3, calcitonin receptor (CTR), and TRAP (Acp5) which were normalized by GAPDH expression. We further evaluated the in vivo effect of MG in osteoclasts formation in a mBSA-induced arthritis model. For this, C57/BL6 mice were used in this study and treated daily with MG (7mg/kg) for 7 days. The knee joint histology was stained for TRAP and the osteoclasts were quantified in femur. One-way ANOVA and Student t-test statistical analysis were performed in the experiments and $p<0.05$ were set for significance. **Results:** MG, at the dose of 30 µM and 100 µM, significantly inhibited osteoclasts differentiation as indicated by reduced number of TRAP-positive osteoclast compared to control. The osteoclast number for MG 30 µM group were 11±15 cells/well, for 100 µM group were 2±1 cells/well and for control group were 297±32 cell/well; similar number of osteoclasts were observed in controls and MG 3 µM and 10 µM treated groups (297±32; 286±32; 295±40 osteoclast per well, respectively). MG was able to inhibit osteoclast fusion in a dose dependent manner, as evidenced by reduced osteoclasts area on MG 3 µM (0.89±0.13 mm^2), 10 µM (0.69±0.10 mm^2), 30 µM (0.05±0.05 mm^2) and 100 µM (0.005±0.006 mm^2) treated group compared to control (1.139±0.069 mm^2). Furthermore, MG treatment also reduced the osteoclasts marker gene expression such as Nfatc1 (71%), beta-integrin 3 (91%) and CTR (98%) compared to control. MG did not alter the expression of Acp5. *In vivo* model of arthritis induced by mBSA showed a reduction in TRAP positive cells number in femur histology when treated with MG (101±21; and 66±14, respectively; $p<0.01$). **Conclusion:** MG has an inhibitory effect over osteoclast differentiation in a dose-dependent manner. The inhibitory effect of MG on osteoclastogenesis was also confirmed in an *in vivo* animal model of arthritis. Altogether, MG seems to be a promising drug for osteolitic diseases treatment. **License number of ethics committee**: 17.1.807.60.3 **Financial support**: FAPESP; CAPES.

01.023 Polarization of mesenchymal stem cells isolated from human teeth with Toll-like Receptors 3 and 4 agonists. Chagastelles PC[1], Medeiros RPS[2], Dagnino APA[2], Goldani E[1], Campos MM[2], Silva JB[3] [1]PUCRS – Medicina e Ciências da Saúde, [2]PUCRS – Toxicologia e Farmacologia, [3]PUCRS – Medicina

Mesenchymal stem cells (MSC) has been the target of studies because it is easy to obtain and maintain compared to other types of stem cells. MSCs play an important role in the maintenance of tissue homeostasis and repair and regeneration processes. Studies describe the polarization of bone-marrow MSC in a proinflammatory phenotype, via toll-like receptor 4; as well as an anti-inflammatory phenotype, via toll-like receptor 3 (Waterman, 2010; Waterman, 2012). The present project aims to test the ability of MSC isolated from the apical papilla of human teeth to polarize into different phenotypes, aiming to improve the therapeutic activity of these cells, as a step prior to its application in cell therapy. This project was approved by the Research Ethics Committee of PUCRS. For the characterization of the isolated cultures, immunophenotyping was performed by flow cytometry and osteogenic differentiation using cell culture medium with specific inducers. Two protocols were performed to induce MSC polarization; Protocol 1: cells treated with the agonists LPS (0.01 or 0.1 µg/ml) and Poly(i:c) (1 or 5 µg/ml) for 1 h. Protocol 2: cells treated with LPS (0.1 or 1 µg/ml) and Poly(i:c) (1 or 5 µg/ml) for 24 h. The levels of IL-6 and CCL5 in the supernatant of the cultures were determined by ELISA after 24 and 48 h of the onset of treatments. Untreated cells were used as controls. In addition, the cell viability was assessed through the enzyme lactate dehydrogenase (LDH) in the culture supernatant (LDH Liquiform). For statistical analysis, one-way ANOVA followed by Tukey's Post Hoc was performed. To date, seven MSC lines have been isolated from different patients. The cultures expressed the CD73, CD90 and CD105 membrane markers in more than 95% of the analyzed cells and were negative for CD14, CD34, CD45 and HLA-DR (<2% of cells) markers. After 30 days in differentiation medium, they differentiated into osteoblasts through the deposition of mineralized matrix. Protocol 1 did not induce IL-6 and CCL5 expression in the cultures after 24 and 48 h. In protocol 2 the response of the cells was quite variable among the different cultures, with a tendency to increased IL-6 production in cells treated with LPS and 5 µg/ml Poly(i:c), although without statistical significance. Expression of CCL5 in the supernatant was observed only for the highest concentration of Poly(i:c) after 24 and 48 h ($P<0.05$). Regarding the activity of the LDH enzyme, no differences were observed between groups for both protocols, demonstrating that the agonists, at the concentrations tested, did not induce cell death. In conclusion, MSC from the apical papilla did not respond in the same way as bone-marrow MSC as previously published, when protocol 1 was applied (Waterman, 2010; Waterman, 2012). Cells submitted to protocol 2 secreted low levels of CCL5, and only at 5 µg/ml of Poly(i:c). Higher concentrations of poly(i:c) will be tested in an attempt to induce an anti-inflammatory phenotype in MSC from apical papilla. Waterman RS, PLoSOne, 5, e10088, 2010. Waterman RS, PLoSOne, 7:e45590, 2012. **License number of ethics committee**: 60389816.7.0000.5336 **Financial support**: CAPES e Fapergs

01.025 Lipopolysaccharide (LPS) alters the functional Alpha-1 adrenoceptor subtype in the rat distal cauda epididymis smooth muscle. Mueller A[1,2], Silva AAS[1], Silva ERJ[1], Pupo AS[1] [1]IBB-Unesp – Farmacologia, [2]UFMT – Ciências da Saúde

Introduction: The distal cauda epididymis (CE) is densely supplied by sympathetic postganglionic neurons that release noradrenaline (NA) to contract the smooth muscle layer through alpha-1A adrenoceptors (alpha1A-ARs) activation. As epididymitis has been associated to damage of CE smooth muscle, we hypothesize that this condition affects the CE via changes in alpha1A-ARs responses. Thus, our aim was to investigate the alpha1-ARs in CE in an experimental model of epididymitis induced by the injection of LPS from *E. coli* into the lumen of the vas deferens. **Methods:** adult male Wistar rats were anesthetized with ketamine/xylazine and epididymitis was induced by an intravasal retrograde injection of 25 □g (25 □l) of LPS from *E. coli.* Sham-injected rats received 25 □l of sterile saline. Animals were killed 6h after treatment (LPS25_6h; Sal_6h), and CE ducts (~1.0 cm length) were isolated and mounted in organ baths. Cumulative concentration-response curves (CRC) to agonists NA, buspirone or oxymetazoline were obtained in the absence and presence of prazosin and BMY7378 (non-selective alpha1 and selective alpha1D-ARs antagonists, respectively). In *in vitro* protocol, segments of CE duct from untreated rats were incubated in a sterile nutrient solution containing or not 100 □M pyrrolidinedithiocarbamate (PDTC, NF-κB inhibitor) for 1h and then challenged with 300ng/ml LPS for 4h, followed by contraction studies. All the CRC were performed in the presence of a cocktail of inhibitors to allow experimental conditions to evaluate antagonist potency (pA_2). Agonist potency was expressed as pD_2 values. **Results:** in LPS-induced epididymitis *in vivo*, NA-induced contractions of CE were competitively antagonized by prazosin with similar affinities (pA_2□9.0) in LPS25_6h and Sal_6h. However, the affinity of BMY7378 in CE duct from LPS25_6h was >50-fold higher than Sal_6h (pA_2□8.70 *vs* 6.90) with a Schild slope less than unity, suggesting that responses are mediated by more than one alpha1-AR subtype under this inflammatory condition. Moreover, in LPS25_6h the selective alpha1D agonist buspirone was ~3-fold more potent, and 30nM BMY7378 produced a rightward displacement, yielding an estimated pA_2□8.67. Supporting this data, after *in vitro* LPS exposure, the CRCs to NA were shifted to the right by 10 nM BMY7378, producing a pA_2□8.50; furthermore, oxymetazoline (alpha-1A partial agonist) was ~6-fold less potent in comparison to control (pD_2□7.00 *vs* 6.22), without changing E_{max}. In tissues that were pre-incubated with PDTC before the LPS challenge, the selective alpha1D-ARs antagonist BMY7378 (up to 30 nM) was ineffective in the CRCs to NA. **Conclusion:** LPS changes the functional alpha1-AR subtypes in the CE smooth muscle, indicating a likely heterogeneous receptor population (alpha1A and 1D-ARs) involved in the CE contractile response. Moreover, the co-participation of alpha1D-ARs provoked by inflammation seems to depend on NF-κB signaling pathway. These data contribute to the understanding the impact of inflammation on male fertility. **License number of ethics committee**: 749/2015 **Financial support**: Fapesp (08/50423-7 and 2015/08227-0), CNPq (479546/2013-4), UFMT/Capes.

01.026 Melanoma-derived microvesicles modulate neutrophils and macrophages polarization. Guimarães-Bastos D[1], Frony AC[1], Saldanha-Gama R[1], Santos-Oliveira R[2], Barja-Fidalgo TC[1], Moraes JA[3] [1]UERJ – Biologia Celular e Molecular, [2]UFRJ – Energia Nuclear, [3]UFRJ – Ciências Biomédicas

Introduction: The understanding on the mechanisms involved in tumor growth runs through the acknowledgement on its microenvironment, formed by extracellular matrix components, growth factors, cytokines and different cell types. Immune cells present in tumor microenvironment may have their pro-inflammatory functions modified to support its growth. Recent studies have provided evidence on pro-tumor phenotypes that neutrophils (PMN) and macrophages may assume in tumor microenvironment: TAN-N2 and TAM, respectively. Evidence also show that tumor microenvironment is rich in membrane-derived microvesicles (MV), which carry molecular information from their cellular origin, capable of modulating activity of target cells. Although tumor-derived MV was shown to interact with different cells, reports on its contribution to modulate TAN and TAM are poorly elucidated. We investigate whether MV produced by a human melanoma cell line would modulate human PMN and macrophages activity, shifting these cells to pro-tumor phenotypes *in vitro*. **Methods:** MV were obtained from human melanoma cell line MV3 cultures and quantified by annexin-V staining and Nanoparticle Tracking Analysis. The systemic biodistribution of MV was evaluated by injecting retro-orbitally radiolabeled MV (200 µg) in 8-weeks nude mice. Human PMN, isolated by Percoll gradient, were incubated in the presence or absence of MV (10%v/v) or LPS (10 µg/mL) at different times. Human monocytes, isolated by Ficoll gradient, were allowed to differentiate into macrophages during 7 days in culture, unless stated otherwise. Macrophages were incubated in the presence or absence of MV (50%v/v), LPS + IFN-γ (1 µg/mL + 100ng/mL, respectively) or IL-4 (50 ng/mL) during 3 additional days. Chemotaxis was assayed in a 48-wells modified Boyden Chamber. Gene expression analyses were obtained by qRT-PCR. Protein expression analyses were obtained by cytometry, immunoblotting and ELISA. **Results:** Initially we characterized MV3-derived MV and challenged mice with these MV, observing bioaccumulation in liver tissue. We also observed that MV induced PMN, monocytes and MV3 chemotaxis in a phosphatidylserine-dependent manner. We also observed that MV augmented the expression of CD206, TAM molecular marker, on the surface of macrophages, as well as their IL-10 and TGF-β protein contents. MV also increased the mRNA content of several TAN-N2 molecular markers, such as arginase, CXCR4 and VEGF on PMN, as well as their CXCR4 and arginase protein contents. Finally, we observed that MV induced different functions in PMN, favoring tumor cell survival. **Conclusion:** Our data indicate that melanoma-derived MV may have an important role on shaping the phenotype of PMN and macrophages in tumor microenvironments towards TAN-N2 and TAM phenotypes, respectively, which show increased expression pro-tumor molecular markers and present low cytotoxic properties. **License number of ethics committee**: 38257914.7.0000.5259 e 01200.001568/2013-87 **Financial support**: CAPES, CNPq, FAPERJ

01.027 Post-Traumatic Stress Disorder (**PTSD**): **Association between neuroinflammation and impairment of epididymal function and sperm parameters**.

Freitas GA[1,2], Scavone C[1], Pinna G[3], Avellar MCW[2] [1]ICB-USP – Farmacologia, [2]Unifesp-EPM – Farmacologia, [3]University of Illinois - Psychiatric Institute

Introduction: Stressfull and traumatic life events result in development of mood disorders, such as depression and anxiety spectrum disorders, which includes post-traumatic stress disorder (PTSD), a debilitating condition that follows trauma exposure. Several studies indicate a relationship between PTSD and its consequences on poorer health outcomes, which include systemically pro-inflammatory state and chronic diseases. How the neurobiological abnormalities in PTSD impact male reproductive function and fertility still remains largely unknown. Behavioral and neuroinflammatory changes found in PTSD can be reproduced by social isolation (SI) in mice. Herein we evaluated the consequences of this protracted stress model on the epididymis and sperm parameters. **Methods:** Adult Swiss mice (90 days) were kept in groups (GH, control) or individually (socially isolated, SI) for 4 weeks. Total RNA was extracted from isolated pre-frontal cortex, hippocampus and epididymal regions (initial segment, caput, corpus and cauda). Cryosections from brain (20 µm) and epididymis (10 µm) were used for immunofluorescence studies (IF) with antibodies against molecular markers for glial cells (microglia, IBA1; astrocyte, GFAP), F4/80-positive epididymal cells (dendritic cells and macrophages) and β-defensin SPAG11C. Proper controls were performed. Image analyses were conducted by fluorescence and confocal microscopy. Testicular and epididymal sperm analysis were evaluated. **Results:** IF revealed that both the number and reactivity of astrocytes and microglia were significantly increased in pre-frontal cortex and hippocampus of SI mice, confirming the expected neuroinflammatory profile induced in the central nervous system (CNS) by this stress condition. Its impact on the epididymis was revealed by the significant increase in the number of F4/80-positive cells immunostained along the entire tissue from SI mice when compared to controls. Although testicular sperm count and daily sperm production were similar in GH and SI mice, the stress condition led to an increase in sperm count (caput/corpus and cauda region) and sperm transit time in the epididymis. The motility of cauda epididymal spermatozoa was also reduced in SI mice, indicating that social isolation impaired both quantitative and qualitative sperm parameters. These changes occurred in the absence of changes in testis and epididymis relative tissue weight. Interestingly, the SPAG11C was immunolocalized in most, but not all, microglia and astrocytes in the brain, as well as in F4/80-positive epididymal cells from GH and SI mice, indicating a potential role for this β-defensin in tissue homeostasis and immune responses during inflammatory conditions. **Conclusions:** Collectively our results support the hypothesis that a neuroinflammatory condition such as PTSD may be related to impaired epididymal function and male fertility, due to systemic inflammation. The data also highlight a role for β-defensins in the modulation of immune responses in the CNS and epididymis. **Financial support**: CNPq, CAPES, FAPESP. **Ethics Approval**: CEUA UNIFESP-EPM #7991170915/2015. **License number of ethics committee**: CEUA UNIFESP-EPM #7991170915/2015 **Financial support**: CNPq (#140172/2017-3); FAPESP (#2014/19378-6).

01.028 Knockdown of Carboxypeptidase A6 in Zebrafish larvae reduces response to seizure-inducing drugs and causes changes in the level of mRNAs encoding signaling molecules. Lopes MW[1,2], Sapio MR[3], Leal RB[1], Fricker LD[3,4] [1]UFSC – Biochemistry, [2]UNIDAVI – Biological Sciences, Medical and Health, [3]Albert Einstein College of Medicine, – Neuroscience, [4]Albert Einstein College of Medicine – Molecular Pharmacology

Introduction: Carboxypeptidase A6 (CPA6) is an extracellular matrix metallocarboxypeptidase that modulates peptide and protein function by removal of hydrophobic C-terminal amino acids. Mutations in the human CPA6 gene that reduce enzymatic activity in the extracellular matrix are associated with febrile seizures, temporal lobe epilepsy, and juvenile myoclonic epilepsy. The characterization of these human mutations suggests a dominant mode of inheritance by haploinsufficiency through loss of function mutations, however the total number of humans with pathologic mutations in CPA6 identified to date remains small. **Methods:** To better understand the relationship between CPA6 and seizures we investigated the effects of morpholino knockdown of cpa6 mRNA in zebrafish (Danio rerio) larvae. **Results:** Knockdown of cpa6 mRNA resulted in resistance to the effect of seizure-inducing drugs pentylenetetrazole and pilocarpine on swimming behaviors. Knockdown of cpa6 mRNA also reduced the levels of mRNAs encoding neuropeptide precursors, bdnf (44%), npy (48%), chga (28%), pcsk1nl (37%), tac1 (50%), nts (49%), edn1 (28%), a neuropeptide processing enzyme (cpe, 30%), transcription factor (c-fos, 78%), and molecules implicated in glutamatergic signaling (grin1a, 40% and slc1a2b, 36%). Treatment of zebrafish embryos with 60 mM pilocarpine for 1 hour led to reductions in levels of many of the same mRNAs when measured 1 day after pilocarpine exposure, except for c-fos which was elevated (150%) 1 day after pilocarpine treatment. Pilocarpine treatment, like cpa6 knockdown, led to a reduced sensitivity to pentylenetetrazole when tested 1 day after pilocarpine treatment. **Conclusion:** Taken together, these results add to mounting evidence that peptidergic systems participate in the biological effects of seizure-inducing drugs, and are the first in vivo demonstration of the molecular and behavioral consequences of cpa6 insufficiency. **Reference:** Lopes MW. PLos ONE. 11(4): e0152905. 2016. Acknowledgments: The authors gratefully acknowledge helpful advice and guidance from Dr. Florence Marlow, Clinton DePaolo, Dr. Sayani Dasgupta, Dr. Roger Walz, and Dr. Peter Lyons. **License number of ethics committee**: Protocol Number: 20140102 **Financial support**: U. S. National Institutes of Health, CNPq, CAPES, FAPESC and INCT.

01.029 Computational studies of bioative peptides with potential interaction with αB-crystalline chaperone. Antunes JE[1], Pereira MBM[2] [1]UFJF – Ciências Farmacêuticas, [2]UFJF – Ciências Básicas

Introduction: Cells have specialized systems for the maintenance of protein homeostasis[1]. Among the mechanisms that modulate this homeostasis are: molecular chaperones and proteolytic systems such as calpain. The failure of these systems is emerging as an important mechanism in various pathological conditions such as cancer, cardiovascular and neurodegenerative diseases[2]. Among the chaperone proteins, αB-crystalline has been highlighted as an important chaperone in different human diseases including cardiac myopathies, malignant neoplasms and others[3]. Previous studies have demonstrated an interaction between the proteins: αB-crystalline and Focal Adhesion Kinase (FAK). In this work, the researchers revealed that the interaction between αB-crystalline and FAK is important for protection of FAK against proteolysis by calpain[4]. Since αB-crystalline protein plays an important role in maintaining protein stability, and that calpain plays an essential role in the modulation of protein activity, it would be reasonable to propose that the mechanism of modulation and protection exerted by αB-crystallin on FAK protein represents a primary protection element between proteins. Therefore, we suggest that other signaling molecules that are targeted for calpain proteolysis may also be protected from degradation by interaction with the αB-crystalline protein. **Methods.** We have searched in scientific articles of the proteins proteolysis targets by calpain. Alignments were made between the C-terminal sequence of the FAK protein and the amino acid sequences of each protein previously chosen. For the alignment was used the platform multalin.toulouse.inra.fr/multalin. Similar sequences between the C-terminal region of FAK and the target proteins of proteolysis by calpain were selected and identified as peptides (P-01, P-02, P-03). Interaction studies between the peptides and αB-crystallin were performed by molecular docking. **Results.** The results showed some consensus sequences between the calpain proteolysis target proteins and the previously identified sequences of C-terminal region of FAK that interact with αB-crystallin. *Molecular docking* studies demonstrated the interaction between the identified peptides (P-01, P02, P03) and αB-crystallin. These sequences may be proposed as bioactive peptides (P-01, P-02, P-03) with the potential of binding to αB-crystallin. **Conclusion.** We concluded that the peptides identified can be considered as binding motifs between the calpain proteolysis target proteins and αB-crystallin. Further studies will be conducted to evaluate the interaction between αB-crystallin and these proteins. Also, the evaluate the ability of these peptides to displace the interaction between αB-crystallin and calpain proteolysis target proteins. This interaction may lead to the degradation of such proteins and have a direct impact on diseases related to protein homeoastase, such as cancer, cardiovascular and neurodegenerative diseases.

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01.031 Structural insights on the galanin receptor-2 (GALR2) interactome and signalling. Goncalves DC[1], Pereira JGC[1], Fonseca MC[1], Morales-Neto R[1,2], Self T[2], Arruda MA[2], Kellam B[3], Holliday N[2], Dias-Neto E[4], Franchini KG[1], Oliveira PSL[1], Stoddart L[2], Hill SJ[2], Trivella DBB[1] [1]CNPEM – Biociências, [2]University of Nottingham – Life Sciences, [3]University of Nottingham – Pharmacy, [4]AC Camargo Cancer Center – Medical Genomics

Introduction: Deficiencies in the G-protein coupled receptor (GPCR) galanin receptor 2 (*GALR2*) mediated signalling have been recently correlated to head and neck cancer progression[1] and the onset of multiple sclerosis (MS)[2]. The molecular structure of this receptor is yet to be resolved and the signalling pathways downstream its activation is not fully understood. Some of us has recently described the first GALR2 mutation in an MS patient[2], which alters an important amino acid in the TM6 molecular toggle switch region (W249L) of GALR2, resulting in constitutive receptor internalization and consequent lack of galanin-mediated signalling through Gq. The aim of this work is to further investigate this effect and the cross-talk between the TM6 mutation and intracellular regions of GALR2 and their importance in the receptor's interactome and signalling. **Methods:** In-situ mutagenesis, guided by *in silico* modelling, were performed in the intracellular loops and the C-terminal region of the GALR2 receptor (WT and W249L) aiming to define the main intracellular regions affected by the clinical mutation. GALR2 (WT and mutants) were transfected in HEK293, and GALR2 cellular localization, its interaction with intracellular signalling components and galanin-triggered signalling were assessed by confocal microscopy, using a set of molecular probes. **Results:** Confocal microscopy analysis allowed us to characterize intracellular calcium mobilization induced by receptor activation mediated by the agonist galanin. C-terminal mutations in critical phosphorylation sites yielded sustained Gq signalling, however with different profiles observed with the wild type and W249L-based constructs. Receptor internalization and persistent signalling could also be controlled by mutations at the C-terminal phosphorylation sites and at one of the intracellular loops, namely intracellular loop 4 (ICL4). **Conclusion:** Besides the local effects of the W249L clinical mutation on TM6, we further verified the impact of the clinical mutation on the C-terminal phosphorylation sites and, unexpectedly, on the intracellular loop 4 (ICL4). These conformational alterations influence both agonist signalling mediated by Gq and interactions with beta-arrestin, thereby fine-tuning receptor signalling and cell effects with implications in disease. **Acknowledgments:** Authors thank the financial support of the School of Pharmacy (The University of Nottingham), LNBio-CNPEM and The Newton Fund Institutional Links through the GPCR-InSITW pump priming grant. This work was also supported by the CAPES-University of Nottingham grant 23038.007775/2014-98, CNPEM and the School of Pharmacy and the School of Life Sciences of the University of Nottingham. DCG, DBBT and RMN also thank CAPES for the international fellowships. 1. Kanazawa et al. (2018) Oncol. Lett. 15 (6): 9043-9050. 2. Garcia-Rosa et al. (2018) Pharmacogenomics J. (*accepted*) **Financial support**: Authors thank the financial support of the School of Pharmacy (The University of Nottingham), LNBio-CNPEM and The Newton Fund Institutional Links through the GPCR-InSITW pump priming grant. This work was also supported by the CAPES-University of Nottingham grant 23038.007775/2014-98, CNPEM and the School of Pharmacy and the School of Life Sciences of the University of Nottingham. DCG, DBBT and RMN also thank CAPES for the international fellowships.