# ABSTRACTS


# 49th Brazilian Congress of Pharmacology and Experimental Therapeutics

**Ribeirão Preto Convention Center**
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## 11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

**11.001 Lipid core nanocapsules modulate quetiapine hippocampal exposure in a neurodevelopmental model of schizophrenia**. Carreño F[1], Helfer VE[1], Staudt KJ[1], Paese K[1], Meyer FS[1], Silva CM[1], Herrmann AP[2], Rates SMK[1], Guterres SS[1], Dalla Costa T[1] [1]UFRGS, [2]UFFS

**Introduction:** High variability in chronic schizophrenia (SCZ) response to treatment may be partially related to blood-brain barrier (BBB) dysfunction caused by the disease[1]. Neurodevelopmental model of SCZ induced by administration of viral mimic polyinosinic–polycytidilic acid (poly i:c) to pregnant rats has substantial face validity. Adult offspring demonstrate sensorimotor gating deficits, enhanced sensitivity to MK-801 hyperlocomotion and morphofunctional alterations in the hippocampus that parallels SCZ neuropathology opening new opportunities for the investigation of better treatments[2]. This study aimed to investigate free and nanoencapsulated quetiapine (QTP) neuropharmacokinetics in a neurodevelopmental model of SCZ. **Methods:** Protocols approved by UFRGS' Ethics Committee in Animal Use (#31001). Pregnant Wistar dams (GD15) received poly (i:c) 4 mg/kg i.v. *bolus* dose. SCZ-like deficits in the adult offspring (PND75) were confirmed by elevated plus-maze, pre-pulse inhibition of the startle response and MK-801 induced hyperlocomotion (*P* groups). QTP lipid core nanocapsules (QLNC, 1 mg/mL) were obtained by nanoprecipitation[3]. Hippocampal implantation[3] of microdialysis probes (CMA 12, 3 mm PAES, 20 kDa cutoff) was used to access active unbound QTP concentrations and jugular vein was cannulated for blood sampling. PK was evaluated in awaken animals after single i.v. dosing of QTP solution (FQ-*P*, 10 mg/kg, n = 5) or QLCN-*P* (5 mg/kg, n = 5) 48 h after brain surgery. Control naive offspring groups (*N* groups) received QTP solution (FQ-*N*, 10 mg/kg; n = 7) or lipid core nanocapsules (QLNC-*N*, 5 mg/kg, n = 6). PK parameters determined using Phoenix® v. 64 software. **Results:** Groups that received QLNC showed increased half-life (QLNC-*N* = 3.7 ± 1.0 h and QLNC-*P* = 5.4 ± 2.4 h) in comparison to groups that received QTP solution (FQ-*N* = 2.9 ± 0.8 h and FQ-*P* = 3.8 ± 1.1 h) due to a significant decrease in clearance (QLCN-*N* = 0.8 ± 0.1 L/h/kg; QLNC-*P* = 0.6 ± 0.1 L/h/kg; FQ-*N* = 1.6 ± 0.2 L/h/kg; FQ-*P* = 1.4 ± 0.4 L/h/kg) ($p<0.05$). Volume of distribution was not altered due to nanoencapsulation. Unbound hippocampal exposure to QTP ($AUC_{0\text{-}8h}$/D) in naive offspring was similar for QTP in solution and QLCN (FQ-*N* = 0.20 ± 0.05 and QLCN-*N* = 0.24 ± 0.04). However, the significant decrease in hippocampal exposure in FQ-*P* group (0.11 ± 0.01) was reverted by drug nanoencapsulation (QLNC-*P* = 0.24 ± 0.02) ($p<0.05$). **Conclusions:** QTP brain exposure is reduced in SCZ-induced rats, supporting the hypothesis that BBB dysfunction contributes to treatment failures. Drug encapsulation on lipid core nanocapsules overcomes BBB disorder, returning drug penetration to the levels observed in normal animals. **References:** [1] Schoknecht, K. et al. Epilepsia. 53, 7-13, 2012. [2] Macedo et al Braz J Med Biol Res. 45(3): 179–186, 2012. [3] Carreño, F. et al. Mol. Pharm. 13, 1289, 2016. **Acknowledgments: Financial Support** CNPq/Brazil and CAPES- PROEX 646-2014.

**11.002 The low contribution of urinary excretion in the elimination of daunorubicin and its metabolite daunorubicinol in patients with acute myeloid leucemia**. Oliveira ML[1], Nardotto GHB[1], Rocha A[1], Simoes BP[2], Lanchote VL[1]
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**Introduction:** Daunorubicin, an anthracycline widely used in the treatment of acute myeloid leukemia (AML), is metabolized to daunorubicinol by the carbonyl reductase enzymes. Interindividual variability in daunorubicin metabolism may have potential implications on the toxicity and efficacy of the drug, making it critical to understand the factors influencing drug response. The present study aims to clarify how metabolism influences daunorubicin pharmacokinetics at steady state including plasma and urine data of patients with AML. **Methods:** Daunorubicin and its metabolite (daunorubicinol) were assessed by a liquid chromatography–tandem mass spectrometry (LC–MS/MS) using aliquots of 50 µL of plasma or urine. The methods were validated according to FDA recommendations and applied in the evaluation of daunorubicin pharmacokinetics in plasma and urine of 12 adult patients with AML after receiving the third infusion of 60 mg/m$^2$ daunorubicin for 1 hour. Serial blood samples were collected until 144 h after the first infusion and the pharmacokinetic parameters were calculated using WinNonlin software. **Results:** The methods of simultaneous analyses of daunorubicin and daunorubicinol in plasma and urine were able to quantify the analytes until 72 h after the third infusion of the drug. The methods are fast, with a chromatographic run time of 6 min, show no matrix effect, are sensitive (LIQ of 0.1 ng/mL of plasma and 0.5 ng/mL of urine), exhibit wide ranges of linearities (0.1-1000 ng/mL plasma and 0.5-3000 ng/mL urine). The WinNonlin noncompartmental analysis was able to predict the following parameters (mean±SD): $t_{1/2}$: 13.8 ±7 h, Cmax: 81 ±28 ng/mL, $AUC^{0-\infty}$ 388 ±103 ng.h/mL, total Cl 298.7 ±60 L/h and Vdss 2514 ±745 L for daunorubicin in plasma and $t_{1/2}$: 15.9 ±2.24 h, Cmax: 107 ±19 ng/mL, $AUC^{0-\infty}$ 2569 ±466 ng.h/mL and Vdss 894 ±250 L for daunorubicinol in plasma. Regarding urinary excretion, the following parameters were obtained (mean±SD): fraction of drug eliminated in unchanged form 4.5±1.0 %; renal Cl of daunorubicin 13.0 ±3.2 L/h; fraction of drug eliminated as metabolite 8.2±2.0 %; formation CL of metabolite 25.0±9.0 L/h. The daunorubicin hepatic CL, representing the drug elimination by metabolism and biliary excretion, was 285.7 L/h. **Conclusion:** The formation clearance of daunorubicinol (25.0±9.0 L/h) represents only 8.3% of daunorubicin total clearance (298 ±60 L/h) showing that urinary excretion of unchanged daunorubicin and daunorubicinol does not represent the major factor in total clearance variability and inferring that daunorubicin and/or daunorubicinol biliary clearance drug transporters-dependent probably have the major contribution. References: GALETTIS, P. Br. J. Cancer. v. 70, p. 324, 1994. LACHÂTRE, F. J Chromatogr B. v.738, p.281, 2000. SAULTZ, J. N. J. Clin. Med., v.5, p.1 2016. VARATHARAJAN, S. Eur J Clin Pharmacol. v. 68, p.1577, 2012. **Financial Support**: National Council for Scientific and Technological Development (CNPq) Approval by the Human Research Ethical Committee: n° 9064/2014 Acknowledgments: To the team of physicians and nurses of hematology sector of HCFMRP-USP.

**11.003 Exposure to the electrophilic air pollutant 1,2-naphtoquinone and health effects in the vascular system**: **Role of transient receptor potential**. Soares AG[1], Florenzano J[1], Rodrigues L[1], Teixeira SA[1], Muscará MN[1], Brain SD[2], Costa SK[1] [1]ICB-USP – Pharmacology, [2]King's College London – Cardiovascular

**Introduction:** We have previously shown that early postnatal, but not late, exposure to chemical ambient pollutant 1,2-naphthoquinone (1,2-NQ) increases susceptibility to pulmonary allergic inflammation at adulthood [1], and that the signalling pathway regulated by transient receptor potential ankyrin 1 (TRPA1) is involved [2]. However, little is known regarding the involved mechanisms and role for TRPs in the health effects of 1,2-NQ in the cardiovascular system. AIM: In this study we aimed to investigate the effects of 1,2-NQ in the vascular beds and the role of TRP channels. MATERIAL AND **Methods**: For the study male C57bl/6 mice were used and both the pulmonary artery (PA) and mesenteric artery (MA) were isolated and mounted on a wire myograph and subjected to pharmacological tests (eg. adrenergic or cholinergic stimuli) in the presence or absence of TRP receptor antagonists. For the study of exposure to the pollutant *in vitro*, increasing or single concentration of 1,2-NQ was performed in the absence or presence of TRP receptor antagonists. **Results**: Increasing concentrations of 1,2-NQ ($10^{-9}$ – $10^{-4}$ M) led to vasoconstriction in a concentration-dependent manner (PA - $E_{Max}$(%) 22.24 ± 1.8 and $pD_2$ 4.79±0.03 and MA - $E_{Max}$(%) 26.45±2.6 and pD2 4.77±0.06). Pretreatment of the PA with 1,2-NQ for 1 hour impaired endothelial-dependent vasodilation in response to acetylcholine (ACh) (PA - $E_{Max}$(%) 35.95±0.59; 12.58±2.10*, VEH and 1,2-NQ respectively, *P<0.05). While MA and PA from TRPA1 knockout mice did not show significant changes in vascular responses to 1,2-NQ compared to the changes vasodilation seen in wild type mice, the vasodilatation responsiveness to ACh in the MA from TRPV1 knockout mice was markedly reduced by prior incubation with 1,2-NQ (P<0.05) when compared to WT mice MA exposed to 1,2-NQ. The gene expression (mRNA) for the nuclear factor erythroid 2-related factor (NrF2) was significantly reduced in PA and elevated in MA in naïve mice, but the TRPV1 mRNA did not change, by exposure to 1,2-NQ in (MA or PA). **Conclusions**: We conclude that the ambient pollutant 1,2-NQ has the ability to directly or indirectly (via oxidant generation) promote vasoconstriction in distinct vascular beds and the activation of TRP vanilloid type 1 (TRPV1) and ankyrin 1 (TRPA1) channels might play a role. **Acknowledgments**: CAPES, CNPq, FAPESP Ethic Comitte: 48/2016-CEUA References: [1] Santos et al., Arch. Toxicol. 88, 1589–605. [2] Florenzano et al., 2011. Inflamm Res 60, 1–321.

**11.004 Comparative toxicological effects of the antiatherogenic drugs LASSBio-788 and simvastatin**. Maia IC[1], Motta NAV[1], Ribas JAS[1], Kümmerle AE[2], Brito FCF[1], Marostica E[1] [1]UFF – Fisiologia e Farmacologia, [2]UFRRJ – Química

**Introduction:** The compound LASSBio-788 is a thienyacylhhydrazone derivative that has an antiatherogenic effect with antiplatelet, anti-inflammatory, vasodilatory, anti-oxidant and lipidic lowering properties already well established (Motta *et al.,* J Pharmacol Sci 123:47, 2013). Therefore, it is important to evaluate the toxic effects caused by this potential drug candidate for the treatment of atherosclerosis. Thus, the aim of this study is to evaluate the possible toxic effects of LASSBio-788 on the different tissues in rats, comparing to simvastatin. **Methods:** (CEUA 695/16) Male Wistar rats (90-day-old) were shared in 3 groups (n=5/group) that received for 15 days: (CO) vehicle i.p.; (LASSBio788) LASSBio-788 100 µmol/kg, i.p. and (SIMVA) simvastatin 10mg/kg, gavage. Animals were anesthetized and blood samples were collected for biochemical and hematimetric analyzes. Testes, liver and kidney from different experimental groups were removed, weighed and processed for morphologic analyze. Spermatic evaluation (progressive motility, vigor, membrane integrity and hypo-osmotic swelling test) was performed using sperm from epididymis cauda. Values are mean±SEM, ANOVA one-way, Newman-Keuls *post hoc* test, $P<0.05$. **Results:** Data are CO, LASSBio788 and SIMVA, respectively. No differences were found in corporal weight (376.6±31.4, 392.8±27.1, 402.6±39.3 g) or in relative weight of testes (0.48±0.05, 0.45±0.04, 0.47±0.02), liver (3.13±0.30, 3.05±0.13, 2.97±0.26) and kidney (0.39±0.02; 0.37±0.03; 0.39±0.02) among the groups. Biochemical parameters (urea: 46±3, 48±7, 46±8 mg/dL; creatinina: 0.5±0.2, 0.5±0.2, 0.5±0.1 mg/dL; uric acid: 2.2±1.1, 2.0±0.4, 1.5±0.2 mg/dL; total protein: 6.1±0.3, 6.7±0.4, 6.2±0.2 g/dL; albumin: 4.0±0.1, 4.1±0.1, 3.8±0.1 g/dL; magnesium: 2.8±0.2, 2.5±0.3, 2.5±0.3 mg/dL; TGO/TGP 4.9±2.7, 6.3±1.4, 4.5±2.0; creatine phosphokinase: 1215±177, 1335±438, 1074±658U/L; calcium (10.2±0.4, 11.7±1.7, 10.5±0.4 mg/dL) were not altered either. However, LASSBio-788 increased phosphorus serum level (7.1±1.0, 10.5±1.2*, 8.0±0.4 mg/dL) and reduced the hematocrit (50.2±1.2, 47.3±1.8*, 49.4±0.5%), as well as both drugs decreased alkaline phosphatase activity (215±83; 140±19*; 158±53* U/L) when compared to control group. Among the others hematimetric parameters (erythrocytes: 9.48±0.47, 8.95±0.59, 9.28±0.16 million/$mm^3$; hemoglobin: 16.8±0.7, 15.9±0.6, 16.9±0.2 g/dL; platelets: 870±110, 925±109, 889±57 thousand/$mm^3$ and leukocytes 5.8±1.5, 6.3±0.7, 5.4±0.7 thousand/$mm^3$) were not detected differences. Morphological analysis of hepatic and renal tissue did not suggest toxic effects induced by LASSBio-788 or simvastatin. Regarding spermatic evaluation, spermatozoid motility, membrane integrity and functionality were also preserved by both drugs at the respective doses used. **Discussion**: Our preliminary results showed that the new compound LASSBio-788 does not have significant toxic effects on the male reproductive tract and gamete, as well as on the hepatic and renal tissues, being a potential candidate for an antiatherogenic drug prototype. However, LASSBio-788 effects on the phosphorus serum concentration and decreased alkaline phosphatase activity remain unclear and shold be investigated. **Financial Support:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

**11.005 Metformin pharmacokinetics and pharmacodynamics evaluation in diabetic rats induced by streptozotocin and nicotinamide**. Braga A, Lima D, Barreto F, Dalla Costa T, Araujo BV UFRGS

**Introduction:** Metformin is the most prescribed drug to treat type II diabetes *mellitus*. There are some studies regarding this drug which describing the pharmacokinetics in plasma samples[1] and its pharmacodynamics[2]; however, some pharmacological features are not still clarified. This study aims to evaluate the free levels of metformin on its target tissue (liver) and in a feasible tissue (leg muscle) in both healthy and diabetic rats. We also investigated the metformin acute antidiabetic effect in diabetic rats induced by streptozotocin and nicotinamide. **Methods:** The diabetes *mellitus* was induced in male Wistar rats according to study[3]. Plasma and tissue pharmacokinetics were carried out in different groups of anesthetized (urethane 1.25 g/kg, i.p.) healthy and diabetic rats (n=5/group) after metformin administration (50 mg/kg, i.v.). Liver and muscle pharmacokinetic were determined by microdialisys (CMA 20); samples were collect during 12 hours. The animals' carotid artery was cannulated for plasma pharmacokinetic study, allowing blood be collect up to 12 hours. Pharmacodynamics study was carried in another group of diabetic rats (n=5-7/group) that received glucose (5g/kg, v.o.) and treated with metformin (50, 100 or 150mg/kg, i.v.) or saline (10 mL/kg, i.v.), rat serum were collected up to 4 hours to determine the glycaemia. All experiments were approved by the Committee of Ethics in Animal Use - UFRGS (25780). The non-compartmental analysis were performed by Phoenix software and compared by ANOVA one-way and pharmacodynamics data by ANOVA two way with repeated measures ($p < 0.05$) using the software SigmaStat®. **Results:** For both healthy states (diabetic and control), liver and muscle showed higher concentration when compared to plasma, increasing the drug exposition ($p<0.05$), $AUC_{0-12}$ from liver *vs* plasma in healthy (116.39 ± 39.71 *vs* 44.98 ± 23.50 µg*h/mL) and diabetic (107.90 ± 48.05 *vs* 47.08 ± 23.79 µg*h/mL) rats. The same result was observed at leg muscle in control animals ($AUC_{0-12}$ 106.26 ± 43.34 *vs* 44.98 ± 23.50 µg*h/mL). These results could be explained by metformin influx transporters expressed in both tissue that increase drug concentration. Metformin at doses 100 and 150 mg/kg decreased the glucose absorption on 0.5h up to 1.5h ($p<0.05$), the effect on high doses could be due to the acute drug administration. **Conclusion:** Metformin showed high free levels in the liver and muscle, suggesting the influx transporter involvement. Acute metformin administration showed decrease on glucose absorption in high doses. This study prospect the pharmacokinetic and pharmacodynamics modeling that will allow predict the drug behavior in scenarios not study yet. **References:** [1] A.K. Madiraju *et al.,Nature,* **26**, 510 (2014). [2] H. Choi *et al*., *J Pharm Sci,* ***95***, 2543 (2006). [3] P. Masielo *et al*., *Diabetes,* **47**, 224 (1998). ***Acknowledgements:*** **Financial Support** from CNPq/Brazil and FAPERGS

**11.006 Biodisponibility study of two propafenone formulations in healthy volunteers**. Iwamoto RD, Moreno RA, de Nucci G Unicamp

**Introduction:** Propafenone is a drug used as an antiarrhythmic agent, acting stabilizing the membrane of miocardic cells. Propafenone is classified as an antiarrhythmic of class 1C, blocks the fast sodium channels, beta receptors and exerts some effect on potassium and calcium channels. The aim of this study was to compare the biodisponibility after administration of two different formulations of propafenone in healthy volunteers. **Methods:** The present study started only after approval by the ethics committee of State University of Campinas. Blood samples were collected from healthy volunteers after administration of formulation 1 or 2. Adverse events were registered. The propafenone in plasma were quantified employing the liquid chromatography coupled to mass spectrometry in tandem. **Results:** The quantification method in plasma was properly validated. The 90% confidence interval of the PK ratio lied between 0.80 and 1.25, with a statistic power superior to 89% for Max Concentration and superior to 97% for Area Under de Curve. Qualitatively and quantitatively no differences were observed in adverse events after administration of each formulation. **Conclusion:** The two formulations were considered bioequivalent, safe and well tolerated. **Keywords:** bioequivalence, biodisponibility, propafenone. **References:** Freemantle N. Journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology. 13(3):329-45, 2011. Grebe SK, Singh RJ. LC-MS/MS in the Clinical Laboratory - Where to From Here? The Clinical biochemist Reviews. 32(1):5-31, 2011. Lafuente-Lafuente C, Valembois L, Bergmann JF, Belmin J. Antiarrhythmics for maintaining sinus rhythm after cardioversion of atrial fibrillation. The Cochrane database of systematic reviews. Vol. 3, 2011. **Financial support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. **Approval number of ethics committee on human research:** 879/2011.

**11.007 Evaluation of the safety and wound healing activity of the aqueous extract of *Sorocea guilleminiana* Gaudich**. **Leaves**. Figueiredo FF, Venturini CL, Pavan E, Oliveira DM, Paes RL, Almeida POA, Martins DTO UFMT – Basic Sciences in Health

**Introduction:** *Sorocea guilleminina*, Moraceae, popularly known as "espinheira-santa-falsa", is a native and endemic Brazilian shrub found in the Cerrado and Atlantic forest, used by traditional medicine in the treatment of wounds among others diseases. Objective: To evaluate the genotoxicity, acute toxicity and wound healing activities of aqueous extract of S. guilleminiana leaves (AESg) in experimental models. **Methods:** AESg was prepared by infusion of 10 g of the powdered leaves in 1000 mL of water for 15 min (yield of 8.2%). Genotoxicity was assessed in Chinese hamster ovarian epithelial cells (CHO-k1) by the micronucleus and comet assays. The acute toxicity of AESg was evaluated by Hippocratic test in female and male Swiss-Webster mice. In order to evaluate the wound healing activity of AESg, excision wound model was carried out in Wistar rats. The animal experiments were only carried out after authorization from the CEUA - UFMT (nº 23108.175769/2016-53). **Results:** Treatment with AESg (10, 30 or 100 µg/mL) did not change the median values of micronucleus (MN), bridges (NP), buds (BD), as well as the nuclear division index (NDI) in relation to the vehicle group. Doxorubicin, used as a standard drug, promoted an increase ($p < 0.001$) of 266.01 % in the number of MN, 385.2 % in the number of NP and 413.3 % in the number of BD, with an NDI of 1.11. In the comet test, pre-treatment with AESg completely prevented (~ 100%, $p < 0.001$) $H_2O_2$-induced damage to DNA at the three concentrations tested, while co-treatment reduced the damage completely only at the lowest concentration. Post-treatment with AESg inhibited ($p < 0.001$) DNA damage at concentrations of 10 (36.37%) and 100 µg/mL (27.27%). In the acute toxicity test, AESg (2000 mg/kg) did not cause changes in clinical signs and symptoms and relative organ weights, except the increase in the stomach weight of female mice (31.07%, $p < 0.05$). In the excision wound model, topical treatment with 0.2 % AESg (w/w) increased the rate of wound contraction ($p < 0.05$) on the $5^{th}$ and $9^{th}$ day (44.64% and 6.77%, respectively), while treatment with 5% AESg increased the rate of wound contraction ($p < 0.05$) at $7^{th}$ (10.88%) and $9^{th}$ day (7.91%) respectively, compared to the vehicle (non-ionic cream base). Fitoscar® (6% w/w) increased the rate of wound contraction on the $7^{th}$ day ($p < 0.05$), reaching the maximum effect on the $9^{th}$ day (8.34%) when compared to the vehicle. The treatment with AESg did not alter the re-epithelialization time in any of the doses tested, the same effect found for the Fitoscar®. **Conclusion:** AESg was non-genotoxic in the micronucleus and comet in vitro assays and showed no acute toxicity in mice. AEGs showed antigenotoxicity activity and potent wound healing effect, confirming the popular use of *S. guilleminiana* leaves remedies to treat wounds. **Financial Support** and Acknowledgments: CAPES/Pró-Amazônia, INAU-INCT and CNPq.

**1111.008 Determination of glyphosate in urine samples derivatisation with 9-fluorenylmethyl chloroformate by liquid chromatography with fluorescence detection**. Melo KG[1], Rosa PCP[1], de Nucci G[1], Trape AZ[2], Garlipp CR[3] [1]FCM-Unicamp – Farmacologia, [2]FCM-Unicamp – Farmacologia e Toxicologia, [3]FCM-Unicamp – Patologia

**Introduction:** Glyphosate had its wide activity discovered in 1970 when Monsanto synthesized the herbicide for the first time. Its success is due to the fact that it presents a wide spectrum of action, which makes possible an excellent control of weeds[3] and for that, it has become the bestselling pesticide in the world, having registered in more than 160 countries and representing 60% Of the world market in non-selective herbicides[2]. Considering the environmental and health risks, it is of paramount importance to develop effective and safe methodologies to assess and determine the level of exposure of farmers and ranchers to direct contact with pesticides. This work consisted in the development and validation of an analytical methodology in Fluorescence HPLC, in order to analyze urine samples from farmers in the northern region of Mato Grosso who had direct exposure to glyphosate. **Methodology:** Working solutions were prepared for ultraviolet water at concentrations of 10.0, 25.0, 50.0, 75.0 and 100 µg/kg, obtained by diluting the standard stock solutions of 1mg/mL. SPE cartridges were preconditioned from methanol acidified with 0.1% formic acid, and then eluted with acetonitrile. Then the solution was derivatized with the Fmoc-Cl derivative and 0.2 mol/L borate buffer pH 9. Stability tests of short and long duration, with the standard and of contaminated samples, were carried out in the time of 6 and 72 hours, respectively. The stability test of three freeze-thaw cycles in the 24 hour interval was also performed. The analytical methodology is being developed and will be validated by evaluating the following parameters: linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy (recovery) and precision (repeatability and intermediate precision) and robustness. **Results:** During the experimental design, the concentration of 0.2 mol/L of the borate buffer solution pH 9 presented a larger area in relation to the lower buffer concentrations. It has been found that increasing the buffer concentration in the reaction medium promotes the reactivity of the amine function and stabilizes the solubility of the derivatizing reagent in acetonitrile favoring the bypass process. Therefore, the sodium borate solution at a concentration of 0.2mol/L was selected for subsequent testing. In addition, preliminary results showed that the analytical method allowed accurate quantification of the target compound of up to 10ppb with excellent repeatability. The results using the extraction with SPE cartridges had recoveries between 60-98% in the matrix. **Financial Support:** National Council for Scientific and Technological Development (CNPQ). **CAAE:** 62715416.1.0000.5404 **References:** 1. VELINI, E.; D*. et al*. Glyphosate. 1. ed. Fepaf. São Paulo,p. 21-23. 2009 2. MONSANTO. Segurança do Glifosato, 2015. Disponível em: http://www.monsanto.com/global/br/produtos/pages/seguranca-glifosato.aspx Acesso em: 03 jun. 2017. 3. SANCHO, J.V.,. *Chromatography.* 737, 75-83, 1996. 4. HOGENDOORN, E.A. *J. Chromatography.* 833, 67-73, 1999.

**11.009 Association of the *ABCB1* c.3435C>T genotypes and required warfarin doses**. Tavares LC[1], Marcatto LR[1], Cassaro-Strunz CM[1], Scanavacca M[1], Krieger JE[1], Pereira AC[1], Santos PCJL[2] [1]FMUSP, [2]Unifesp – Farmacologia

**Introduction:** In the last 65 years, warfarin has been the most widely prescribed oral anticoagulant for treating and preventing thromboembolic disorders. However, due to its narrow therapeutic index and high variance of pharmacokinetics and pharmacodynamics, pharmaceutical interpatient responses are largely variable. Among other factors, genotype is well established to influence the ideal dose of warfarin to achieve therapeutic anticoagulation. A determinant of interindividual variability is the single nucleotide polymorphism (SNP) c.3435C>T (rs1045642) present in the *ABCB1* gene, which codes for the known multidrug efflux pump P-glycoprotein (P-gp), commonly associated with development of resistance to many drugs. **OBJECTIVE** The aim of this study was to assess the association between *ABCB1* c.3435C>T genotypes and required doses of warfarin. **Methods** For this retrospective study, DNA samples from 1,128 patients, enrolled in the Arrhythmia and Pacemaker Clinical Units of Heart Institute (InCor), University of São Paulo Medical School (FMUSP), were genotyped for *ABCB1* c.3435C>T by polymerase chain reaction followed by melting curve analysis (HRM-PCR). From all included patients, 344 of them presented stable warfarin dose, which was considered when the three last measures of standardized prothrombin time (INR) reached the target range 1.8 to 3.2. All statistical analyses were carried out using the SPSS software (v. 20.0). P value < 0.05 was set as level of significance. **Results** The observed genotypic distribution for the *ABCB1* c.3435C>T polymorphism was: 35.5% CC wild-type, 45.7% CT heterozygous, and 18.9% TT variant homozygous (n=1,128). For the sample of patients with stable warfarin treatment (n=344), the observed required dose of warfarin ranged from 6 to 105 mg per week. By analyzing the mean warfarin stable dose required for each *ABCB1* 3435C>T genotype, we found no significant differences (mean stable dose, in mg/week: CC = 29.7 ± 12.4, n=107; CT = 28.4 ± 12.7, n=168; TT = 26.3 ± 9.4, n=69). **Conclusion** In conclusion, our results suggest c.3435C>T polymorphism in the *ABCB1* gene may not have a potent impact in warfarin required dose variability. **Financial Support**: São Paulo Research Foundation (FAPESP), Process Number: 2013/09295-3. This study was approved by the ethics committee and all subjects included provided written informed consent (SDC: 3554/10/143, CAPPesq: 0804/10).

**11.010 Molecular Mechanisms Associated with Palmitate Induced Atrophy in Cells C2C12**. Paixão AO, Rodrigues AC ICB-USP – Farmacologia

**Introduction:** Obesity is a multifactorial and complex disease that is defined as excess fat in the body. In addition to the expansion of adipose tissue, there is ectopic accumulation of fat in non-fat tissues, such as skeletal muscle, leading to insulin resistance in this tissue. The accumulation of fatty acids and/or their derivatives may be the reason not only for insulin resistance but also for the decline of muscle mass in obesity. Objective: The effect of palmitic acid, the most abundant saturated fatty acid in the body, on muscle atrophy and regeneration in C2C12 cells was investigated. Methodology: For all assays, C2C12 cells were treated for 1-5 days with palmitic acid (100æM and 150µM) or vehicle (0.5% ethanol). The MTT and LDH assay were evaluated for cell viability and cytotoxicity, respectively. Also, it was performed in vitro healing assay for cell regeneration analysis; immunofluorescence for identification of myosin heavy chain (MyHC, Sigma), DAPI for cell differentiation analysis, quantification of mRNA (MyoD, Myostatin, Miomesin, Myosin heavy chain 7 (MyH7) and MyF5 genes) and microRNA expression (miR-1) by real-time PCR. **Results:** The MTT and LDH assay showed that palmitic acid (100µM and 150µM) did not affect cell proliferation/viability in the C2C12 lineage, but both concentrations reduced the cell differentiation process. The cells who were treated with Palmitic acid had smaller diameter, size and number of mature myotubes, as indicated by measurement of mature myotubes marked with MyHC slow contraction. In the healing assay, we observed that the cells treated with 100µM palmitic acid kept the migration ability and regenerated as faster as the vehicle treated cells; however, cells treated with 150µM palmitic acid could not regenerate even after 16 hours of injury. Concerning the gene expression of genes related to muscle development and miR-1, MyH7 and myomesin genes, had a significant change in all treatments from day 4, the expression of these genes were increased, whereas MyF5 and myostatin did not have significant change. In addition, there is an increase in MyoD expression in the control group when compared to palmitic acid treatments. Mir-1 is responsible for the differentiation of myoblasts and it was observed that the expression of miR-1 was increased from the 3rd day of differentiation in all the groups, however, 150µM palmitic acid obtained a higher expression of miR-1 in comparison to vehicle and 100µM palmitic acid group. **Conclusion:** The present study shows that palmitic acid (100µM and 150µM) induces atrophy in C2C12 cells probably affecting cell differentiation process. **Financial support:** FAPESP(2016/09173-3). Bryner, W. ISRN Obes. 2012(2012). Chavez, A. J. Biol. Chem. 280: 20148(2005). Frias F. Front Endocrinol. 7:76(2016).

**11.011 Angiotensin converting enzyme inhibitors enhance the hypotensive effects of propofol by increasing nitric oxide production**. Oliveira-Paula GH[1], Pinheiro LC[1], Ferreira GC[1], Garcia W[2], Lacchini R[3], Garcia LV[2], Tanos-Santos JE[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Anestesiologia, [3]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas

**Introduction:** Propofol anesthesia is usually accompanied by hypotension. Studies have shown that the hypotensive effects of propofol increase in patients treated with angiotensin-converting enzyme inhibitors (ACEi). Given that both propofol and ACEi affect nitric oxide (NO) signaling, the present study tested the hypothesis that ACEi treatment induces pronounced hypotensive responses to propofol by increasing NO bioavailability. **Methods:** We evaluated 65 patients, divided into three groups: hypertensive patients chronically treated with ACEi (HT-ACEi; n=21), hypertensive patients treated with other antihypertensive drugs instead of ACEi (HT; n=21), and healthy normotensive subjects (NT; n=23). Venous blood samples were collected at baseline and after 10 minutes of anesthesia with propofol 2 mg/kg administrated intravenously by bolus injection. Hemodynamic parameters were recorded at each blood sample collection. Plasma ACE activity was evaluated by using a fluorometric assay. Plasma nitrite levels were determined by using an ozone-based chemiluminescence assay. Additionally, we used experimental approaches to validate our clinical findings. **Results**: Decreased ACE activity was found in HT-ACEi group as compared with NT and HT groups (P<0.0001), confirming that patients from HT-ACEi group really adhered to ACEi treatment. Higher decreases in systolic and mean blood pressure after propofol anesthesia were observed in HT-ACEi group as compared with those found in NT and HT groups (P<0.05). Enhanced increases in nitrite levels after propofol anesthesia were observed in HT-ACEi patients compared with NT and HT groups (HT-ACEi=30.5±33.5 versus NT=10.3±17.3 and HT=9.7±19.5 nmol/l, respectively; P<0.05). Adjustment for age in multiple linear regression analysis did not change these findings. Finally, we found a negative correlation between systolic blood pressure and nitrite levels (r=-0.818; P=0.046). These clinical results were recapitulated in experimental approaches. Wistar rats treated with the inhibitor of NO synthases Nω-nitro-L-arginine methylester (L-NAME) showed decreased hypotensive responses to propofol (1, 2 and 4 mg/kg, i.v.) compared with rats treated with phenylephrine (P<0.05). In addition, rats treated with the ACEi enalapril 20 mg/kg by gavage for seven days showed a highly pronounced hypotensive response to propofol compared with controls (P<0.05). **Conclusion:** Our data show that ACEi enhance the hypotensive responses to propofol anesthesia and increase nitrite concentrations. These findings suggest that increased NO bioavailability may account for the enhanced hypotensive effects of propofol in ACEi-treated patients. **Financial support:** CNPq and FAPESP This study was approved by the Institutional Review Board at the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo (Process 1129/2013) and by the Institutional Animal Care and Use Committee of the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo (Process 63/2017).

**11.012 Ceftriaxone population pharmacokinetics in adults and limited sampling strategy in paediatric patients**. Neves DV[1], Lanchote VL[1], Oosterholt S[2], Pasqua OD[2] [1]FCFRP-USP – Análises Clinicas, Toxicológicas e Bromatológicas, [2]University College London – Clinical Pharmacology & Therapeutics

**Introduction:** Ceftriaxone is a third-generation cephalosporin approved for use in adults and children for the treatment of a range of infections. A particular feature of ceftriaxone is its long elimination half-life, approximately 6 h, and extensive binding to plasma proteins. It is eliminated in urine as unchanged drug (0.33-0.44), but biliary excretion transporters-mediated seems to be one of the major elimination pathway of the drug. Considering that the pharmacokinetic characteristics of a drug change over the course of a child's development, the aim of the current investigation was to evaluate the population pharmacokinetics of ceftriaxone in healthy adults and to simulate a limited sampling strategy for future characterization of ceftriaxone pharmacokinetics in the paediatric population. **Methods:** Data from 12 healthy adults receiving ceftriaxone (intravenous infusion of 1 g over to 3 min) were used for model building purposes. A population pharmacokinetic analysis was performed using NONMEM v.7.2. Allometric scaling and renal functional maturation were included to extrapolate ceftriaxone pharmacokinetics to neonates and children. Internal and external validation procedures implemented before sampling optimization were performed using the ED-optimality criterion of PopED. **Results:** A two-compartment model was found to best describe the pharmacokinetics of ceftriaxone. Allometric scaling was implemented to address the effect of body weight on clearance and central volume of distribution across all age groups. On the other hand, the inclusion of a renal maturation function accounted for the effect of organ maturation in neonates and toddlers. Means central and peripheral volume of distribution were 5.83 L and 2.97 L, systemic clearance was 0.98 L/h and intercompartmental clearance was 1.14 L/h. Actual body weight was the most important covariate for the volume of distribution. A sparse sampling system with no more than four samples per patient (t = 0.21, 0.95, 5.35, 11.75 h post dose) proved to be sufficient to describe the pharmacokinetics of ceftriaxone. **Conclusion:** A limited sampling strategy using only 4 times sampling (0.21, 0.95, 5.35 and 11.75 h) following drug administration should be considered to characterize ceftriaxone pharmacokinetics in paediatric patients. **References**: Nahata MC, Drug Intell Clin Pharm, 19, 900, 1985. Li N, Pediatrics, 133, e917, 2014. Patel IH, Antimicrob Agents Chemother, 20, 634, 1981. Brogard JM, Schweiz Med Wochenschr, 117, 1549, 1987. Kato Y, Drug Metab Dispos, 36, 1088, 2008. **Financial Support** and acknowledgements: The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2014/06526-7) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for **Financial Support**. Human Research Ethical Committee: The study was approved by the Research Ethics Committee of the Hospital das Clínicas of the Medical School of Ribeirão Preto, University of São Paulo (CEP-HCFMRP-USP 942889/2015).

**11.013 Microsatellite polymorphism in the heme oxygenase-1 promoter is not associated with antihypertensive therapy response in preeclampsia**. Sandrim VC[1], Lacchini R[2], Coeli-Lacchini FB[3], Cavalli R[4] [1]IBB-Unesp – Farmacologia, [2]FMRP-USP – Enfermagem Psiquiátrica e Ciências Humanas, [3]FMRP-USP – Medicina Interna, [4]FMRP-USP – Ginecologia e Obstetrícia

**Introduction:** Although of the etiology of preeclampsia is not completely known, it is well recognized that an unbalance in oxidative stress, proinflammatory and antiangiogenic factors in found. An important mechanism of oxidative stress regulation is the expression of the enzyme heme oxygenase encoded by the *HMOX-1* gene which is highly induced in response to stress, catalyzing the decomposition of heme into CO, ferrous iron and biliverdin. These products have antioxidant, anti-inflammatory, vasodilatory, and angiogenic functions. Importantly, the promoter region of *HMOX-1* present a guanine-thymine (GTn) microsatellite polymorphism; and higher expression is associated with ≤25 repeats compared to long allele (> 25 repeats). The hypothesis of this study is that preeclampsia pregnant and mainly those not responsive to antihypertensive therapy (poor clinical outcome) present higher frequencies of long allele of GTn polymorphism which may impair HMOX-1 expression and consequently their functions. Therefore, our objective was compared allele frequencies of this polymorphism between healthy and preeclampsia pregnant; and between preeclampsia responsive and not responsive to antihypertensive therapy. **Methods:** The subjects were recruited from the *Hospital das Clinicas de Ribeirao Preto*, University of Sao Paulo. All participants provided written informed consent. We enrolled 94 healthy and 81 preeclampsia (44 responsive and 37 non-responsive to antihypertensive therapy). Preeclampsia was defined following guidelines of National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. The patients included in this study were monitored closely for signs and symptoms of PE, with careful fetal surveillance and laboratory tests at least twice weekly. Responsiveness to therapy was based on the evaluation of clinical and laboratory parameters in response to the administration of antihypertensive drugs. Genomic DNA was extracted from whole blood and genotyping was done using electrophoresis capillary methodology. We subgrouped the genotypes in the short (S) GTn allele (≤25 repeats) and to the long (L) allele (>25 repeats). Categorical variables were analyzed using the $\chi 2$ test and continuous variables were performed using the Mann–Whitney *U* test or t-test. A *P* value <0.05 was considered significant. **Results:** As expected preeclampsia pregnant present higher systolic and diastolic blood pressure levels and lower gestational age of delivery and newborn weight (all P<0.05). Regarding genotype frequencies, we found similar frequencies between healthy and preeclampsia pregnant (48%, 46% and 6% *vs* 59%, 35% and 6% respectively to LL, SL and SS genotypes, P>0.05). Moreover, lack of difference was found among preeclampsia responsive and nonresponsive to antihypertensive therapy (52%, 36% and 11% *vs* 53%, 36% and 11% respectively to LL, SL and SS genotypes, P>0.05). **Conclusion:** We conclude that the microsatellite polymorphism located in *HMOX-1* is not related with preeclampsia development neither antihypertensive therapeutic response. **Financial Support:** CNPq and FAPESP-Brazil **Research Approval:** Clinical Process reference 4682/2006 (approved by the IRB-HCRP*).*

**11.014 Study of the oral acute toxicity of zinc oxide nanoparticles**. Sousa RV[1], Abreu CPV[1], Oliveira CP, Andrade PVD, Gern JC, Wouters F, SIlva SR, Brandão HM – [1]UFLA – Medicina Veterinária

The nanomaterials have had a great development and the nanotechnological industry is in continuous growth. Some nanomaterials, like zinc oxide nanoparticles (ZnO NP), are used in the cosmetic, pharmaceutical and even in the food industry. The toxicological potential of ZnO NP is described in the literature. In the study of Wang et al with two different size of the ZnO powder, 20 nm and 120 nm, one mice died after ingesting one oral dose of 2000 mg.Kg-1 of both NP. Considering that the toxicological effects of the inorganic NP is not totally stablished and the importance to know the safety of them, this work aimed to evaluate the acute oral toxicity of ZnO NP at four different doses following the OECD Guideline 423 for Testing of Chemicals. Healthy female BALB/c isogenic micewild-type mice (3/group) were treated with increasing doses of ZnO NP (5, 30, 300 and 2000 mg.Kg-1). The experiment was approved by the Local Animal Care and Use Commitee (CEUA/UFLA), number 020/16. ZnO NP (~450 nm by FEG-SEM) were suspended in bovine serum albumin and homogenized for 16 minutes to disperse (400 Watt Branson Sonifier S-450D ). Each animal received one dose of ZnO NP by gavage using a suitable intubation cannula. After dosing they were observed for clinical signs on the first 4 hours, each 12 h during 48 h and each 24 h until the 14th day. The body weight was determined before the administration of the nanoparticles (day 1), and after 7 and 14 days of the experiment. In the 14th day, the animals were humanely killed by cervical dislocation and samples of kidney, liver, heart, lung, spleen, pancreas, intestine, muscle, brain, lymph nodes were collected for histopathological analyses. In this trial, we did not observe mortality or clinical signs indicating a toxic effect of ZnO NP, even in the highest dose. The only significant ($p<0.05$) effect was an increase in the body weights after seven days; however, this happened in all groups, including the control. Histopathological analysis did not show any visible lesions in the different organs. Similar results were observed with by ZnO NP until the doses of the 2000 mg.Kg-1. In conclusion, the results of this work showed that an oral administration of ZnO NP was not toxic on single doses until 2000 mg.Kg-1 and did not cause significant clinical signs. Acknowledgements: Fapemig, CNPq, Rede agronano, NanoReg

**11.015 Populational pharmacokinetic modeling of cefazolin prophylatic doses to morbidly obese patients undergoing bariatric surgery**. Palma EC[1], Meinhardt NG[2], Heineck I[1], Fischer MI[3], Stein AT[2], Araújo BV[4], Dalla Costa T[4] [1]UFRGS – Pharmaceutical Sciences, [2]Hospital Nossa Senhora Conceição, [3]UFRN – Pharmacy, [4]UFRN – Pharmaceutical Sciences

**Introduction:** Bariatric surgery is an effective therapy for treating morbidly obese patients (BMI ≥ 40 kg/m2)[1]. Cefazolin (CFZ) is used to prevent surgical site infections (SSI)[1,3]. A prophylactic standard 2 g dose is used for obese and normal weight patients undergoing abdominal surgery[3]. A prophylactic CFZ 3 g dose for obese patients has been suggested recently[4]. The aims of this work was to propose a populational pharmacokinetic (POPPK) model to describe CFZ concentrations in plasma and subcutaneous tissue of patients undergoing bariatric surgery. **Methods**: Ethics in Research Committee approval from UFRGS (572-000) and HNSC (12-058). Before incision, patients received 2 g or 3 g CFZ i.v. *bolus* dose and blood and abdominal subcutaneous microdialysate (CMA 66 probe, 30 mm) samples were collected up to 4 h post-dose. Total and free (ultrafiltration, Millipore Centrifree®) CFZ plasma levels, and free tissue levels were assayed by validated LC/UV method. Microdialysis probes recovery was determined by retrodialysis previous to drug dosing. Pharmacokinetic (PK) profiles were evaluated by non-compartmental analysis (NCA) (Phoenix®) and parameters compared by Student´s "t" test. POPPK analysis was performed in Monolix v.4.3.3 (Lixoft®) using SAEM algorithms as the first-order conditional estimation. **Results and Discussion**: Patients received 2 g (n = 4, BMI 49.7 ± 5.4 mg/h$^2$) or 3 g (n = 5, BMI 44.0 ± 5.1 mg/h$^2$) of CFZ. Probes recovery was 17.7 ± 4.84 %. Individual recoveries were used to determine real free tissue levels for each patient. Body exposure to CFZ was proportionally increased with dose. No statistical differences on CFZ plasma PK parameters determined by NCA were observed between 2 g and 3 g doses: $t_{1/2}$ of 2.9 ± 1.6 h and 2.8 ± 1.2 h; $CL_{tot}$ of 5.3 ± 2.3 L/h and 5.2 ± 1.7 L/h; and $Vd_{SS}$ of 16.0 ± 1.7 L and 17.2 ± 4.5 L, respectively ($p > 0.05$). The same holds true for parameters determined from free plasma and tissue profiles. A 2-compartment POPPK model with fluctuating and saturable protein binding in plasma and subcutaneous tissue was developed to simultaneously describe these three sets of data. Interindividual variability was attributed to volume of distribution of the central compartment, inter-compartmental clearance, clearance from the central compartment and maximal binding capacity to tissue albumin. No covariates tested reduced significantly the -2-likelihood function. **Conclusion**: The POPPK model proposed, that accounts for protein binding saturation in plasma and tissue, should improve CFZ prophylactic dosing to prevent SSI in patients submitted to bariatric surgery. **Acknowledgements: Financial Support** from PPSUS/FAPERGS 2013 (#1298-255/13-8). **References:** 1. ECHOLS J. Prof. Case Manag. 15(1): 17-26, 2010. 2. ALEXANDER, J.W. Surg. Infect. 10: 53-7, 2009. 3. BRATZLER D.W. Am. J. Health-Syst. Pharm. 70: 195-283, 2013. 4. BRILL, M.J.E. et al. J. Antimicrob. Chemother. 69(3): 715-23, 2014.

**11.016 Evaluation of the association between 3435 C>T ABCB1 gene polymorphism and response to clozapine treatment**. Ghedini PC, Brito RB UFG – Farmacologia

**Introduction:** Genetic and environmental factors contribute to the interindividual variability in clozapine (CLZ) treatment response. P-glycoprotein 1 also known as multidrug resistance protein 1 (MDR1) or ATP-binding cassette sub-family B member 1 (ABCB1) is an important protein of the cell membrane that pumps many foreign substances out of cells. Several single-nucleotide polymorphisms (SNPs) of ABCB1 gene are known to affect transporter activity of drugs, causing changes in the pharmacokinetics of them (Consoli *et al*., Pharmacogenomics J., 10(8): 1267, 2009). Therefore, the aim of the study was to investigate the possible association between C3435T polymorphism in the ABCB1 gene and therapeutic response to CLZ treatment.

**Methods**: The study group comprised 47 men (69.1%) and 21 women (31%) and the median age of all patients was 39.2 ± 9.9 years (range 18-60 years). Genomic DNA was extracted from blood samples of patients who were born and living in the Goiás State, Brazil, diagnosed with refractory (RS; 34 patients) or super-refractory schizophrenia (SRS; 34 patients) on stable CLZ treatment for at least 1 year. The polymorphism 3435 C>T was detected by PCR and sequencing technique. The allelic and genotypic frequencies and relationship with symptomatology and treatment response were determined. Statistical analyses ($X^2$ and Fischer exact test) were performed using SPSS version 21 and Hardy Weinberg equilibrium was analyzed with Arlequin version 3.5. A $P < 0.05$ was considered to indicate statistical significance. All experimental procedures were approved by the Ethics and Research Committee of the Federal University of Goiás (protocol number CEP/UFG 039/13). **Results:** The frequency of C allele was 63% (RS) and 66% (SRS) and for T mutated allele was 37% (RS) and 34% (SRS), respectively. The genotype frequencies in RS group were 38.2% (CC), 50.0% (CT), and 11.8% (TT), and in SRS group were 52.9% (CC), 26.4% (CT), and 20.6% (TT), respectively. Statistical analysis not found differences between RS and SRS groups for both allele (P= 0.13) and genotype frequencies (P= 0.86). The CLZ mean dose was 548.4 ± 128.7 (CC), 559.6 ± 151.0 (CT), and 518.2 ± 132.8 mg (TT), respectively, showing no influence of allele mutated on the administered CLZ dose (P= 0.869). The alcohol and cigarette uses were similar in both SR and SRS groups (P= 0.44 and 0.31, respectively) and without association between genotype and alcohol or tobacco users (P>0.05). **Conclusions**: These preliminary results suggest that polymorphism 3435 C>T of ABCB1 gene, at least individually, is not associated with refractoriness to schizophrenia treatment. More studies using a large number of patients and analyzing the interaction of several ABCB1 SNPs can lead to a better understanding of the association of ABCB1 polymorphisms and CLZ therapeutic response. **Financial Support:** Fapeg, Capes and CNPq

**11.017 Pharmacological inquiry of negative results associated with medications for hypertension and diabetes in primary care facilities in Manaus**, **AM**. Pinto EO[1], Cruz LO[1], Albuquerque NR[1], Correa JWN[2] [1]UFAM – Enfermagem, [2]UFAM – Pharmacology

**Introduction:** Systemic Arterial Hypertension (SAH) and Diabetes Mellitus (DM) are public health problems. Due to the high prevalence and incidence of such diseases, the HIPERDIA program was created, with the purpose of organizing care, preventing illness and promoting health in a systematic way through the link of users to the health network. **Methods:** This is a quantitative, descriptive and prospective study, aiming to verify the occurrence of Negative Results Associated with Medication (NRM) in hypertensive and diabetic patients, treated in the primary health care network, using the Pharmacotherapeutic Follow - up Dader method. **Results:** The study involved 382 users, the majority being female (58%). Cases of isolated hypertension were the most prevalent (47.6%) when compared to other pathologies (DM1 4.5%, DM2 14.4% and DM + HAS 33.5%). The Effectiveness category was the most prevalent among the observed NRMs, with the most common being quantitative inefficiency (54.9%), a usual reflex of low adherence to therapy. The non-quantitative inefficacy was 23.1%, which reflects the need for modification in the therapy, which is only possible from medical care. Quantitative insecurity was observed in 15.4% of the patients, being this variable related to the manifestation of side effects. On the other hand, 4.5% of the patients were affected by non-quantitative insecurity, since it reflects misuse of the drugs, either by overdose or forgetfulness in the administration of the drug. As for the need category, only 1.9% of the patients presented an untreated health problem and 0.3% were taking drugs not required. **Conclusion:** Treatment of hypertension and diabetes causes adverse effects due to the use of various medications. The occurrence of adverse reactions contributed to non-adherence to treatment. In this context, the lack of knowledge about the health framework itself and low schooling were aggravating factors. It is observed that a large part of the users received adequate treatment, since the statistics of need while evaluating the NMRs were less representative. On the other hand, it reinforces the need for frequent monitoring of users, to reduce side effects, guarantee information about the disease and promote the rational use of medicines. Key Words: Negative Results Associated with Medication, Drug-Related Problems, Arterial Hypertension, Diabetes Mellitus, Basic Health Unit. **Financial Support** and acknowledgments: PROEXTI-UFAM, FAPEAM, PREFEITURA DE MANAUS/AM CAAE: 49066815.0.0000.5020.

**11.018 Influence of cetirizine on gabapentin kinetic disposition and pharmacodynamics in patients with neuropathic pain**. Costa ACC[1], Yamamoto PA[2], Benzi JRL[1], Lauretti GR[3], Moraes NV[2] [1]FCFRP-USP, [2]FCFar-Unesp-Araraquara, [3]FMRP-USP

**Introduction:** Gabapentin (GBP), used to treat epilepsy and neuropathic pain, is mainly eliminated as the unchanged drug in urine. Its elimination is partially dependent on the active secretion by organic cation transporter 2 (OCT2). Cetirizine (CET), an anti-allergic of second generation has been recommended by the International Transporter Consortium for use as OCT2 inhibitor in clinical studies. Our objective was to evaluate the effect of cetirizine on GBP pharmacokinetics and pharmacodynamics in patients with neuropathic pain. **Methods:** An open-label, two-period, crossover, non-randomized clinical trial was conducted in patients with neuropathic pain higher than 4, in a 0-10 analogue pain scale (n=8). Patients were treated with 300 mg GBP (Treatment A) and 20 mg/day of CET for five days and 300 mg GBP on the last day of CET treatment (Treatment B). Blood samples were collected up to 36 hours after drug administration and pain attenuation was assessed up to 36 hours after GBP administration. **Results:** CET treatment resulted in reduced $AUC^{0-\infty}$ and $C_{max}$, and higher apparent total clearance and volume of distribution in patients treated with single dose GAB. The geometric mean ratio (90% confidence intervals) of $AUC^{0-\infty}$ and $C_{max}$ (B vs. A) were 78 (68% – 90%) and 73.6 (59-96%), respectively. The geometric mean (geometric coefficient of variation) of the concentrations required to achieve 50% of maximum effect ($EC_{50}$) were similar for treatments A [996.4 ng/mL (43.7%)] and B [756 ng/mL (49.2%)] ($p>0.05$). **Conclusion:** Although cetirizine treatment reduced the systemic exposure to gabapentin, this pharmacokinetic interaction has no clinical relevance because no differences were found in terms of neuropathic pain attenuation. **Financial Support**: CNPq, Programa de Apoio ao Desenvolvimento Científico (PADC-FCF-UNESP) Research was approved by the Ethics Committee of the School of Pharmaceutical Sciences of Ribeirão Preto and School of Medicine of Ribeirão Preto, University of São Paulo (USP) on April 16th, 2015 (CAAE: 34175314.3.0000.5403).

**11.019 Study of the oral acute toxicity of titanium dioxide nanoparticle**. Abreu CPV[1], Brandão HM, Oliveira CP, Wouters F, Pereira MM, SIlva SR, Sousa RV[1] [1]UFLA – Medicina Veterinária

Titanium dioxide ($TiO_2$) is used as an additive in the food industry, and it is also present in personal care products, like toothpastes. $TiO_2$ is chemically inert, but nanoparticles (NP) possess different physicochemical properties and can have negative health effects. Because there is some evidence that TiO2 NP are absorbed in the gastrointestinal tract, the present study evaluates the acute oral toxicity of four different doses of these nanoparticles. We followed the protocol established by the OECD Guideline 423 for Testing of Chemicals. This experiment was carried out at Department of Veterinary Medicine of University of Lavras. In brief, healthy female BALB/c isogenic mice wild-type mice (3/group) were treated with increasing doses of $TiO_2$ NP (0; 5; 30; 300; and 2000 mg.Kg-1).The experiment was approved by the Local Animal Care and Use Committee (CEUA/UFLA), number 020/16. TiO2 NP (510nm by DLS) were suspended in bovine serum albumin and homogenized for 16 minutes to disperse (400 Watt Branson Sonifier S-450D). Each animal received one dose of TiO2 NP by gavage using a suitable intubation cannula. After dosing they were observed for clinical signs on the first 4 hours, each 12 h during 48 h and each 24 h until the 14th day. The body weight was determined before the administration of the nanoparticles (day 1), and after 7 and 14 days of the experiment. In the 14th day, the animals were humanely killed by cervical dislocation and samples of kidney, liver, heart, lung, spleen, pancreas, intestine, muscle, brain, lymph nodes were collected for histopathological analyses. In all groups, including the control group, the body weights increased ($p<0.05$) after seven days. No adverse clinical sign was observed during the observation period and there were no visible lesions in the histopathological analyses. The results of this study demonstrated an absence of toxicological effects in concordance with the study of Warheit et al. We concluded that, in our conditions, a single dose of $TiO_2$ NP until 2000 mg.Kg-1 has no oral toxicity to mice. Acknowledgements: Fapemig, CNPq, Rede agronano, NanoReg

**11.020 Chronic treatment with paracetamol impairs vascular relaxation in rats**. Porto HKP, Rocha ML UFG – Ciências Farmacêuticas

**Introduction:** Paracetamol (PAR) is one of the most consumed drugs in the world; however its indiscriminate use causes severe organ damages. Early studies have shown the several hepatic and kidney damages, particularly induced by PAR. The probable mechanism of these damages is the high oxidative stress induced by PAR, either in high dosages or due long expositions. Due this fact and knowing that the cardiovascular system is extremely sensitive to oxidative stresses, the continuous exposition to PAR could cause vascular cells damages and cardiovascular dysfunction. This work aimed to evaluate the possible vascular dysfunction caused by chronic treatment with PAR in isolated arteries from rats. **Methods:** Male rats (210 ± 30g) were exposed to a dosage of 400mg/Kg/day of PAR in drinking water for 14 days. The arterial pressure was constantly verified by tail-cuff method. On the $15^{th}$ day, the rats were euthanized, the thoracic aorta was removed, cleaned, sectioned in rings (~4mm) and set up to isometric recordings in an organ bath. The endothelium of some arteries was mechanically removed. After stabilization (50 min, resting tension of 1,5 g), cumulative concentration-effect curves for phenylephrine ($E^-$, 0.1 nM to 10 µM), Ach ($E^+$, 0.1 nM to 10 µM) and SNP ($E^-$, 0.1 nM to 1 µM) were carried out. These protocols also were performed in presence (30 min) or absence of Vitamin C (100 µM). **Results:** The level of blood pressure remained unchanged after treatment with PAR. The vascular contraction induced by phenylephrine was similar between PAR (2.46 ± 0.25g, n=5) and control group (2.39 ± 0.27g, n=7). The endothelium dependent relaxation induced by ACh was impaired in PAR group (58.2 ± 4.9%, n=8) vs. control group (100.3 ± 4.1%, n=8). The treatment with Vitamin C significantly ($p<0.001$) improved the vascular relaxation to PAR group (83.45 ± 4.51%). The relaxation induced by SNP presented impaired potency in PAR group (pD2: 6.5 ± 0.2, n=7) as compared to control group (pD2: 7.8 ± 0.4, n=8). The treatment with Vitamin C significantly ($p<0.01$) improved the potency to SNP after treatment with PAR (pD2: 7.3 ± 0.3, n=5). **Conclusion:** These findings showed that chronic treatment with PAR caused impairment in the vascular relaxation dependent and independent of endothelium. This vascular injury probably occurs due to the high oxidative stress caused by the treatment with PAR, since the treatment with antioxidant (vitamin c) is able to reverse the vascular dysfunction induced by PAR. **Financial support:** FAPEG. Research approval by Animal Research Ethical Committee (CEUA/UFG: protocol 083/2016).

**11.021 PK-PD modeling of triazoles against *Cryptoccocus neoformans* using time-kill curves and simulation**. Alves IA[1], Staudt KJ[1], Kuhn K[2], Dalla Costa T[1], Araújo BV[1] [1]UFRGS, [2]URI-Santo Angelo

**Introduction:** The treatment of cryptococcal meningitis is a big challenge in poor countries where limited availability of amphotecin B and flucytocine results on the recommendation of azoles in monotherapy using very high doses[1]. The use of static data such as the MIC to define susceptibility to antifungal is limited, the potential use of PK/PD modeling for a better understanding of the relationship between PK and PD by defining parameters such as $EC_{50,}$ k, $k_{max}$, N, $N_{max}$. Based on experimental data derived from kill curve experiments these models then serve as a basis to simulate different dosing scenarios[2,3]. In the present study we evaluate the effect of fluconazole (FCZ) and voriconazole (VCZ) against *Cryptococcus neoformans* using time-kill curves modeled with the modified $E_{max}$ equation and simulate the clinical outcomes in different scenarios of doses and modifications of the fungal susceptibility. **Methods:** *C. neoformans* ATCC 28957 was used, the MICs values were determined and time-kill curves was performed using multiple MIC of FCZ (0.062 - 64 x MIC) or VCZ (0.031 - 64 x MIC). An $E_{max}$ model was used to model the curves (Scientist®). The Monte Carlo simulation (MC) (Berkley Madonna®) was done using the free levels expected in the brain of HIV patients after administration of 200, 800 and 2000 mg of FCZ q24h or 200 and 400 mg q12 of VCZ for ten weeks. To simulation an effect with the most frequent MICs, FCZ (4 µg/ mL) and VCZ (0.06 µg/mL), the equation proposed by Mueller et al. (2004) were used to define the value of $EC_{50}$. All experiments were approved by the Ethics Committee of Animal Use of Federal University of Rio Grande do Sul (Protocol # 26605). **Results:** The MIC values were 0.5 µg/mL for FCZ and 0.032 µg/mL for VCZ. The mean value of k = 0.385 $h^{-1}$, $EC_{50}$ = 1.26 ± 0.18 and 0.325 ± 0.059 µg/mL and $k_{max}$ = 0.948 ± 0.204 and 0.639 ± 0.123 $h^{-1}$ to FCZ and VCZ. The $EC_{50}$ calculated to FCZ to MIC of 4 µg/mL was 11.20 µg/mL, for VCZ to MIC 0.06 µg/mL was 0.45 µg/mL. These values were used in the MC simulations showed to FCZ even for the high dose (2000 mg) there is a 10% who do not respond to the treatment and this pattern is associated to MIC=0.5 µg/mL. Simulating the same doses in another scenario wich higher value of MIC (=4.0 µg/mL) the result of simulation is worst. For the smaller dose only 10 % of patients respond to treatment and a time of three weeks is enough to eradicate the yeast. These percentages were similar to failure rates in treatment with FCZ monotherapy in patients with cryptococcal meningitis. The use of VCZ predict a completely yeast eradication in short dose before one week of treatment. **Conclusions:** The results present in this study show the applicability of PK/PD modeling to a better comprehension about the effect of azoles against *C. neorformans*. The analysis indicates the poor effectiveness of FCZ in monotherapy and a variable perceptual of patients who do not response to the treatment that is related to MIC values, but VCZ presents an excellent effectiveness against the yeast for both doses investigated and may a smaller dose could be administered to the patients. **Financial Support:** CNPq/449972/2014-3. **References:** 1. Larsen et al. *Ann Intern Med.* 113,1990. 2**.** Mueller et al. *Antimicrob Agents Chemother* 48, 2004. 3**.** Schmidt, et al . *Expert Opin Drug Discov.* 2,2007.

**11.022 Markers of sildenafil responsiveness in the treatment of erectile dysfunction**: **asymmetric dimethylarginine related genes**. Milanez-Azevedo AM[1], Viana-Figaro F[2], Belo VA[3], Molina CAF[4], Andrade MF[5], Muniz JJ[6], Tanus-Santos JE[1], Tucci Jr S[4], Lacchini R[2] [1]FMRP-USP – Farmacologia, [2] EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas, [3]UFMG – Instituto de Ciências Biológicas, [4]FMRP-USP – Cirurgia e Anatomia, [5]FMRP-USP – Cirurgia, Ortopedia e Traumatologia, [6]Univas

**Introduction:** Erectile dysfunction (ED) is a disease related to deficient nitric oxide (NO) signaling. NO is produced from L-arginine by three nitric oxide synthases (NOS). Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of all NOS, and may be a plasma marker of endothelial function and a risk factor for cardiovascular disease. ADMA is mainly metabolized by the enzymes dimethylarginine dimethylaminohydrolase 1 and 2 (DDAH1 and DDAH2). Several studies have associated alterations in genes, expression or activity of DDAH enzymes with disorders in with impaired NO signaling. The aim of this study was to evaluate whether the response to ED treatment with sildenafil may be associated with polymorphisms of the *DDAH1* (rs1554597 and rs18582) and *DDAH2* genes (rs805304 and rs805305), as well as the haplotypes formed by these polymorphisms. **Methods:** Were included seventy post-prostatectomy (PPED) and seventy clinical ED (CED) patients from the Urology Clinic of the University Hospital of the Faculty of Medicine of Ribeirao Preto. Erectile function was evaluated using the International Index for Erectile Function (IIEF) questionnaire. To evaluate the response to sildenafil, the difference between the pre- and post-treatment scores (ΔIIEF) and the percentage reached from the maximum possible response (ΔIIEF%) of each patient were calculated. Also, patients from each group were divided into good and bad responders to sildenafil according to the median values of ΔIIEF%. The genotypes of rs1554597, rs805304 and rs805305 were obtained by the polymerase chain reaction (PCR) technique followed by restriction fragment length polymorphism (RFLP), and rs18582 by the allele-specific PCR technique. The PHASE 2.1 software was used to estimate the haplotypes in each group. The statistical analyses were made by: unpaired t test, Mann Whitney, Kruskal-Wallis with Dunn post-test or contingency tables, when appropriated. In order to correct the results found by variables of clinical relevance (age, smoking, ethanol consumption, diabetes and the use of antihypertensive medications), multivariate linear regression and multivariate logistic regression analyzes were performed. **Results:** The variant A allele of rs18582 showed a tendency to be associated with a greater chance of worse responses to sildenafil in the CED group (P=0,058). In the PPED group, carriers of the variant alleles A of rs805304 and G of rs805305 were associated with better responses to sildenafil (ΔIIEF, P=0,007; ΔIIEF%, P=0,025; and post-treatment IIEF score, P=0,014). We haven't found any other significant associations. **Conclusion:** These results show that the genetic markers rs805304 and rs805305 of *DDAH2* may influence the responses to sildenafil in patients with ED. **Financial Support**: CNPq, FAPESP, CAPES. Approval at the Human Research Ethics Committee: CAAE 51398915.1.0000.5393

**11.023 Estimation of *In vivo* hepatic extraction ratio of doxorubicin in breast cancer patients**. Pippa LF[1], Rocha A[1], Andrade JM[2], Lanchote VL[1] [1]FCFRP-USP, [2]FMRP-USP

**Introduction:** The aim of this study is to describe the pharmacokinetics, protein binding, metabolism and renal excretion of doxorubicin, an anthracycline used in breast cancer treatment, in order to estimate its in vivo hepatic extraction ratio. Doxorubicin is metabolized to doxorubicinol by carbonyl reductase 1 and 3, and aldo-keto reductase enzymes [1]. **Methods:** Twelve breast cancer patients with indication of adjuvant or neoadjuvant treatment were assessed during the first cycle of doxorubicin administration (60 mg/m2, iv-infusion, 30 min). Serial blood samples were collected up to 48 hours after the start of iv-infusion; urine was collected in 4-hour intervals, during 48 h. Methods for simultaneous quantification of doxorubicin and doxorubicinol in urine, as well as total and unbound plasma concentrations were developed applying LC-MS/MS. Doxorubicin pharmacokinetic parameters were calculated based on total plasma concentration versus time curves applying Phoenix® WinNonlin® (Certara USA, Inc., Princeton, NJ, USA) software by tricompartmental model analysis, and doxorubicinol was assessed by non-compartmental analysis. **Results:** Distribution, fast elimination and slow elimination half-lives were observed to be 0.10, 2.55 and 40.87 h, respectively. Unbound fractions were 16.05% for doxorubicin and 17.34% for doxorubicinol. The fractions of doxorubicin dose recovered in urine (0-48 h) were 2.35% for the unchanged drug and 1.35% for doxorubicinol. The clearance mean values for the assessed population were 58.07 L/h for total clearance, 1.45 L/h for renal clearance, 56.62 L/h for hepatic clearance and 0.71 L/h for doxorubicinol metabolite formation clearance, suggesting that doxorubicin elimination is carried out mainly by biliary excretion. Initial total clearance values demonstrated 95.32% variation coefficient, while total clearance values corrected to weight or body surface area presented similar variation coefficients, respectively 88.74% and 89.84%. **Conclusion:** The detailed pharmacokinetics study of doxorubicin, assuming blood/plasma concentrarion ratio is 1 [2], made it possible to estimate the in vivo hepatic extraction ratio (E = 0.63) [3], categorizing doxorubicin as an intermediate hepatic-extraction-ratio drug with total clearance values depending on protein binding, carbonyl reductase 1 and 3, and aldo-keto reductase enzymes activities and hepatic blood flow. **References:** [1] Lal, S. Pharmacogenetics of target genes across doxorubicin disposition pathway: a review. Curr. Drug Metab. 11, 115, 2010. [2] Mehvar, R. Application of Organ Clearance to Estimation of the In Vivo Hepatic Extraction Ratio. Curr. Clin. Pharmacol. 11, 47, 2016. [3] Benet, L. Z. Clearance (née Rowland) concepts: a downdate and an update. J. Pharmacokinet. Pharmacodyn. 37, 529, 2010. **Financial support:** FAPESP, process No 2014/06846-1; CNPq. This research project was approved by the School of Pharmaceutical Sciences of Ribeirão Preto Human Research Ethical Committee on April 7th, 2015, processes CEP/FCFRP No 358. **Acknowledgments:** We would like to thank the staff from HCFMRP-USP for the support during the clinical phase, as well as the patients for their precious collaboration.

**11.024 Effects of the CYP2D6 and CYP3A4/5 genetic polymorphisms on the population pharmacokinetics of tamoxifen and its main metabolites in breast cancer patients**. Ximenez JPB[1], D'Agate S[2], Pereira MPM[1], Andrade JM[1], Suarez-Kurtz G[3], Della Pasqua O[2], Lanchote VL[1] [1]USP, [2]University College London, [3]INCa

**Introduction:** Tamoxifen is considered a pro-drug of its active metabolite endoxifen (END). The major metabolic enzymes involved in END formation are CYP2D6 and CYP3A, whose activity variability influences END exposure and consequently clinical outcome [1]. In this context, the aim of this study was to develop a population pharmacokinetic model for tamoxifen and its metabolites, and its subsequently use to explore opportunities for treatment personalization. **Methods:** Tamoxifen (TAM), endoxifen (END), 4-OH-tamoxifen (4OHT) and N-desmethyl-tamoxifen (NDMT) plasma concentrations were sampled at steady-state, during a 24h dose interval, from 40 breast cancer patients treated with 20 mg of tamoxifen every 24 h. TAM and its metabolites were quantified by LC-MS/MS. PK modelling was performed using NONMEM 7.3. One and two-compartment models with first-order absorption and elimination were evaluated based on previous publications. Additional compartments were appended to the model to allow characterization of the different metabolites. Selection of the best hierarchical model was based on standard model diagnostic criteria [2]. The selection of covariates was performed through a forward selection and backward elimination method. Final model performance was assessed by bootstrapping and visual predictive checks (VPC). Simulations (n=100) were performed using different metabolic constant rates in order to mimic CYP2D6 and CYP3A4/5 poor metabolizer patients. **Results:** The PK of TAM and its metabolites was best described by a four compartment models. $K_a$, $CL_{TAM}$, $V_{TAM}$, $CL_{END}$ and $V_{END}$ were assumed based on published results following single doses. A proportional residual model error was used to describe residual variability. Estimated apparent TAM to NDMT ($K_{23}$), TAM to 4OHT ($K_{24}$), NDMT to END ($K_{35}$) and 4OHT to END ($K_{45}$) metabolic constant rates were (mean and CV) 0.0116 (11%), 0.0019 (4.4%), 0.00029 (1.7%) and 0.0079 (9%), respectively. Inter-individual variability (IIV) was identified on $K_a$ (14.1%), $K_{23}$ (33%), $K_{24}$ (33%), $K_{35}$ (46%), $K_{45}$ (13.2%) and $CL_{END}$ (42.2%). Simulations showed that CYP3A5 PM metabolizers had END levels below the efficacious END levels (6 ng/mL) compared to wild type, in another hand CYP2D6 poor metabolizer showed usually smaller differences relative to the wild type. **Conclusion:** Although previous models have been developed for TAM, our study is the first to describe tamoxifen metabolism *in vivo*, including information about CYP2D6 and CYP3A genotypes. The proposed parameterization allows the possibility to discriminate the contribution of different moieties and explore dosing algorithms. [1] Province MA, et al. Clin Pharmacol Ther. 2014;95(2):216. [2] Ter Heine R, et al. Br J Clin Pharmacol. 2014; 2:1. **Financial Support**: This project was funded by FAPESP (2014/16360-9) Human Research Ethical Committee (FCFRP-USP), CAAE: 35539714.7.0000.5403 Acknowledgments: We would like to thank physicians and nurses from HCFMRP-USP for giving us support during the clinical phase as well as the patients for their precious collaboration.