15. Pharmacology: Others

15.001 Activation of δPKC and AKT mediates inhibition of platelet aggregation of rats 6h after lipopolysaccharide injection. Frade-Guanaes JO[1], Lopes-Pires ME, Marcondes S[1], Antunes E[2] [1]Unicamp – Farmacologia, [2]Unicamp – Farmacologia e Inflamação

Introduction: Protein kinase C (PKC), PI3K and AKT modulate platelet activity in physiological conditions, but there are no studies evaluating the role of these enzymes in platelet aggregation in sepsis. Previous work of our group showed that treatment of rats with lipopolysaccharide (LPS) decreases platelet aggregation, which is reversed by AKT inhibition and by the nonspecific PKC inhibitor GF109203X. Besides PI3K, reports have shown that AKT may be phosphorylated by other kinases, including PKC. The protein kinase C (PKC) family is composed of serine/threonine protein kinases of at least 12 isoforms in mammals and they are in a variety of signal pathways. Therefore, in the present work we investigate the role of εPCK and δPKC isoforms on AKT activation as well as on platelet aggregation of LPS-injected rats. **Methods**: Wistar rats were injected i.p. with saline or LPS (from *E. coli*, 1 mg/kg) and after 6h or 48h arterial blood was collected. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min and the platelets were washed using citrated buffer (pH 6.0). ADP (5 μM)-induced platelet aggregation was evaluated using a two-channel aggregometer in absence or presence of enzymatic inhibitors. AKT activation was determinate by western blotting assays. **Results**: ADP-induced platelet aggregation of rats 6h after LPS injection was 70% lower than aggregation in saline injected-rats, which was accompanied by increase of AKT phosphorylation at Thr 308 residue (increase of 40% compared to the platelets of saline-injected rats). Incubation of platelets of LPS-injected rats at 6h with PI3-K inhibitors, wortmannin (100 nM) or LY29004 (10 μM), or εPKC inhibitor sc-3095 (1 μM) did not affect either platelet aggregation or AKT phosphorylation. However, inhibition of AKT by API-1 (20 μM) reverted platelet inhibition. Similarly, δPKC inhibition by rottlerin (5 μM) reverted platelet aggregation, which was accompanied by significant reduction of AKT phosphorylation. Platelet aggregation of rats 48h after LPS injection was reduced by 53% compared to the platelet aggregation of saline-injected rats, but this effect was not affected by inhibition of AKT, δPKC or εPKC. AKT phosphorylation in platelets of LPS-injected rats at 48h was not inhibited by δPKC or εPKC inhibition. **Conclusions**: δPKC phosphorylates AKT that mediates inhibition of platelets aggregation in LPS-injected rats at 6h. On the other hand, δPKC or AKT do not take place on inhibition of platelet aggregation of rats 48h after LPS injection. **Supported**: CAPES The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 3316-1).

15.002 Creatine-loaded liposomes on oxidative stress parameters in model hyperphenylalaninemia. Borin DB[1], Mezzomo NJ[1], Dotto B[2], Amaral RG[2], Dias JB[2], Rech VC[1], Boeck CR[1] – [1]Unifran – Nanociências, [2]Unifran – Biomedicina

Introduction: Phenylketonuria (PKU) is an inborn error of metabolism caused by a deficiency of phenylalanine hydroxylase, the enzyme that catalyzes the hydroxylation of phenylalanine (Phe) to tyrosine. As a result of this deficiency, Phe and its metabolites accumulate in tissues causing hyperphenylalaninemia (HPA). The effects of PKU are linked to high concentrations of Phe, mainly in brain. Untreated patients develop severe neurological dysfuction, profound mental retardation, behavioral disorders, and autism characteristics. If the disease is detected early and with proper treatment the symptoms can be minimized, however some individuals may have high levels of Phe typically by trouble following the strict dietary Phe. Excess Phe is toxic to the brain because it reduces the activity of important enzymes of energy metabolism which also generates oxidative stress due to this energy deficit. To avoid this energy deficit the purpose of the study is using creatine-loaded liposome with intention to facilitate its delivery into the brain, because creatine induces energy homeostasis, and has antioxidant effects. Objective: To evaluate the efficiency of front creatine-lodead liposomes to a model of HPA, analyzing oxidative stress parameters. Methodology: HPA was induced by daily subcutaneous administration of Phe 5.2 mmol/g body weight plus α-methylphenylalanine 2.4 mmol/g body weight, from the 7th to the 19th day of life of animals. Creatine in free form or liposome was administered at a dose of 0.4 mg/g in young rats. Parameters of oxidative stress were evaluated as thiobarbituric acid reactive species (TBARS), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). **Results**: GSH content in cerebral cortex was increased by free liposomes and creatine-lodead liposomes in non-HPA rats when compared to controls. CAT activity was unchanged in rats subjected to treatment. Creatine-lodead liposome administration increased the activity of SOD in non-HPA rats. TBARS was not changed in any of the treatments. **Conclusion**: The model of HPA did not change the parameters from evaluated oxidative stress. However, the administration of creatine-lodead liposomes to animals non-HPA, caused an increase in GSH levels and increased SOD activity. Similarly, administration of free liposomes in non-HPA animals also increased the levels of GSH. Other parameters will be analyzed to better understand the mechanism by which the liposomes induce increase in GSH levels. Acknowledgements: Research supported by FAPERGS, CAPES and CNPq. All animal procedures were approved by the Animal Ethics Committee from the Centro Universitário Franciscano (protocol number: 006/2014).

15.003 Pharmacological activity extract ethanolic Cyperus articulatus **var.** *Nodosus***.** Silva EBS[1], Machado IR[2], Barata LES[2], Arévalo MR[2], Silva AS[2], Vieira LQ[3], Castro W[3], Ruiz ALTG[4], Torre AD[4], Castro KCF[2], Moraes WP[1] – [1]UFOPA – Farmacologia, [2]UFOPA – Produtos Naturais Bioativos, [3]UFMG – Gnotobiologia e Imunologia, [4]CPQBA-Unicamp

Introduction: Priprioca (*Cyperus articulatus var nodosus*) is a medicinal plant used mainly as an anti-inflammatory; however, there are few studies on this property. Studies carried out with this essential oil has led to identification of many terpene compounds. Little is known about the use of solid waste generated after the essential oil extraction. **Objective**: Investigate the chemical composition and evaluate the anti-inflammatory, antioxidant and anticancer efficiency of the ethanol extract of solid waste from the extraction of oil of *C. articulatus*, in experimental models, *in vitro*, in peritoneal macrophages of mice and human tumor cell lines. **Methods**: Cell viability was performed according to the MTT method, the determination of the production of reactive oxygen species (ROS) by luminescence, measurement of the production of nitric oxide (NO) by the Griess method, determination of the activity of arginase by the Kropf method. Number of CEUA approval: 07004/2013). The chemical composition analysis of the ethanol extract was performed in a Gas Chromatograph Agilent, HP-6890 model equipped with a selective mass Agilent detector, HP-5975 using a Model HP-5MS capillary column. This evaluation of anticancer activity was performed using the *in vitro* anticancer activity assay on human tumor cell lines U251 (glioma CNS), MCF-7 (breast cancer), NCI-H460 (lung). **Results**: It was observed the presence of monoterpenes, sesquiterpenes and ketones sequiterpênicas as major compounds. The ethanolic extract of C. articulatus (EECA) reduced production of ROS, and in the groups treated production rates remained below the baseline (15,000 relative light units) decreased by 85% EECA NO production, the activity enzyme arginase decreased by 90%. The value of the Total Growth Inhibition (TGI) in antiproliferative activity for the extract was 62.7 mg/mL active in the U251 glioma tumor cells. For other tumor cells such as MCF7 breast and NCI-H460 lung, TGI EECA was 100 and 135.7 g/ml and also not demonstrated cytotoxicity at concentrations of 50 and 25 mg/ml, so that the percentage the cell viability of the groups treated with EECA exceeded 100%. **Conclusion**: The results indicate that treatment with EECA exerts a powerful antioxidant activity and has promoted inhibition of cancer cell proliferation and inhibition of inflammatory mediators *in vitro* experimental models. **Financial support**: Fundação de Apoio à Pesquisa do Estado do Pará (*Foundation for Support of the Pará State Research*) - FAPESPA

15.004 Effect of swimming training on neurogenic contraction and stock of intracellular calcium concentration in spontaneously hypertensive rats. Pena-Garcia M[1], Miranda-Ferreira R[1], Castro Musial D[1], Jurkiewicz A[1], Da Silva R[2], Cezaretti M[2] [1]Unifesp – Farmacologia, [2]Unifesp

Introduction: Calcium (Ca^{2+}) is an important second messenger cell; Changes in Ca^{2+} concentrations in the intracellular stocks were linked to the pathogenesis of hypertension. Studies have shown that exercise training has beneficial effects in both humans and animals, such as lowering blood pressure (BP), decreased sympathetic activity, decreased peripheral vascular resistance, and it is known that Ca^{2+} participates in these functions. However it is not known whether the exercise is able to modify the Ca^{2+} concentration of intracellular stores as sarcoplasmic reticulum (SR) and mitochondria (MIT) before the onset of the disease, thus leading to an improvement in blood pressure levels in SHR young and adults. **Aims**: To study the effect of swimming training (ST) on BP, neurogenic contraction in vas deferens (VD) and the concentration basal of calcium in adrenal gland SHR youth and adults. **Materials and Methods**: All experimental procedures were approved by Ethical Committee of Federal University of São Paulo, Brazil (7866020315). The ST consisted of swimming sessions of 60 minutes, 5 days per week, for 8 weeks. Using animals NWR and SHR Young (12 weeks) and adults (20 weeks), n = 8; We study: 1) BP Measurement. 2)Neurogenic Contraction through electrical stimulation (0.2 Hz, 60 V) of the sympathetic nerves of the vas deferens (VD); 2a) Using CRT mixture of caffeine 10^{-2}M, Ryanodine $3x10^{-6}$M and thapsigargin 10^{-6}M to depletion of Ca^{2+} RE. 2b) Neurogenic Contraction using CCCP 10^{-6}M to depletion of Ca^{2+} MIT. 3) Fluorescence microscopy with Fura-2AM, fluorescent probe to measure intracellular Ca^{2+} basal unstimulated, in adrenal gland slices in trained and sedentary animals. **Results and Conclusion**: Compared to the sedentary animals trained animals both NWR and SHR youth and adults showed: 1) Decreased BP (mmHg): Young = NWR (s) 145.5 ± 1.0; NWR (t) 128.6 ± 1.2 and SHR (s) 199.5 ± 1.3; SHR (t) 141.2 ± 1.5; Adults = NWR (s) 151.4 ± 0.56; NWR (t) 144.4 ± 1.1 and SHR (s) 228.5 ± 2.0; SHR (t) 173.5 ± 1.9 2)) Decrease in neurogenic contraction in training groups; 2a) Decrease in neurogenic contraction (expressed in g of tension / g of tissue) using CRT: Young = NWR (s) 33.9 ± 2.1; NWR (t) 25.17 ± 2.1 * and SHR (s) 43.57 ± 0.8; SHR (t) 26.9 ± 1.3 ***; Adults = NWR (s) 28.5 ± 3.7; NWR (t) 18.41 ± 0.7 * and SHR (s) 48.06 ± 4.2; SHR (t) 26.96 ± 1.3. 2b) Decrease in neurogenic contraction using CCCP those from group.3) Decreased basal intracellular Ca^{2+} expressed in [Ca2 +]i basal (UAF): Young = NWR (s) 0.95 ± 0.08; NWR (t) 0.89 ± 0.11; SHR (s) 1.3 ± 0.1; SHR (t): 1.0 ± 0.2. Adults = NWR (s) 1.3 ± 0.12; NWR (t) 0.99 ± 0.23; SHR (s) 2.4 ± 0.19; SHR (t): 1.99 ± 035. With these results we can infer that the ST was able to improve the condition of these animals by reducing all parameters studied before the development of hypertension in young animals as well as in adults, showing the importance of physical activity, because it leads to improved quality and the life expectancy of animals, results could be extrapolated to humans. **Financial Support**: FAPESP, CAPES, CNPq.

15.005 Unfractionated heparin effect on wound healing. Nascimento AS[1], Borges PA[2], Nogueira TA[1], Gomes JPM[1], Garcia TA[1], Calil-Elias S[1] [1]UFF – Farmácia, [2]UFRJ – Farmacologia e Química Medicinal

Introduction: The wounds affect the population in general, regardless of gender, age or ethnicity, determining a high rate of people with alterations in skin integrity, thus constituting a serious public health problem. The wound healing process involves distinct overlapping phase of homeostasis, inflammation, proliferation and tissue remodeling. The inflammation stage begins immediately after injury, first with vasoconstriction that favors homeostasis. Furthermore, the extravasation of blood constituents provides the formation of the blood clot reinforcing the hemostatic plug. Components of the coagulation cascade, inflammatory pathways and the immune system are necessary to prevent loss of blood and fluid, removing necrotic tissue and to prevent infection. The proliferative phase is characterized by epithelialization, angiogenesis, granulation tissue formation, and collagen deposition. The final phase of wound healing is the maturation phase. Type III collagen formed in the granulation tissue is gradually degraded and is replaced by collagen type I. Growth factors are responsible for regulating cellular functions such as adhesion, proliferation, migration and differentiation, and is therefore fundamental in the tissue repair. Heparin is well known for its anticoagulant properties. In addition, heparin possesses anti-inflammatory effects and binding of growth factors. Studies have shown that heparin can be used in the treatment of burns, accelerating the wound healing process. Some studies have shown the effect of heparin on the healing of other tissues. In this sense, our proposal is to investigate the function of low molecular weight heparin during wound. **Methods**: The animals used were adult albino Swiss mice with 25 ± 3 g (n = 4/group), which throughout the experiment received food and water "ad libitum" and were maintained in natural light cycle. Manipulation and procedures with animals followed the principles of Evaluation Committee on the Use of Animals in Research (CEUA-UFF nº 410). The skin lesions were made with metal *punch*. The control group received physiological saline solution and the treated group received heparin solution, both treatments made topically, for five days. The animals were sacrificed 1, 3, 7, 14, 21, 30 and 60 days after lesions, with overdose of anesthetic (enflurane), in order to remove the skin for histological processing. Histopathological evaluation was made with H&E and picrosirius staining. Immunohistochemical analysis was performed for marking of collagen type I and III and Western blotting for quantification of collagen III. **Results**: The evaluation of wound contraction area showed that heparin accelerated the contraction of the lesion. Through histological analysis it was found that the treatment of lesions with heparin accelerated the reepitheliazation process and proved to be important in the formation of a granulation tissue, with collagen deposition and qualitatively amount of inflammatory infiltrates (D7). Furthermore, heparin gave better organization of dermis, making of the damaged skin resembles the intact skin. The quantification of collagen III by Western blotting and total collagen by picrosirius in D30 showed no significant difference between the groups, but the quantification on D21 showed that heparin increased total collagen deposition. In this study, we showed that heparin was able to accelerate the wound healing of mice.