Setor 11. Farmacologia Clínica, Farmacocinética, Farmacogenômica e Toxicologia Pré-Clínica/Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Preclinical Toxicology

11.001

Evaluation of liver function in LPS-induced sepsis and flunixin meglumin-treated rats. Ávila TV[1], Bastos-Pereira AL[1], Christoff AO[1], Eler GJ[2], Bracht A[2], Zampronio AR[3], Acco A2[3]
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Introduction: Flunixin meglumine (FM) is a non-steroid anti-inflammatory drug largely used in Veterinary Medicine for the treatment of muscle-skeletal diseases and endotoxemic shock. However, the effects of FM in sepsis, including effects on the liver, are still not clear. The aim of this study was to investigate the hepatic actions of FM on the LPS-induced endotoxemia in rats, by means of liver perfusion technique. **Methods:** The protocols were submitted to the Ethics Committee in Animal Experimentation (CEEA), Sector of Biological Sciences of UFPR for approval of methods and agree with the ethical principles of the use of laboratory animals established by the Brazilian College of Animal Experimentation (COBEA) and were approved with certificate 279. The drugs were injected intraperitoneally in Wistar rats divided into 5 groups: 1) Control, injected with PBS; 2) LPS, injected with 10 $mg.kg^{-1}$ lipopolysaccharides (LPS); 3) FM 1.1 $mg.kg^{-1}$ injected 2 hours before LPS; 4) FM 1.1 $mg.kg^{-1}$; and 5) FM 2.2 $mg.kg^{-1}$. Twelve hours after the LPS injection, blood samples were collected for determination of ASAT and ALAT plasmatic levels. Additionally, monovascular liver perfusion using 2,5 mM alanine as a substrate was performed on rats starved for 18 h. **Results:** 12h after the LPS injection, the ASAT and ALAT plasmatic levels had increased significantly as compared to the control group, while the FM pre-treatment showed reduction of both enzymes. It was clearly observed that LPS reduced gluconeogenesis, ureagenesis and oxygen uptake when the liver was stimulated with 2,5 mM alanine. FM pre-LPS normalized glucose production and recovered ureagenesis and oxygen consumption, at least partially. Pyruvate and ammonia production in both FM groups (1.1 and 2.2 $mg.kg^{-1}$) were compromised and urea production was reduced in FM 1.1 $mg.kg^{-1}$ group. Lactate production in all groups did not experience any effect. **Discussion:** The results showed that LPS has significant effects in liver metabolism and that FM can protect the liver against damage caused by endotoxemia, especially when administered prior to the LPS. However, FM also can change the liver function, as shown in pyruvate, ammonia and urea production. **Financial support:** CAPES and CNPq

11.002

Association study of eNOS haplotype and chronic renal failure (CRF). Ishizawa MH, Metzger IF, Marson B, Izidoro-Toledo TC, Tanus-Santos JE FMRP-USP - Farmacologia

Introduction: Chronic Renal Failure (CRF) is a complex condition, with multiple causes and associated with high mortality rate. It is indeed a quite prevalent condition that's high cost for the public health. Nitric Oxide is one of the most important key for cardiovascular homeostasis and therefore plays an important role in CRF pathogenesis. It's produced by Endothelial Nitric Oxide Synthase (eNOS) which shows that genetic polymorphisms may be associated with diseases predisposition. Our study tries to elucidate the role of eNOS polymorphisms and CRI predisposition throughout the comparison of haplotypes distribution involving three relevant polymorphisms of eNOS gene: $T^{-786}C$ (promoter region), Glu298Asp (exon 7) and VNTR of Intron 4 in healthy volunteers and patients with Chronic Renal Failure. **Methods:** Were studied 110 healthy volunteers (control group) and 127 patients with CRF between 18 and 75 years old. Genomic DNA was isolated for determination of genotypes. The $T^{-786}C$ and Glu298Asp polymorphisms were determined by Taqman® Allele Discrimination assay and VNTR of Intron 4 by PCR and fragment separation by electrophoresis. Haplotipic frequencies were estimated using PHASE 2.1 software. **Results:** We compared the distribution of each alleles, genotypes and haplotypes between the two groups, control and CRF. The haplotypes frequencies for the control and CRF groups were, respectively: H1 (T 4b Glu)= 51.6% and 42.5%; H2 (T 4b Asp)=8.9% and 11.2%; H3 (T 4a Glu)=2.0% and 7.7%; H4 (T 4a Asp)=0.2% and 0.3%; H5 (C 4b Asp)=5.8% and 5.3%; H6 (C 4b Asp)=20.9% and 20.5%; H7 (C 4a Glu)=10.6% and 10.6%; H8 (C 4a Asp)=0.0% and 1.8%. We found non-significant differences between the frequencies of the genotypes and haplotypes in the control and CRF groups. **Discussion:** Our results suggest that eNOS gene polymorphism do not present any association with Renal Insufficiency because haplotypic, genotypic and allelic frequencies were the same between the two groups. (Obs.: Projeto Aprovado pelo CEP - HCRP, nº 10125/2007). FAPESP, CNPq and Capes

11.003

Matrix metalloproteinase-9 genotypes and haplotypes are associated with multiple sclerosis and with the disability degree of the disease. Fernandes KS[1], Brum DG[2], Sandrim VC[1], Guerreiro CT[3], Barreira AA[2], Tanus-Santos JE[3] [1]FMRP-USP - Farmacologia, [2]FMRP-USP - Neurociências e Ciências do Comportamento, [3]FMRP-USP - Biomecânica, Medicina e Reabilitação do Aparelho Locomotor

Introduction. Multiple sclerosis (MS) is an autoimmune demyelinating disease and a common cause of neurological disability in young adults. Two functional polymorphisms, the $C^{-1562}T$ and the microsatellite $(CA)_{13-25}$, both in the promoter region of the MMP-9 gene have been associated with several diseases. The aim of this study was determine whether these genetic variants predispose to MS or modify clinical status of patient. **Methods.** A total of 356 subjects were enrolled in the study. Genomic DNA was extracted from whole blood and genotypes for the $C^{-1562}T$ and the microsatellite $(CA)_n$ polymorphisms were determined by PCR using TaqMan genotyping assays. The distribution of genotypes for each polymorphism was assessed for deviation from the Hardy–Weinberg equilibrium, and differences in genotype frequency and in allele frequency between groups were assessed using χ2-tests. A value of $P < 0.05$ was considered statistically significant. Haplotypes were inferred using the Bayesian statistically based program PHASE version 2.1 (http://www.stat.washington.edu/stephens/software.html) (Stephens et al, Am J Hum Genet 68, 978, 2001). **Results.** In our study the majority of individuals presented lower EDSS scores (0-2 and 2.5-4.0 groups). Moreover, some patients were scored as 8.5 and 9.0. While no difference was observed to $C^{-1562}T$ polymorphism between healthy and MS groups, a significant difference occurred to genotypes and allele frequencies of the $(CA)_n$ polymorphism. In addition, we have demonstrated that the haplotypes for the two polymorphisms are not associated with the presence of MS, however these haplotypes seems be relevant to the MS clinical status. **Discussion.** This study was the first to evaluate the association of MMP-9 haplotypes and multiple sclerosis. While single locus analysis showed that (CA)n polymorphism may modify the susceptibility to develop MS, the main finding of this study was that a combination between this variant and $C^{-1562}T$ may modulate the clinical status ("severity") of disease. Indeed, haplotype analysis has been valued as a more powerful approach than the analysis of single polymorphisms (Crawford and Nickerson, Annu Rev Med 56, 303, 2005), because the study of haplotypes could eliminate inconsistencies commonly found in studies analyzing single polymorphisms one at a time (Sandrim et al, Atherosclerosis 186, 428, 2006; Sandrim et al, Atherosclerosis 189, 241, 2006). In conclusion, we found that the (CA)n and C-1562T polymorphism may modify drastically the clinical status of MS, measured here by EDSS. The combination between T and H variants, increase consistently the disability status of patients. Moreover, we observed that the higher the CA repeating higher the risk to development MS. **Supported by**: Fapesp, CNPq and CAPES.

11.004

Mercury exposure increases net matrix metalloproteinase (MMP)-9 activity in environmentally exposed subjects. Jacob Ferreira ALB[1], Barbosa Jr F[2], Tanus-Santos JE[3]
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Introduction: Mercury (Hg) exposure is widely recognized as a serious environmental health problem and it has been shown that this exposure may promote cardiovascular diseases. Increased expression and activity of gelatinases (MMP-2 and MMP-9) have been reported in a variety of pathological conditions, including cardiovascular diseases. In the present study, we investigated whether there is an association between circulating levels of matrix metalloproteinase (MMP) -9, tissue inhibitor of metalloproteinase (TIMP)-1, the MMP-9/TIMP-1 ratio and the concentration of mercury in plasma from mercury-exposed individuals. **Methods**: We studied 245 volunteers environmentally exposed to Hg. Plasma Hg and blood concentrations were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). MMP and TIMP concentrations were measured in plasma samples by gelatin zymography and ELISA, respectively. Volunteers were divided into 3 groups with basis on the plasma Hg concentrations: A) below 1.8µg/L (N= 49); B) between 1.9 and 7.0 µg/L (N=107); and C) above 7.0µg/L (N=89). These plasma Hg concentrations corresponded, respectively, to blood Hg concentrations below 10µg/L (considered the reference value for Hg), between 10 and 50µg/L, and above 50µg/L (considered as a toxic concentration). RESULTS: In a multivariate regression model including variables susceptible to influence either MMPs, TIMPs, or Hg levels (plasma Se, MDA, smoking habits, age, gender and body mass index, we found that log Hg plasma does not affect MMP-9 (β=0.0072325; P=0.9151), TIMP-1 (β=-26.2507; P=0.3980), and MMP-9/TIMP-1 ratio (β=0.0001137; P=0.7018) in the A group. However, we found that plasma Hg tended to increase MMP-9 (β=0.2672447; P=0.0712), but not TIMP-1 (β=-4.294076;P=0.9405) or MMP-9/TIMP-1 ratio (β=0.0010796;P=0.1059) in the B group, and increased MMP-9 (β=0.3968959;P=0.0363) and MMP-9/TIMP-1 ratio (β=0.0018157;P=0.0346) but not TIMP-1 in the C group. **Discussion**: The association between Hg in plasma and net MMP-9, as revealed by MMP-9/TIMP-1 ratio depended on the degree of Hg contamination, and this association suggests that increased MMP-9 activity is a mechanism by which mercury may increase the susceptibility to cardiovascular diseases in Hg exposed individuals. Approval was obtained from Ethics Committee of the University of São Paulo at Ribeirão Preto (Brazil). Protocol number CEP/FCFRP #71. Financial support by: FAPESP, CNPq.

11.005

Endothelial nitric oxide synthase (eNOS) polymorphisms and haplotypes in white normotensives and hypertensives. Luizon MR[1], Izidoro-Toledo TC[1], Sandrim VC[1], Coelho EB[2], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]HC-FMRP-USP

Introduction: Nitric oxide (NO) is a major regulator of the cardiovascular system. Abnormalities in the activity of the enzyme that synthesizes NO in endothelial cells (endothelial nitric oxide synthase; eNOS) may lead to NO deficiency and cause clinical hypertension. Controversial results regarding the association of eNOS gene (NOS3) polymorphisms with hypertension have been reported. These inconsistencies may derive from consideration limited to only one rather than combinations of polymorphisms. We have studied the haplotypes formed by three genetic polymorphisms in the eNOS gene; a single nucleotide polymorphism (SNP) in the promoter region (T-786C, rs2070744), in exon 7 (Glu298Asp, rs1799983), and a variable number of tandem repeats (VNTR) in intron 4 (b/a) in white hypertensives and normotensives (Sandrim et al. *Atherosclerosis* 186: 428, 2006). Our results suggested that two eNOS haplotypes were associated with a protective effect against hypertension and one eNOS haplotype (H7, "*C Asp b"*) conferred susceptibility to hypertension in white subjects. In the present study, we studied two additional SNPs of the NOS3 gene (C>T, rs3918226 and A>G, rs743506) and examined a possible association between the haplotypes formed by these five polymorphisms and hypertension in a sample of the Brazilian white population. **Methods**: This study was approved by the institutional review committee of the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo; CEP 9944/2003. A total of 86 Brazilian patients with hypertension and 84 healthy controls were included in the study. The NOS3 gene intron 4a4b VNTR polymorphism was analyzed by PCR and the SNPs by Real-Time PCR. The haplotype frequencies were estimated with the software PHASE. Statistical analysis was performed using GraphPad Prism v.5. **Results and Discussion**: The distribution of genotypes for the five polymorphisms studied showed no deviation from Hardy-Weinberg equilibrium. No differences were observed in the frequencies of genotypes and alleles of the five polymorphisms when white hypertensives and normotensives were compared (all P>0.05). The haplotypes "C A *C Asp b*" and "T G *C Asp b*" contain the *same alleles* of that susceptibility haplotype first observed; H7. These haplotypes were also more commonly found in white hypertensives (5 and 7%, respectively) than in normotensives (one and 3%, respectively), but these differences were not statistically significant (P>0.05). No statistically significant association was found between the two new SNPs and the haplotypes formed by them and hypertension, suggesting that they do not play a major role in the development of essential hypertension. **Financial support**: Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP and CNPq.

11.006

Cigarette smoke exposure on fertility and possible protective effect of zinc supplementation in male rats. Sankako MK[1], Garcia PC[2], Piffer RC[2], Pereira OCM[1] [1]UNESP-Botucatu - Farmacologia, [2]UNESP-Botucatu - Clínica Médica/Farmacologia

Introduction: According to World Health Organization the infertility reaches 15% of active sex life population and 50 % of these cases are caused by male factor as primary factor or in association with female ones. About one-third of reproductive age people smoke cigarettes and it can also be involved with male and female infertility. Some investigations have proposed a detrimental effect of smoking on sperm concentration and quality. Adverse effects of cigarette smoke on Leydig cell function in animals have been also reported. Zinc can be an antioxidant and may be a cofactor for the cellular division so important for reproduction. The aim of this study was to evaluate, in an animal model, the effects of cigarette smoke exposure on semen and fertility parameters and to identify possible protective effect of zinc supplementation. **Methods:** Male Wistar rats (60 days old) were divided into three groups (n=10/group): control (G1), cigarette smoke (G2; 20 cigarettes/day/9 weeks by inhalation) and cigarette smoke plus zinc (G3; 20 cigarettes/day/9 weeks; zinc chloride 20mg/kg- daily by gavage for 9 weeks). After finishing the treatment, the males were housed in a large cage with fertile untreated female rats (2 females/male). Vaginal smears were examined daily for up to 15 days for the presence of spermatozoa (indicating the first day of pregnancy). On 20th day of pregnancy, the rates of pre- and post-implantation losses, implantation and fetal viability were quantified. The spermatozoa were collected from vas deferens and the semen parameters (concentration, vitality, motility and morphology) were analyzed. Statistics: the values were expressed in median ($IQ_{25\%}$-$IQ_{75\%}$) or mean±SD, G2 was compared to G1 and G3, $p<0.05$. This study was approved by the Committee of Animal Experiments # 430. **Results:** The fetal viability decreased in control females mated with G2 when compared with G1 and G3 [G1:95.65(90.90-100.00)/ G2:85.28(71.68-96.42)/ G3:94.73(91.30-100.00)].The rates of pre- and post-implantation losses and implantation were not significantly altered. We also observed reductions on sperm concentration in million/ml in G2 when compared with G3 (G1:66.20±15.56/ G2:56.00±46.43/ G3:127.30±27.03), on percentage of live spermatozoa in G2 when compared with G1 and G3 [G1:98.00(97.00-98.00)/ G2:91.00(89.50-92.50)/ G3:97.50(97.00-98.75)], on percentage of morphologically normal spermatozoa in G2 when compared with G1 and G3 [G1:98.00(97.25-98.75)/ G2:96.00(93.00-97.00)/ G3:98.00(97.25-98.75)] and on percentage of motile spermatozoa in G2 when compared with G3 [G1:26.00(14.00-34.00)/ G2:17.50(14.00-19.00)/ G3:22.00(19.00-24.00)]. **Discussion:** These results indicate that the cigarette smoke exposure really damages male fertility and can injure the offspring. Probably the reduction on fetal viability occurs due to decrease of sperm concentration and quality. The protective effect of zinc supplementation was observed in some of these parameters. It is important to consider that human infertility can cause emotional disorders (guilt, anxiety, stress, low self-esteem), and frequently the individuals have to undergo to expensive procedures. Thus, this study can also suggest an alternative treatment for this kind of infertility. **Financial Support:** CNPq

11.007

Specific cochlear or vestibular toxicity induced by new semi-synthetic aminoglycoside antibiotics(AGAs): implications for the treatment of Ménière's disease (MD).

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Introduction: AGAs are potent bactericidal agents that share general range of antibacterial activity, pharmacokinetic behavioral and important side-effects, represented by impairments of renal and inner ear functions. The ototoxicity includes the concomitant damage of cochlear and vestibular hair cells. However, there are AGAs mainly cochleotoxic such as neomycin, and fundamentally vestibulotoxic such as streptomycin and gentamicin. In the MD, a cochlear and vestibular damage can taking place which unable episodes of vertigo, progressive deafness and humming. The vertigo may disappear by chemical labirintectomy in the sick ear using AGAs that are mainly vestibulotoxic, however an undesirable hearing loss side-effect can occur by the use of non-specific vestibulotoxic AGAs. Therefore the present study concerns the obtention of new AGAs with selective vestibulotoxicity with cochleotoxic-dissociated effect. **Methods:** From neomycin, we obtained the three molecular fractions: neamine(N), methyl-neobiosamine-B(NMB) and 2-deoxy-streptamine(2-DS) and from neamine(N): (I-N)tetra-azido-neamine, (II-N)tetra-N-acetyl-neamine, (III-N)tetra –N-carboxymethyl-neamine, (IV-N)tetra-N-carboxybenzyl-neamine and (V-N)tetra-p-methoxybenzylimino-neamine. The all fractions were evaluated in cochlear and vestibular assays: (1) the brainstem evoked auditory potential(BEAP), (2) distortion product otoacoustic emissions(DPOAE) and (3) scanning electron microscopy(SEM). The experimental model was performed on guinea pigs that were kept in the animal facilities as recommended by the guidelines for the care and use of laboratory animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, of the US National Research Council and was approved by the Institutional Ethical Committee (protocol No. 070/2005). **Results and Discussion:** Only (NMB) showed to be selective vestibulotoxic agent, without damage of inner and outer hair cells proved by SEM, DPOAE and BEAP in all animals studied. This is the first compound with this characteristic to be able to use in clinical trials for the treatment to MD. The 2-DS, I-N, II-N and III-N were unable to induced ototoxicity. Interestingly IV-N and V-N caused only cochleotoxicity at the outer and inner hair cells respectively. Therefore, beside the discovery of a new therapeutic compound relevant for the treatment of MD, the acquisition of selective cochleotoxic and vestibulotoxic compounds certainly will open new perspectives to the study of the mechanism of AGAs-induce ototoxicity. Financial Support: CNPq

11.008

Phenotyping of CYP2D6 in elderly cardiopatic classified as extensive or poor metoprolol metabolizers. Neves DV[1], Souza L[2], Hayashida M[3], Lanchote VL[1], Cesarino EJ[1] [1]FCFRP-USP - Análises Clínicas, Toxicológicas Bromatológicas, [2]FMRP- USP - Medicina Social, [3]EERP-USP - Enfermagem Geral

Introduction: These days, elderly people constitute the most important part of the increasing population. This population segment requires frequent medical and therapeutic cares, and spends three times more drugs than the young individuals. The objective of this study was to phenotype CYP2D6 in elderly cardiopatic patients classified as extensive or poor metoprolol metabolisers, to develop and validate the method for analysis of metoprolol tartrate and its metabolite in urine and to identify possible correlations between anthropometric factors, racial, comorbidities, risk factors and number of medicines used with the urine *metabolic* ratio of *metoprolol/α-OH*-metoprolol. **Methods***:* The project was approved by the Research Ethics Committee of the Faculty of Pharmaceutical Sciences of Ribeirão Preto – USP (protocol n°91/2007). The casuistry was composed by 130 elderly individuals over 60 years old, volunteer, carrying any type of previously identified cardiomyopathy, with normal renal and hepatic functions. All patients were submitted to a collection of urine from 0-8h after administration of one tablet of 100 mg of metoprolol tartrate, using HPLC with detection by fluorescence to quantify the metoprolol and its metabolite α-OH metoprolol. Mann-Whitney test and Kruskal-Wallis test were used for comparison between anthropometric factors, race, comorbidities, use of alcohol and quantity of drugs used with metabolic ratio of the concentration of metoprolol /α-OH metoprolol in urine. **Results***:* Three patients (2.3%) were phenotyped as poor metoprolol metabolisers. Mostly studied patients percentage age was 60-70 years old (mean: 71.8 ± 6.2). One hundred and one patients (77.7%) were female. The studied patients used approximately 5.9 ± 2.1 types of drugs, varying of 1 to 11 concomitant drugs used. According to Mann Whitney test, there was a statistically significant difference in the amount of drugs used by older ($p = 0.017$) and intake of alcohol ($p = 0.045$). *Discussion:* The percentage of elderly patients found as poor metabolisers of metoprolol (2.3%) differed from 7 to 10% of poor metabolisers in the Caucasian population, 1% of the Asian population in general and about 1.4% of the Arab population. The validation of the method of analysis of metoprolol and α-OH metoprolol in urine was considered precise, accurate, sensitive and selective and compatible with the application in kinetic studies of provision. The statistically significant difference found in the metabolic ratio metoprolol / α-OH according to the quantity of medicines used by the elderly could be related to the saturation of the CYP2D6 enzyme to metabolize substrates other than metoprolol. The statistically significant difference found in the use of alcohol, could be related to the induction of CYP3A by alcohol thus could have changed the metabolism of metoprolol, because this isoform of enzyme also participates of the metabolism this beta-blocker. Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

11.009

Two MMP-9 gene polymorphisms (C-1562T and CAn) and preeclampsia. Palei ACT[1], Sandrim VC[1], Cavalli RC[2], Gerlach RF[3], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]FMRP-USP - Ginecologia e Obstetrícia, [3]FORP- USP - Morfologia

Introduction: Matrix Metalloproteinases (MMPs) are Zn^{2+} endopeptidases that break down the extracellular matrix macromolecules. Several members of this enzyme family, especially MMP-9, are present at the fetal-maternal interface. The inability to produce sufficient MMPs may reflect abnormal placentation and endothelial dysfunction leading to preeclampsia. Moreover, some studies showed that a microsatellite $(CA\ repeats)_{13\text{-}25}$ localized in the promoter region of MMP-9 gene can alter gene expression and affect MMP-9 activity. Other studies showed that a single nucleotide polymorphism (SNP: $C^{-1562}T$) in MMP-9 gene may affect the transcription rate and amounts of enzyme synthesized. Here, we analyzed whether these polymorphisms are associated with preeclampsia and/or gestational hypertension. **Methods:** We studied 202 healthy pregnants (HP), 134 pregnants with gestational hypertension (GH) (blood pressure ≥ 140/90 mmHg after 20 weeks of gestation) and 155 pregnants with preeclampsia (PE) (gestational hypertension plus proteinuria > 0.3 g/L in 24 h). Genomic DNA was extracted from whole blood and genotyping for CAn and $C^{-1562}T$ polymorphisms were done by polymerase chain reaction without or with restriction (PCR-RFLP), respectively. This study was approved by the local Ethics Review Board (CEP HCRP n° 4682/2006). **Results:** The alleles for the CAn polymorphism were grouped in 14 or lower CA repeats (L) and with 21 or higher CA_{21} repeats (H). Therefore, the genotypic frequencies were 37% HH, 42% HL and 21% LL in PE patients; 34% HH, 48% HL and 18% LL in HG; and 38% HH, 44% HL and 18% LL in HP. The allelic frequencies were 58% H and 42% L in PE; 58% H and 42% L in GH patients; and 60% H and 40% L in HP group. The frequencies of genotypes for the $C^{-1562}T$ polymorphism were 76% CC, 23% CT and 1% TT in PE; 68% CC, 31% CT and 1% TT in GH; and 84% CC, 16 CT and 0% TT in HP. The frequencies of alleles were 88% C and 12% T in PE; 83% C and 17% T in GH; and 92% C and 8% T in HP. All the distributions followed Hardy-Weinberg equilibrium. **Discussion:** Our results suggest that the two MMP-9 gene polymorphisms are not associated with preeclampsia or gestational hypertension, thus suggesting that variations in this gene (MMP-9) probably do not contribute to the susceptibility to these hypertensive disorders of pregnancy. **Financial support**: FAPESP and CNPq.

11.010

Determination of phenytoin in human plasma by LC method and its application to a bioequivalence study. Dalmora, SL[1], Nogueira DR[1], Dalla Lana AJ[1], D'Ávila, FB[1], Santana D[2], Gonçalves, TM[2] [1]UFSM - Farmácia Industrial, [2]UFPE - Ciências Farmacêuticas - Ciências Farmacêuticas

Introduction: Phenytoin is one of the most commonly prescribed anticonvulsant drugs for the treatment of epilepsy. It has three pharmacologic characteristics associated with the risk of non-equivalence after generic substitution: poor water solubility, nonlinear kinetics and a narrow therapeutic window. The aim of the present study was to develop and validate a liquid chromatography (LC) method for the determination of phenytoin in human plasma supporting a pharmacokinetic and bioequivalence study. **Methods:** Phenytoin and phenobarbital (internal standard) were extracted from plasma by liquid-liquid extraction using tert-butyl-methyl ether as solvent and separated on a Phenomenex Synergi 4µ MAX - RP 80Å analytical column (150 x 4,6 mm) maintained at 35 ºC, with water: acetonitrile: methanol (58.8:15.2:26 v/v/v) as mobile phase. The flow rate was 1.2 mL/min and the detection was carried out by photodiode array detector (PDA) set at 205 nm. The bioequivalence study was an open, randomized, two period crossover design with a one-week washout interval between the doses. Twenty eight male healthy volunteers aged between 18 and 45 years and within 15% of the ideal body weight were selected by clinical evaluation and laboratory tests. The clinical protocol was approved by the local Ethic Committee (CAAE-0386.0.172.000-07 and Register No CEP/CCS/UFPE 369/07) and the volunteers given written informed agreement to participate in the study. During each period, a single oral dose of phenytoin (1 tablet-100 mg) was given after an overnight fast of at least 10 hours, and the blood samples were collected up to 72 hours post dosing. **Results and Discussion:** The method validation investigated the parameters recommended for the bioanalytical methods and yielded good results with limit of quantization of 50 ng/mL. The chromatographic separation was obtained within 12 min, and the response was linear in the concentration range of 50-2500 ng/mL (r^2= 0.9999). The mean extraction recoveries were 96.98% for phenytoin and 96.01% for phenobarbital. Intra and inter days relative standard deviation (%RSD) were less than 4.48% and the accuracy was within 98.71 and 100.17%. Moreover, the samples were stable (Bias% < 4.38%) after short-term, long-term and three freeze-thaw cycles. The proposed method was successfully applied for the bioequivalence study of two tablet formulations (test and reference) of phenytoin 100 mg after single oral dose administration to 28 healthy volunteers. The geometric means ratios of C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-inf)}$ were 111.67, 104.67 and 102.77%, respectively, with both the confidence intervals between 99.97–118.40%, demonstrating that the two formulations showed similar bioavailability profiles and therefore are considered bioequivalent with regard to the extent and rate of absorption and, interchangeable as well, for clinical and therapeutic purposes. **Acknowledgments:** Cristália Produtos Químicos Farmacêuticos (São Paulo, Brazil).

11.011

Relevance of polymorphisms of endothelial nitric oxide synthase (eNOS) gene for the circulating nitrite in healthy black subjects. Metzger IF[1], Rios-Santos F[2], Carvalho WA[3], Tanus-Santos JE[4] - [1]FMRP-USP - Farmacologia, [2]UESC - Saúde, [3]Hospital São Rafael - Patologia Clínica e Toxicologia, [4]FMRP-USP - Farmacologia

Introduction: Nitric oxide (NO), mainly produced by endothelial nitric oxide synthase (eNOS), plays a major role in cardiovascular homeostasis. eNOS exhibits genetic polymorphisms which has been associated to NO biodisponibility. However, the effects of eNOS polymorphisms, $T^{-786}C$ and Intron 4, on the circulating concentrations of nitrite (a sensitive marker of NO formation) in black subjects were not yet evaluated. **Methods:** It was evaluated the effect of eNOS haplotype on Nitrite levels in 198 healthy black subjects (males, non-smokers, 18-60 years old, and not taking any medication). Nitrite levels (Plasma and Whole blood) were compared in three relevant eNOS polymorphisms ($T^{-786}C$ in the promoter region; 4b4a in intron 4, and Glu298Asp in exon 7). The $T^{-786}C$ and Glu298Asp polymorphisms were determined by Taqman® Allele Discrimination assay and the Intron 4 by PCR and fragment separation by electrophoresis. To assess NO formation, the plasma concentrations of nitrite were determined using an ozone-based chemiluminescence assay and an enzyme immunoassay. **Results and Discussion:** The frequency of genotypes “TT”, “TC” and “CC” of $T^{-786}C$ polymorfism were 59.1, 36.4 and 4.5 %, respectively. The genotypes “4b4b”, “4b4c”, “4a4b”, “4a4a”, “4a4c” and “4a4y” of Intron 4 polymorfism were 43.4, 4.0, 43.9, 7.1, 1.0 and 0.5 %. And the genotypes “GluGlu”, “GluAsp” and “AspAsp” of Glu298Asp were 70.7, 27.3 and 2.0%. We found non-significant differences on circulating nitrite and the genotypes of each polymorphism of the eNOS gene. The Plasma Nitrite mean$\pm$SD were: $T^{-786}C$ - “TT” = 171.6$\pm$183.6, “TC” = 180.0$\pm$216.7, and “CC” = 229.4$\pm$200.4 nmol/L; Intron4 - “4b4b” = 160.9$\pm$160.6, “4b4c” = 145.7$\pm$96.23, “4a4b” = 188.5$\pm$234.6, “4a4a” = 226.5$\pm$200.8, “4a4c” = 191.6$\pm$185.3 and “4a4y” = 107.8 nmol/L; and Glu298Asp - “GluGlu” = 160.4$\pm$168.4, “GluAsp” = 199.6$\pm$229.2, and “AspAsp” = 440,3$\pm$400.3 nmol/L, $P>0.05$. Our results suggest that these polymorphisms do not affect circulating nitrite in black healthy subjects as they do in white healthy subjects. (Project Approved by Research Ethics Committee of São Rafael Hospital - Salvador-BA, Project number 04/06). Financial support: FAPESP-CAPES-CNPq

11.012

Generation of a pharmacogenetic-based *in vitro* model to investigate the read-through of premature termination codons. Fuchshuber-Moraes M[1], Carvalho MA[2], Suarez-Kurtz G[1]
[1]INCa - Farmacologia, [2]INCA-CEFETEQ - Divisão de Farmacologia,

Introduction: Nonsense mutations are responsible for several genetic diseases. It has been shown that compounds such aminoglycosides induce the readthough of premature termination codons. The aim of this work is to develop a *CYP2C19*3*-expressing *in vitro* model to test read-through inductors, since that allele is characterized by a nonsense mutation codon and present a clear genotype-phenotype correlation. **Methods:** The *CYP2C19*3* was generated by direct-site mutagenesis using the *CYP2C19*WT* as template. Both fragments were fusioned to eGFP in C-terminus portion, subcloned in an expression vector and stably transfected into HEK-293T cell line. The mRNA expression was measured by RT-PCR and the protein expression by Western blotting and protein fluorescence assays. **Results**: mRNA assays showed that transfection worked well leading to a high CYP2C19 mRNA expression. After that, we treated the CYP2C19*3 stably transfected cells with aminoglycosides, such as gentamicin and G418, and then we observed an eGFP expression, suggesting the premature termination codon readthrough. **Discussion:** CYP2C19 is one of the main enzymes of the human CYP450 complex and has a large number of pharmacological substrates. There is a clear genotype-phenotype relation and good and easy technical approaches to measure the enzyme activity, suggesting that it would be a good model to our purposes. Although the results suggest that aminoglycosides are capable to induce the CYP2C19 readthrough *in vitro*, the data are preliminary and, to establish such model as a feasible tool, more data is required about the expression/function of the post-readthrough translated CYP2C19. **Financial Support**: CAPES / INCA / FAF

11.013

Influence of quinidine on the enantioselective metabolism of tramadol in rats. Godoy ALPC[1], Moraes NV[1], Carvalho TMJP[1], Lanchote VL[1] [1]FCFRP-USP - Análises Clínicas, Toxicológicas e Bromatológicas

Tramadol (T), a centrally acting analgesic, is available as a racemic mixture of (+)-*trans*-T and (-)-*trans*-T. T is metabolized mainly to the pharmacologicaly active O-desmethyltramadol (M1) and to the inactive N- desmethyltramadol (M2). The formation of M1 metabolite is mainly via CYP2D6 in humans and, therefore, subject to polymorphism, whereas M2 formation is catalyzed by CYP2B6 and CYP3A4. Its analgesic action is related to the opioid activity of (+)-T and (+)-M1 and to the monoaminergic mechanisms related to (+/-)-T. The present study investigated the influence of quinidine (CYP2D inhibitor) on the enantioselective metabolism of T in rats. The study was approved by the Ethics Committee for the Use of Animals of the Ribeirão Preto Campus (Protocol 07.1.435.53.6). Male Wistar rats (n=6 per collection time) received a single oral dose of 20 mg/kg racemic-T after 4 hours pretreatment with 80mg/Kg quinidine i.p. or vehicle (control). Serial blood samples were collected up to 12 h after T administration. The enantiomers of T and its metabolites M1 and M2 were analysed in plasma samples by LC-MS-MS using the chiral column Chiralpak AD. Pharmacokinetic analysis was performed by using WinNonlin 4.1 computer program. Area under the plasma concentration-time curves ($AUC^{0-¥}$) values are reported as medians. Pharmacokinetics of T was enantioselective in both groups with the observation of higher plasma concentrations of (+)-T when compared to (-)-T ($p<0.05$, Wilcoxon test) ($AUC^{0-¥}$ 2243.10 *vs* 828.44 ng.h/mL and 527.88 *vs* 116.38 ng.h/mL, respectively for quinidine and control groups). AUC values were different (Mann-Whitney test, $p<0.05$) between control and quinidine groups, respectively for (-)-T 116.38 *vs* 828.44 ng.h/mL, (+)-M2 1210.90 vs 4732.40 ng.h/mL and (-)-M2 225.34 *vs* 1582.80 ng.h/mL. Pharmacokinetic analysis indicated that quinidine increased significantly the systemic exposure of (-)-T. Plasma concentrations of both enantiomers of M2 are increased probably due to the quinidine inhibition of M1 formation and elimination via CYP2D. **References:** Wang, N., *Yao Xue Xue Bao,* v. 37, p.169-74, 2002; Garrido, M.J.; *J.Pharmacol. and Exp. Therapeutics, v.* 305, p. 710-18, 2003. Parasrampuria, R.; *Chirality,* v. 19, p. 190-196, 2007. **Financial Support:** CNPq.

11.014

L-arginine reduces dapsone-induced methemoglobinemia in rats in multiple dose regimens. Moraes NV[1], Bergamaschi MM[1], Bragheto JB[1], Bianchi, MLP[1], Queiroz RHC[1]
[1]FCFRP-USP - Análises Clínicas, Toxicológicas e Bromatológicas

Introduction: Dapsone (DDS) is clinically used in the treatment of leprosy, dermatitis herpetiformis, pneumonia caused by *Pneumocystis carinii* and also in the prevention of malaria. Its use is being associated to hematological adverse effects such as methemoglobinemia and hemolytic anemia, which are related to N-hydroxylation mediated by cytochrome P450 enzyme system. Methemoglobinemia is characterized by increased quantities of hemoglobin in which the iron of heme is oxidized from its ferrous (Fe^{2+}) to the ferric form (Fe^{3+}). Considering the potency of nitric oxide (NO) as an antioxidant agent, we propose to evaluate for the first time L-arginine (ARG), a NO precursor, on DDS-induced methemoglobinemia. **Methods**: The study was approved by the Ethics Committee for the Use of Animals of Ribeirão Preto Campus, University of São Paulo (Protocol 06.1.461.53.6). Male Wistar rats were treated with ARG (gavage) in 5, 15, 30, 60 and 180 mg/kg doses for five days. In the fifth day, rats received an intraperitoneal administration of 40 mg/kg DDS 2 hours after ARG administration. Methemoglobin levels were assayed in blood samples by spectrophotometry. DDS plasma concentrations were analysed by high-performance liquid chromatography using a C8 analytical column and a UV detector. **Results**: A reduction on DDS-induced methemoglobinemia was observed when 5 or 15 mg/kg ARG was administered for 5 days prior to DDS administration (3.74 and 3.69%, respectively) compared to animals treated just with DDS (25.84%). However, absence of inhibition of methemoglobin formation was observed when higher doses of ARG (30, 60 and 180 mg/kg) were administered (18.64, 28.91 and 26.83%, respectively). DDS plasma concentrations in rats treated with DDS and 5 or 15 mg/kg ARG were 3 fold lower (5.30 and 6.10µg/mL, respectively) than those observed in the group that received DDS (16.50µg/mL). No statistical difference in DDS plasma concentration was observed when higher doses of ARG were administered to animals treated with DDS. **Discussion**: NO is considered a potent antioxidant agent *in vitro* and *in vivo* by several mechanisms: suppressing iron-induced generation of hydroxyl radicals (·OH) via the Fenton reaction; interrupting the lipid peroxidation chain reaction; increasing the glutathione antioxidant potency; and inhibiting cysteine proteases (Chiueh 1999). NO also present inhibitory effects on cytochrome P450 mediated drug metabolism. According to Vuppugalla & Mehvar (2004a,b) these effects are rapid, concentration-dependent and isoenzyme-selective, mainly in the isozymes 2C11 > 2B1/2 > 2E1 = 3A2 > 1A1/2. This could be a hypothesis for ARG reducing effect on methemoglobin levels in animals treated with 5 and 15 mg/kg ARG and DDS. Finally, we conclude that low dose ARG has an antioxidant effect on DDS-induced methemoglobinemia. However the mechanisms related to this interaction are still not clear. Chiueh CC. *Ann N Y Acad Sci*, v.890, p. 301, 1999. Vuppugalla R. *J Pharmacol Exp Ther*, v. 310, p. 718, 2004a. Vuppugalla R. *Drug Metab Dispos*, v. 32, p. 1446, 2004b. **Financial support:** CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior); FURP (Fundação para o Remédio Popular).

11.015

Clinical assessment of safety phytotherapic tincture jalapa in volunteers with functional constipation. Santos LKX[1], Cunha GH[1], Fechine FV[1], Justa, GCG[1], Leite IO[1], Frota Bezerra FA[1], Citó MCO[2], Silva FCC[2], Moraes MO[1], Moraes MEA[1] [1]UNIFAC-UFC - Fisiologia e Farmacologia , [2]UFC- Departamento de Fisiologia e Farmacologia,

Introduction: The Tincture Jalapa is a phytotherapic extracted from plant Operculina alata (Ham) Urban; know popularly as *Aguardente Alemã*® and extensively used by the population. This phytotherapic has laxative and purgative properties and belongs to the family of Convolvulaceae composed of 51 genders, with wide distribution in tropical and subtropical regions. It is ancient plant use in folk medicine in the Northeast of Brazil. This study aimed to evaluate the clinical safety of the acute use of hydroalcoholic extract of *Operculina alata* in patients with functional constipation. **Methods:** The clinical trial consisted in a randomized study, double blind, placebo-controlled, with 76 volunteers, who were administered an oral dose of 15 mL of tincture Jalapa or placebo for one time a day for seven consecutive days. The volunteers were divided into two groups, Jalapa and placebo (with 38 volunteers each). Both products contained 50% hydroalcoholic vehicle and caramel coloring. The Jalapa Product contained in addition, the root of Jalapa as powder. The volunteers were included only if considered healthy, after clinical examination and exams complementary preceding the study. The laboratory evaluation included hematological analysis, and liver biochemistry. The assessment was made during the run-in period and post-study. Comparisons were made between the two groups in each phase of the study (intergroup analysis) as well as between the two phases in the same group (intragroup analysis). The research project, with the experimental protocol and the term of free informed consent, were submitted to the Research Ethics Committee of the Federal University of Ceará, which approved the protocol of nº 33/08. **Results:** In the intergroup analysis, no statistically significant differences were found between the two groups at any stage of the study, nor in the intragroup analysis, there were statistically significant differences between the periods. Some adverse events were observed in 13 (34.21%) volunteers in the test group, the most frequent were dizziness (13,16%), abdominal pain (13,16%) and headache (5,26%). In the group placebo 13 (34,21%) volunteers reported some adverse event, the most frequent were dizziness (13,16%), headache (10,53%) and nausea (10,53%). **Discussion:** Despite the phytotherapic Tincture Jalapa is widely used for its laxative activity, there is a very large deficit of pharmacological and toxicological studies on Operculina alata. This study showed that this phytotherapic was well tolerated by volunteers. The clinical and laboratory exams did not evidence toxicity signs. Some adverse events were observed with the use of the tincture of Jalapa, and classified as possible, probable and not be attributed to the phytomedicine. Regarding the degree of severity adverse events were of mild intensity. Results showed that, under the conditions of the present study, there was no difference between groups treated with placebo and phytotherapic tincture Jalapa regarding the incidence of adverse events. **Financial support:** CAPES, FINEP, Institute Claude Bernard.

11.016

Pre-clinical investigation of oral pharmacokinetics and tissue distribution of benzaldehyde semicarbazone free and complexed with B-cyclodextrin. Kaiser M[1], Azeredo FJ[2], Uchôa, FDT.[3], Beraldo H[4], Dalla Costa T[1] [1]UFRGS - Ciências Farmacêuticas, [2]FF-UFRGS - Produção e Controle de Medicamentos, [3]UFRGS - Bioanalítico de Medicamentos, [4]UFMG - Química

Introduction: Epilepsy is a neurology disorder characterized by the periodic and unpredictable occurance of convulsive seizures. Benzaldehyde semicarbazone (BS) is an arylsemicarbazone with good antiepileptic activity in the maximal electroshock screen model (MES) in rats[1]. Its complexation with cyclodextrins (b-CD and hydroxypropyl-b-CD) decreased in approximately 70% the minimum dose necessary to produce similar anticonvulsant effect observed for the free drug (100 mg/kg). In addition, whereas free BS exhibits no activity 4 h after administration, the BS complexed protected 60% of the animals against seizures, indicating a possible change in pharmacokinetic (PK) parameters[2,3]. In this context, this study aimed to investigate the oral PK of the BS as a free drug or complexed with b-CD and to characterize the tissue distribution for both formulations after different routes of administration to rodents. **Methods:** PK studies were approved by UFRGS Ethics in Research Committee (#2007794). BS was administered orally to Wistar rats (250-350 g) at 50 and 100 mg/kg doses (10 mg/mL) as free drug suspension prepared with 10% Tween® 80 and 25% DMSO in saline solution, and as 50 mg/kg dose complex suspended in distillated water (5 mg/mL) (n = 8/group). Blood samples were collected from the lateral tail vein at pre-determined time points and plasma samples were frozen for posterior drug quantification by HPLC-UV method previously validated. For the tissue distribution, samples of different organs were removed and weighted after intravenous (10 mg/kg) and oral (50 mg/kg) dosing for both formulations (n = 3 animals/time point). The samples were homogenized using 2 mL of methanol per gram of tissue and further processed for drug quantification using the same HPLC-UV method. PK parameters were determined using non-compartmental (Scientist®) and compartmental (Excel®) analysis. Pharmacokinetic parameters were compared by ANOVA (a = 0.05). **Results and Discussion:** A one-compartmental model adequately described the individual plasma profiles of all groups investigated. After free BS dosing the compound showed linear PK and bioavailability (*f*) around 20%. When the BS/b-CD was administrated, the *f* (37%) was approximately 2-fold of the free drug. For the BS/b-CD, *Vd* and Cl_{tot} (2.2 ± 0.8 L/kg and 1.8 ± 0.5 L/h×kg, respectively) were higher than those obtained for the free drug, but the $t_{1/2}$ (0.8 ± 0.1 h) was similar ($p < 0.05$). The brain penetration and the mean residence time in this organ for the complex after intravenous (2.8) and oral (2.5) dosing were higher when compare to free BS, regardless of the administration route. The drug penetration in all tissues investigated increased after complexed drug dosing. **Conclusion:** BS is rapidly and incompletely absorbed, rapidly distributed and eliminated from the body following oral administration of free or complexed drug. The higher brain penetration shown by b-CD explains the pharmacodynamic results previously published. **References:**[1]Dimmock, *J. Med. Chem***.** 36: 2243, 1993. [2]Beraldo, *Biochem. Biophys. Res. Commun.* 296: 241, 2002. [3]Teixeira, *J. Incl. Phenom. Macr. Chem.* 47: 77, 2003. **Acknowledgments:** CNPq/IM-INOFAR (#420015/05-1)

11.017

Preclinical pharmacokinetic evaluation of thiazolidinone PG15: an anti-inflammatory candidate. Uchoa FDT[1], Silva TG[1], Lima MCA[1], Pitta IR[1], Galdino SL[1], Dalla Costa T[2] [1]UFPE - Antibióticos, [2]UFRGS - Ciências Farmacêuticas

Introduction: Inflammation is associated with a wide range of human diseases and conditions which are often treated with anti-inflammatory drugs. Novel 5-benzilidene thiazolidinones have been synthesized and exhibited anti-inflammatory activity[1]. In this work one compound from this chemical series, (5Z,E) -3- [2- (4- chlorophenyl)-2-oxoethyl] -5- (1H-indol-3-ylmethylene)-thiazolidine-2,4-dione (PG15), previously synthesized by a short and easy synthetic pathway, was investigated aiming to determine the drug pharmacokinetics (PK) in Wistar rats. **Methods:** The animal experiments were approved by UFRGS Ethics in Research Committee (2006/608). PG15 was administered to Wistar rats (290–325 g) intravenously (i.v.; 3 mg/kg, n = 5) and orally [p.o.; 3 mg/kg (n = 3) or 6 mg/kg (n = 3)] as a 4 mg/mL suspension prepared with 10% ethanol, 10% polisorbate 80 in a 5% glucose solution. After dosing, blood samples were collected from the lateral tail vein at pre-determined time points up to 16 h. The plasma was separated by centrifugation (10 min, 6800 g, 4 ºC) and 100 µL aliquots were stored at -20 ºC until analysis. On the analysis day, samples were thaw, spiked with 10 µL of internal standard (IS) (chlortalidone 100 µg/mL); deproteinized with acetonitrile (300 µL) and centrifuged (10 min, 15000 rpm, 4 ºC). 20 µL of the supernatant was used to quantify PG15 using an LC-MS/MS method previously validated according to FDA guidelines[1]. PK parameters were determined from individual plasma profiles by compartmental and non-compartmental approaches. **Results and Discussion:** After i.v. dosing, PG15 was rapidly distributed, with plasma levels falling from 9 µg/mL, 5 min after dosing, to 300 ng/mL in less than 30 min. After distribution, elimination was slow characterizing drug disposition as a two-compartment model. Pharmacokinetic parameters in rats were: Cp_0 = 43397 ± 33384 ng/mL, l = 0.16 ± 0.09 h^{-1}, $t_{1/2}$=5.9 ± 3.8 h, CL_{tot} = 0.9 ± 0.5 L/h/kg and $AUC_{0-\infty}$ = 4025 ± 1496 ng·h/mL. After PG15 3 mg/kg p.o. dosing the drug was rapidly absorbed showing peak levels in 0.5-1 h. The $t_{1/2}$, l and total CL_{tot} were statistically similar to those determined after i.v. dosing, with 28% bioavailability. The PK for the higher p.o. dose (6 mg/kg) of PG15 was also investigated. Plasma profiles for this dose were erratic and present a higher variability than that observed for the lower dose, showing similar concentrations. No PK model was capable of fitting these oral profiles and parameters were estimated only by non-compartmental approach. The variability observed after p.o. dosing was not observed after i.v. administration, and was attributed to an erratic absorption of PG15, caused by its poor water solubility at the site of absorption due to its high lipophilicity (LogP = 4.05). The low solubility of the drug in aqueous media, even in different pHs, probably render the drug to be poorly and non-homogenously absorbed along the gastrointestinal tract. Further studies viewing to improve drug absorption should be conducted. **References:** [1]Uchoa, F.D.T. *et al*., J. Amer. Org. Anal. Chem. 2009, *accepted.* **Acknowledgments:** IM-INOFAR/CNPq (420.015/2005-1), CNPq/Brazil

11.018

Evaluation of the therapeutical efficacy of the tincture of jalapa in the treatment of the functional constipation. Cunha GH, Santos LKX, Fechine FV, Justa, GCG, Oliveira, JC, Nascimento DF do, Moraes MO, Frota Bezerra FA, Moraes MEA UNIFAC-UFC Fisiologia e Farmacologia

Introduction: The Tincture of Jalapa is a phytotherapic produced from the roots of *Operculina alata*, known as Aguardente Alemã®, used by the population because of its laxative effect, but not had their effectiveness evaluated in clinical trials. **Methods:** Double-blind clinical trial, placebo controlled, randomized, and parallel evaluated the therapeutic efficacy of tincture of Jalapa in the treatment of functional constipation in the Unit of Clinical Pharmacology, Ceara, Brazil. There were two treatment groups, the Jalapa and Placebo, both consisting of 38 volunteers with functional constipation. The study had 3 phases, seven days in each phase, in which the volunteer data recorded in a diary of bowel movements. During the pre-treatment there was the selection of volunteers. In the treatment occurred the randomization and administration of 15 mL of tincture Jalapa or placebo. The post-treatment were observed after the voluntary suspension of tincture of Jalapa or placebo. The effectiveness was evaluated through the primary variables: average frequency of evacuations (AFE), average consistency of faeces (ACF) and global improvement of constipation (GIC). Secondary variables were the proportion of evacuations with pain (PED), proportion of effort evacuations (PEE), number of consecutive days without evacuation (DWE) and degree of improvement in constipation. The research project, with the experimental protocol and the term of free and informed consent, were submitted to the Research Ethics Committee of the Federal University of Ceara, which approved the protocol of nº 221/07. **Results and discussion:** All volunteer participants were female. In the pre-treatment, Jalapa and Placebo groups showed statistically similar in age, body mass index and ACF, but differed in the AFE, where the Placebo group (0321 ± 0108) showed that the largest group Jalapa (0262 ± 0132). In the treatment and post-treatment, the AFE and ACF were significantly higher in Jalapa group than in the Placebo group, with 55.26% of volunteers of the Jalapa group showing increased values of AFE and ACF, which was represented by the GIC with 3.5 times more likely to improve the constipation with the tincture of Jalapa that with placebo. The values of the PED, the PEE and the DWE in Jalapa group were lower and statistically significant than those in the placebo group during treatment and post-treatment. The proportion of volunteers reported that the Jalapa group reasonable and complete relief of symptoms of constipation during treatment and after treatment was significantly higher than in the placebo group. There were not significant changes in systolic and diastolic blood pressure or heart rate. Furthermore, it was not observed serious adverse events or abnormalities in electrocardiography. This study found that the tincture of Jalapa is effective in the acute treatment of functional constipation. **Financial support:** CAPES, FINEP, Instituto Claude Bernard.

11.019

Inhibitory activity under alpha-glucosidase and alpha-amylase of carbohydrates-derived 1H-1,2,3-triazoles. Montefusco-Pereira CV[1], Lima ES[2], Ferreira, SB[3], Ferreira VF[4] [1]UNIP-Manaus - Farmácia e Bioquímica, [2]FCF-UFAM, [3]UFRJ - Química, [4]UFF - Química Orgânica

Introduction: At the digestive process, the breaking of starch by enzymes is essential to nutrition and from that it results on monosaccharide, as glucose. Those attacks occur on different spots, although at the small intestine, particularly at the brush border microvilli, there are hydrolases to do that function: glucosidase. Those enzymes do the breaking on alpha and beta carbon glycosidic bonds, there so acting on polysaccharides and disaccharides. With the importance of those hydrolases on biologic and pathologic functions – intestine digestion, lysosomal catabolism glycoconjugates, high frequency on oncologic plasma and for its influence in viral membrane glycoproteins – there is a reason to investigate and search for natural and synthetic means of inhibition. From the medicinal chemistry, aza-heterocyles compounds has uprising, for instance, the alpha-glycosidase, including 1,2,3-triazoles that possess properties against diseases as cancer, AIDS, Parkinson and Alzheimer. Its activity depends on the functional groups orientation and also referring to the conjugation to carbohydrates. At this introduction, this work had the aim to analyze the inhibitory effect under alpha-glycosidase and amylase of synthetic carbohydrates-derived 1H-1,2,3-triazoles. **Methods**: Using colorimetric-enzymatic methods, two assays were done: inhibition of alpha-glucosidase and alpha-amylase. The substances were previously diluted on Dimethyl Sulfoxide (DMSO) at different concentrations from a range of 0,5 to 1000 µmol/L. To a-glucosidase the reaction with 4-nitrophenil-a-D-glycopiranoside was used (Matsui, 1996), where this reagent is chromogenic when interacting with the enzyme, producing color to be read at 405 nm. To a-amylase, the assay based on starch-substrate consumption was used added to an iodine reagent (modified Caraway, 1959), producing color to be read at 605 nm. The 50% inhibitory activity concentration (IC50) was executed by non-linear regression of its sigmoid curve on software Microsoft Origin 6.0. **Results:** From the screening of 73 triazoles, 4 (four) of them were highlighted at the results, what directs us to continue on the evaluation of its biological effect. Where the compounds: 4-phenyl-1H-1,2,3-triazole-5-(1-O-methyl-2,3-O-isopropylidene)-beta-D-ribofuranose; 4-(1-cyclohexene)-1H-1,2,3-triazole-5-(1-O-methyl-2,3-O-isopropylidene)-beta-D-ribofuranose; 4-phenoxymethyl-1H-1,2,3-triazole-5-(1-O-methyl-2,3-O-isopropylidene)-beta-D-ribofuranose; 4-(1-hydroxycyclohexil)-1H-1,2,3-triazole-5-(1-O-methyl-2,3-O-isopropylidene)-beta-D-ribofuranose, respectively presented an IC50 to inhibit a-glucosidase of 14,9 ± 4,2, 3,8 ± 0,5, 5,7 ± 0,3, 5,2 ± 0,9, µmol/L. To a-amylase those substances presented inhibition (%) of 17,14, 14,09, 13,48 e 12,56 at the concentration of 10 µmol/L, respectively. **Conclusion:** With those results, the mentioned triazoles are likely inhibitors of alpha-glucosidase; as for alpha-amylase, there were no meaningful inhibition, what might indicate less production of adverse effect if directed to a possible in vivo assay. **Support Agency**: Fundação de Amparo à Pesquisa do Estado do Amazonas - FAPEAM and Conselho Nacional de Pesquisa e Desenvolvimento - CNPq.

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11.020

The influence of genetic factors on plasma/whole blood lead ratio in pregnancy. De Rezende V[1], Amaral JH[2], Quintana S[3], Barbosa Jr F[2], Gerlach RF[4], Tanus-Santos JE[2] [1]UNICAMP - Farmacologia, [2]FMRP-USP - Farmacologia, [3]FMRP-USP - Ginecologia e Obstetrícia, [4]FORP-USP - Morfologia

Introduction: Genetic factor is important to toxicokinetics of lead (Pb), especially in pregnancy. Pregnant women are one of the most sensitive populations to the toxic effects associated with lead (Pb) exposure. This study aimed at examining if haplotype of three polymorphisms (*BsmI*, *ApaI* and *FokI*) in VDR gene affects Pb-B (Blood-lead concentrations in µg/dL); Pb-P (Plasma lead concentration in µg/dL) and %Pb-P/Pb-B ratio in 256 environmentally exposed pregnants and their umbilical cord. **Material and Methods:** Genotypes for the VDR (*FokI, ApaI* and *BsmI*) polymorphism were determined by PCR and restriction fragment length digestion (RFLP). Pb-P and Pb-B levels were determined by inductively coupled plasma mass spectrometry. Haplotype groups are estimated by Phase v2. **Results:** Mean±SE in pregnants: (Pb-S 1,915±0,06µg/dL; Pb-P 0,040±0,001µg/dL; %Pb-P/Pb-S: 2,533±0,109). Pregnants with *f, a, b* haplotype group (H8) presented lower Pb-P/Pb-B (%) (1,849±0,127 µg/dL) when compared with subjects with other groups. (H1:3,660±0,220 µg/dL; H2: 2,760± 0,175 µg/dL; H4: 2,260± 0,168 µg/dL; H5: 2,475 ± 0,213 µg/dL, respectively) (P<0.05). However, no significant differences were found in these parameter of umbilical cord among haplotypes groups. **Discussion:** The lower %Pb-P/ Pb-B in H8 haplotype group (*f, a, b*) carriers compared with non-carriers indicate that this genetic factor maybe help to recognize women with elevate risk to lead toxicity. **Financial support:**CAPES, FAPESP, CNPq. **References:** 1. Onalaja A. Environ Health Perspect 108; 23 (2000); 2. Uitterlinden A. Gene 338; 143 (2004); 3. Morrison N. Proc Natl Acad Sci 89**;** 6665 (1992); 4. Schwartz B. Environ Health Perspect 108; 199 (2000); 5. Rezende VB Arch toxicol. 2008*; 6*- Gulson, B.L. J. Lab. Clin. Med. 1997. **Comitê de Ética:** ofício n.3233/2004

11.021

Comparison between pharmacokinetic and pharmacodynamic of single-doses furosemide. Bragatto MB[1], Santos[1], Manfio JL[1], Pinto AMP[1], Prado AW[1], Gomes E[1], Viezzer WF G[2], Donaduzzi CM[3] [1]Biocinese - Estudos Biofarmacêuticos-Bioequivalência, [2]Labclinic [3]Prati, Donaduzzi

Introduction: Furosemide has been widely used because of its rapid diuretic action, especially in acute cases (Dias *et al*., Lecta, v. 22, p. 19, 2004). Its absorption is rapid and peak levels occur 60-90 minutes after the dose. It has a high plasma protein binding (97-98%) and the elimination half-life is relatively fast (0.5-2 h), however, the biphasic elimination kinetics is slow (20-30 h). (Wenk *et al* J. Pharm. and Biom. Analysis, vol. 41,p. 1367, 2006). The aim of this study was to evaluate and compare the pharmacokinetic and pharmacodynamic behavior of two formulations of furosemide 40 mg, administered as a single dose to healthy subjects. **Methods:** To assess the quality of form and rate and to simulate the absorption velocity *in vitro*, was performed a dissolution profile of the formulation of furosemide following the test and reference method pharmacopoeias. Twenty-eight male volunteers aged between 18 and 44 years was recruited to take part of *in vivo* study . The inclusion of volunteers in the study was determined by the state of healthy, evidenced by medical history, physical examinations and laboratory tests. The study protocol was approved by license number 101/2007 in the Ethics Committee in Human Research of Assis Gurgacz School, accredited by the National Health Council / MS. The study conducted was an open, randomized and crossover, two internment (crossover 2x2). The volunteers received a single dose of 40 mg of each formulation in fasting and were collected 21 blood samples until 15 hours after administration. Plasma concentrations of furosemide were determined with a validated method by liquid chromatography coupled to mass spectrometry (LC-MS/MS) using chlortalidone as internal standard. We obtained the parameters: AUC_{0-t}, $AUC_{0-\infty}$, K_{el}, $T_{1/2}$, $C_{máx}$ and $T_{máx}$ through the use of analysis of variance (ANOVA) for the crossover model. For the analysis of pharmacodynamics of the drug, the urine was collected in intervals until 12 hours after administration. Through selective Electrode method / automated were determined excretion of sodium, potassium and chlorine, and was measured the total volume excreted in each period. **Results and Discussion:** In the *in vitro* analysis by assessing the profile of dissolution were found that the test and reference products are similar. In the *in vivo* study the formulations of furosemide have been well tolerated in the administered dose. The pharmacokinetic parameters found for the test and reference formulations were, respectively, $T_{máx}$ of 1.82 and 1.54 h, $C_{máx}$ of 879.32 and 884.36 ng/mL, K_{el} of 0.24 and 0.24, t½ 4.70 and 3.86 h, AUC_{0-t} 2137.78 and 2107.38 ng/mL.h and $AUC_{0-\infty}$ 2233.80 and 2183.24 ng/mL.h. Statistical analysis confirmed the similarity of the parameters for urinary volume, excretion of sodium, potassium and chlorine and assuming that both formulations reach the same plasma levels, we expect that the pharmacological effect is also the same. Whereas the rate and extent of absorption as required by existing regulations both products can be considered bioequivalent and, therefore, equal treatment in medical practice. **Acknowledgments:** The authors wish to thank Prati, Donaduzzi for the sponsorship and support.

11.022

Assessment of matrix metalloproteinase (MMP)-2, MMP-8, MMP-9, and their inhibitors, the tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2 in obese children and adolescents. Belo VA[1], Souza-Costa DC[2], Lana CMM[2], Gerlach RF[3], Caputo FLD[2], Marcaccini, AM[3], Bastos GM[4], Tanus-Santos JE[5] [1]UNICAMP - Farmacologia, [2]UFJF - Farmacologia, [3]FORP-USP - Morfologia, [4]UFJF - Clínica Médica, [5]FMRP-USP - Farmacologia

Introduction: There is clear evidence that inflammatory mechanisms play a role in atherogenesis. In this context, several members of the matrix metalloproteinase (MMP) family and their endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs), have been implicated as mediators of this pathology. The aim of this study was to compare the circulating levels of matrix metalloproteinase (MMP)-8, pro-MMP-2, pro-MMP-9, and total MMP-9, their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMP)-1 and TIMP-2, and the MMP-8/TIMP-1, MMP-9/TIMP-1, and MMP-2/TIMP-2 ratios in normontensive obese children and adolescents with those found in non obese children and adolescents. **Methods:** Approval for use of human subjects in this cross-sectional study was obtained from the Institutional Review Board at the Federal University of Juiz de Fora, Brazil (process number: 2006.052.2007).We studied 40 obese and 40 non obese (controls) children and adolescents in this cross-sectional study. MMPs and TIMPs concentrations were measured in plasma samples by gelatin zymography and ELISA. **Results:** Obese children and adolescents had higher circulating MMP-8 concentrations, lower plasma TIMP-1 concentrations, and higher MMP-8/TIMP-1 ratios than non obese controls ($P<0.05$). We found no differences in pro-MMP-9 or total MMP-9 levels, or in MMP-9/TIMP-1 ratios between groups ($P>0.05$). While we found no significant differences in pro-MMP-2 levels ($P>0.05$) obese subjects had higher TIMP-2 concentrations and lower pro-MMP-2/TIMP-2 ratios ($P<0.05$) than non obese controls. **Discussion**: In conclusion, we found evidence indicating higher net MMP-8 (but not MMP-9 and MMP-2) activity in childhood obesity. The increased MMP-8 levels found in obese children suggest a possibly relevant pathophysiological mechanism that may be involved in the increase of cardiovascular risk associated with childhood obesity. **References:** Van Gaal, LF, Nature;444:875, 2006. Libby, P. Nature;420:868, 2002. Glowinska-Olszewska, B. Metabolism;56:799, 2007. **Support:** FAPESP and CNPq.

11.023

Acute vascular relaxation effects of tamoxifen and its metabolites. Montenegro MF[1], Ceron CS[1], Desta Z[2], Flockhart DA[2], Salgado MCO[1], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]Indiana University - Clinical Pharmacology

Introduction: Tamoxifen has been showed to produce acute relaxation effects, although the mechanisms for these effects are not fully known. Moreover, although tamoxifen is widely metabolized and its metabolites may exhibit 30-100 fold higher affinity by estrogen receptor than the parent drug, no previous study has assessed the effects produced by tamoxifen metabolites on vascular relaxation. Here, we examine the acute vascular responses to tamoxifen and its three main metabolites, *N*-desmethyl-tamoxifen, 4-hydroxy-tamoxifen, and endoxifen. Methods: This study was approved by our institutional review committee (number 136/2006). Concentration-response curves (10^{-14} to 10^{-5}M) to tamoxifen, *N*-desmethyl-tamoxifen, 4-hydroxy-tamoxifen, and endoxifen, were performed in endothelium-intact aortic rings pre-contracted with phenylephrine (0.1μM) using standard muscle bath procedures. In addition, we studied the role of L-NAME and estrogen receptor (ER) on effects induced by such drugs. Results: Tamoxifen and its metabolites induced aortic vascular relaxation in a concentration-dependent manner (~88-101% from initial contraction). While 4-hydroxy-tamoxifen induced similar maximum relaxation effect to tamoxifen (92.6±1.3 versus 88.5±1.3%, respectively; P>0.05), *N*-desmethyl-tamoxifen and endoxifen induced higher maximum effect than tamoxifen (101±1.8 to both versus 88.5±1.3% from tamoxifen; P<0.05). In addition, *N*-desmethyl-tamoxifen was more potent than tamoxifen (pD_2 9±0.14 and 8.5±0.12, respectively, P<0.01). While rings pretreatment with L-NAME had no effect on maximum relaxation induced by tamoxifen and 4-hydroxy-tamoxifen (P>0.05), L-NAME significantly reduced the maximum effect induced by *N*-desmethyl-tamoxifen and endoxifen. The estrogen receptor antagonist ICI 182.780 (10μM) induced no change in vasorelaxation responses to tamoxifen and 4-Hydroxy-tamoxifen (P>0.05), but significantly reduced the maximum relaxation responses observed to N-desmethyl-tamoxifen and endoxifen (P<0.05). These results shown for the first time that tamoxifen metabolites *N*-desmethyl-tamoxifen, 4-hydroxy-tamoxifen, and endoxifen has acute vascular effects, and suggest that tamoxifen metabolites *N*-desmethyl-tamoxifen and endoxifen produces its relaxant effects, in part, by nitric oxide-, and estrogen receptor- dependent mechanism. Provided that these findings can be extrapolated to humans, it is possible that these differential effects might to be responsible, at least in part, by wide interindividual variability in the pharmacotherapy of tamoxifen. FAPESP and CNPq

11.024

Artificial membranes for prediction of human drug absorption in Franz cell method. Teixeira LS, Chagas TV, Rezende KR UFG - Farmácia

Introduction: The permeability is indirectly based in the extent of absorption of a drug substance in humans and it is directly based in measurements of the mass transfer rate across human intestinal membrane. Alternatively, nonhuman systems are capable of predicting the extent of drug absorption in humans (e.g., parallel artificial membrane permeability assay - PAMPA). In this technique, the permeability is determined through a microfilter previously impregnated with a solution of phospholipids and cholesterol in octanol. Since the majority of drugs are mainly absorbed through passive transportation, the use of artificial membranes which mimic passive diffusion offers a potentially effective high throughput approach for the assessment of drug absorption potential. An *in vitro* method based in the use of artificial membranes for prediction of the absorption properties of two formulations of betamethasone (BET), an injectable drug constituted of BET dipropionate (5 mg/ml) and BET sodium phosphate (2 mg/ml), was studied and compared to the results from a BET bioequivalence study. **Methods:** Permeation experiments were performed using vertical diffusion cell system from Hanson Research Corp. (USA). To acquire the typical lipophilic properties of biological membranes, the nitro-cellulose support was impregnated with a lipidic phase (cholesterol 2.10%; Lipoid® E 80 1.70%; n-octanol 96.2%). The artificial membrane was inserted in the diffusion cell connected to the donor and receptor compartments. Phosphate buffer pH 7.4 solution was used in the receptor compartment. The system was thermostated at 37±0.5 ◦C. For demonstrating the suitability of the method, model drugs (manitol, metoprolol, naproxen), that represent a range of low (e.g., < 50%), and high (≥ 90%) absorption were studied. Twenty-six healthy volunteers were selected for the bioequivalence study. The study was a single dose, two-way randomized crossover design with a 3-week washout period between the doses. Blood samples were collected before the medication dosing and 10; 20; 30; 45; 60; 90; 120; 150; 180; 210; 240 min and also 5; 6; 8; 10; 12; 24; 48; 72 h post-dosing. This study was approved by the HGG-GO Ethical Review Board. **Results and Discussion:** The resulting permeation coefficients (*P*) were calculated and expressed as mean ± standard deviation. The P values obtained for the BET reference and test formulations were 2,07 x 10-5 ± 1,1 x 10-5 cm/s and 3,94 x 10-4 ± 8,6 x 10-6 cm/s, respectively. These values obtained for two formulations of betamethasone (BET) injectable drug were correlated with the results of the bioequivalence study. The geometric mean and respective 90% confidence interval (CI) of BET percent ratios were 128.35% (121.25–135.87%) for AUC_{last}, 126.89% (120.16–133.99%) for $AUC_{0\text{-}inf}$ and 90.70% (85.49–96.22%) for C_{max}. We found significant correlations between *P* results and AUC_{last} of BET ($P<0.05$). In the present paper we evaluated the actual bio-mimetic capability of such artificial membrane in drug absorption prediction. The results of this study show that the *P* results obtained with the artificial membrane method can be good predictors of the oral absorption of passively absorbed drugs in humans. References: Amidon, G. L. *et al. Pharm Res*, 12, 413, 1995. Corti, G. *et al. Eur J Pharm Sci*, 27, 346, 2006. Lobenberg, R.; Amidon, G.L. *Eur J Pharm Biopharm*, 50, 03, 2000. Sugano, K. *et al. Int J Pharm*, 228, 181, 2001. Acknowledgement: The authors wish to acknowledge the Instituto de Ciências Farmacêuticas – ICF and Universidade Federal de Goiás, Brazil. This paper was sponsored by ICF.

11.025

Brazilian hospital care assistance to patients participating in research: review. Teodoro AR PUCCamp - Ciências Farmacêuticas

Introduction: The pharmaceutical care in hospitals can contribute to improving the quality of treatment and reduce its cost, especially in patients participating in clinical research, but also with the reduction of public health problems, and reducing adverse events often associated with poor therapeutic use. The objective of this research was to describe and evaluate the progress of knowledge and pharmaceutical assistance to participants of clinical research in Brazilian hospitals. **Methods:** Literature review was conducted following the publications available in virtual libraries: PubMed and BIREME using the following descriptors of subject in Portuguese, and their counterparts in Spanish and English: Clinical Pharmacology, Pharmaceutical Services, Hospital Pharmacy, pharmacotherapeutic follow-up. **Results and Discussion:** The sample consisted of 112 publications, however, the objectives were not compatible with the criteria for inclusion in the study. The lack of Brazilian publications (6.6%) may indicate a lack of standardization of concepts related to pharmaceutical care for patients in clinical research in Brazilian hospitals. Several papers were published, however, they were considered unsuitable for the pharmacotherapeutic monitoring of patients in clinical research of the Brazilian hospitals. Pharmaceutical care, can resolve and clarify all doubts of patients in therapeutic trials. This may occur because the relationship between the actions of the pharmacist and the user, ensuring a better quality of life, avoiding the worsening state of health of individuals and thus reducing costs to the hospital. **Bibliography:** BRODY, T. M. et. al. Farmacologia Humana. 4th edition. Rio de Janeiro: Elsevier, 2006. p. 217. RANG, B.P. et. al. Pharmacology. 5th edition. Rio de Janeiro: Elsevier, 2004. p. 72-77. BISSON, MP Clinical Pharmacy Services and Pharmaceuticals. 2nd edition. Barueri: Manole, 2007. p. 158 - 173. Thanks: Carolina T. Garcia - review.

11.026

Interethnic differences in the distribution of clinically relevant vascular endothelial growth factor (*VEGF*) genetic polymorphisms. Muniz JJ[1], Metzger IF[2], Sandrim VC[2], Izidoro-Toledo TC[2], Tanus-Santos JE[2] [1]UNICAMP - Farmacologia, [2]FMRP-USP - Farmacologia

Introduction: Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein produced mostly in endothelial cells (ECs), and its transcription is up-regulated during hypoxia and by a variety of growth factors and cytokines. VEGF causes proliferation and migration of ECs, and vasodilatation through increased nitric oxide and prostacyclin production, thus leading to inhibited leukocyte adhesion and platelet aggregation [1]. Three polymorphisms in *VEGF* gene (two in promoter region "C-2578A, G-1154A" and one in 5´untranslated region(UTR) "G-634C") were related with many vascular diseases [2,3], and recent studies have suggested that there are interethnic differences in the distribution these variants [4]. Here, we examined these possible interethnic differences in the Brazilian population. **Methods:** Approval for use of human donors was obtained from the Institutional Review Board at Faculty of Medicine of Ribeirão Preto (HCRP-10090-2002) and at State University of Santa Cruz (04/06). We examined the distribution of genetic variants of three clinically relevant *VEGF* SNPs (C-2578A, G-1154A and G-634C) in 175 white and 185 black subjects from the Brazilian population. We also estimated the haplotype frequency, and evaluated associations between these variants. **Results:** The A-2578 and A-1154 variants in the promoter region of *VEGF* gene were more common among whites (16 and 29.4%, respectively) than blacks (9.7 and 16.2%, respectively) (both $P<0.05$). The haplotype including the C-2578, G-1154, and G-634 variants was the most common in each ethnic group, and was also more common among blacks than whites ($P<0.05$). The haplotype including the variants C-2578, A-1154, G-634 and the haplotype including the variants C-2578, A-1154, C-634 were more common among whites than blacks (both $P<0.05$). **Conclusions:** These results show marked interethnic differences in the distribution of clinically relevant genetic variants of *VEGF* in the Brazilian population. These differences may explain, at least in part, the interethnic disparities in vascular risk and response to drugs. **Supported by:** CNPq, CAPES and FAPESP. [1] Ferrara. N, *et al.* Nat Méd, 9(6):669-76 (2003) [2] Sandrim. VC, *et al*. Mol Hum Reprod, 15(2) :115-20 (2008) [3] Lambrechts. SD, *et al*. Nat Genet, 34(4) : 383-94 (2003) [4] Girnita, DM, *et al*. Transplantation, 85(11):1632-9 (2008)

11.027

Avaliação da segurança e genotoxicidade do chá de *Alpinia zerumbet* em voluntários sadios. Santana APM, Nascimento DF do, Leite ALAS, Wong DVT, Pontes AV, Ferreira JRO, Uchôa, CRA, Mariz MPV, Tagliapietra JI, Fechine FV, Frota Bezerra FA, Moraes MO, Moraes MEA UNIFAC-UFC - Fisiologia e Farmacologia

Introdução: *Alpinia zerumbet* (AZ), conhecida popularmente como Colônia, é uma planta usada tradicionalmente, na forma de chá, no tratamento da hipertensão arterial. Entretanto, ainda não foram realizados estudos de toxicologia clínica com o chá dessa planta, tampouco o risco de eventuais danos ao DNA foi avaliado, mediante o emprego de ensaios de genotoxicidade. O objetivo deste estudo foi avaliar a segurança clínica e possíveis efeitos genotóxicos do chá de AZ em voluntários sadios. **Método:** O estudo foi conduzido conforme o protocolo (Nº 38/08) aprovado pelo Comitê de Ética em Pesquisa da UFC. Realizou-se um ensaio clínico randomizado, duplo-cego, controlado por placebo, em paralelo, com 36 voluntários sadios de ambos os sexos, distribuídos em dois grupos: grupo Colônia, formado por 24 sujeitos; grupo Placebo composto por 12 voluntários. O estudo compreendeu três fases. Na fase pré-estudo o sujeito era submetido à avaliação clínica e laboratorial (análise hematológica e bioquímica, sorologia para HIV, hepatite B e C, sumário de urina, além de teste sorológico para gravidez para as mulheres), que definiam a sua inclusão no ensaio. Na fase de tratamento, os sujeitos receberam, via oral, 3 doses diárias de 180 mL do chá de colônia ou do chá placebo durante 28 dias consecutivos, ao longo dos quais efetuou-se o monitoramento da freqüência cardíaca (FC) e da pressão arterial (PA). A fase pós-estudo correspondeu à semana pós-tratamento. Realizaram-se avaliações clínicas e exames laboratoriais no 14° e 28° dias de tratamento e no término da fase de pós-estudo. Para o ensaio de genotoxicidade, coletaram-se 5 mL de sangue de 18 voluntários do grupo Colônia, nos períodos de pré e pós-estudo, para avaliar o efeito genotóxico em linfócitos periféricos humanos através do teste do cometa. A análise estatística envolveu comparações intergrupos em cada fase, assim como comparações dos diversos momentos num mesmo grupo (intragrupo). **Resultados e discussão:** A idade média dos voluntários foi 29,96 ± 9,12 anos no grupo Colônia e de 27,42 ± 7,79 anos no Placebo. Não houve variação estatisticamente significante do índice de massa corporal (IMC) nas três fases do estudo. Nas comparações intergrupos, verificaram-se alterações estatisticamente significantes apenas nos leucócitos totais ($P<0,05$), eosinófilos ($P=0,0167$), AST ($P=0,0170$) e triglicerídeos ($P=0,0427$), enquanto na análise intragrupo, constataram-se diferenças significantes nas concentrações de creatinina ($P<0,05$), glicose ($P<0,05$), fosfatase alcalina ($P<0,05$) e albumina ($P<0,05$). As diferenças estatisticamente significantes encontradas corresponderam a variações pequenas nos valores médios, que se mantiveram dentro dos limites de normalidade, não tendo, por conseguinte, relevância clínica. O monitoramento da FC e da PA sistólica, diastólica e média revelou variações significantes, mormente na análise intragrupo, todavia sem relevância clínica. Pelo teste do cometa, não foram observados danos ($P<0,05$) nos linfócitos periféricos dos voluntários tratados com o chá de colônia. Não se observaram toxicidade clínica nem indução de genotoxicidade em voluntários sadios após administração do chá de AZ. **Apoio:** CNPq, FINEP, DECIT/MS, InCB.

11.028

Computational evaluation of pharmacokinetics and bioactivity of new inhibitors of phosphodiesterase Type-5. Antunes JE[1], Freitas MP[2], da Cunha EFF[2], Ramalho TC[2], Rittner R[3] [1]UNICAMP - Farmacologia, [2]DQI-UFLA, [3]IQ-UNICAMP

Introduction: Development of new drugs requires new technologies. Ligand-based approaches, such as MIA-QSAR (multivariate image analysis applied to QSAR),[1] have been applied in the design of new drug candidates. This work reports the development of novel PDE-5 inhibitors for the treatment of erectile dysfunction. This issue is of great interest because erectile dysfunction affects about 50% of 40-70 years old men. The main drugs produced against erectile dysfunction are Sildenafil, Vardenafil and Tadalafil (Viagra®, Levitra® and Cialis®, respectively). Thus, the goal of this work lies on proposing new potent PDE-5 inhibitors, which are miscellany of substructures of the above commercial actives, by using MIA-QSAR and docking studies. **Results and Discussion:** A congeneric series of 48 PDE-5 inhibitors obtained from the literature[2-4] was drawn by using the ChemSketch program. Each molecular structure (2D images) was superimposed to each other by a congruent moiety (two fused rings), and then read by using Matlab; the pixels (binaries) were used as molecular descriptors, where the variant portion (pixels corresponding to the substituents) accounted for the variance in bioactivities. The *I ´ J ´ K* three-way array built (*I* compounds, *J* and *K* coordinates of a 2D image) was unfolded to a *I ´* (*J ´ K*) two-way array (**X**-matrix), and then regressed against the **Y**-block (the activities column vector) through PLS. The calibration yielded an r^2 = 0.97 (using 8 PLS components). The model was validated through leave-one-out and leave-20%-out cross-validation (q^2 = 0.69 and 0.68, respectively). The activities of two novel compounds, derivatives of Sildenafil, Vardenafil and Tadalafil, were predicted using the MIA-QSAR model built. The high bioactivities calculated for the new compounds were corroborated by docking studies. Pharmacokinetic parameters, obtained through Molinspiration and PharmaAlgorithms programs, suggest that such compounds are potentially useful for the treatment of erectile dysfunction, because of the calculated log*P*, TPSA, molecular weight, solubility (in water and buffer), etc., which are comparable to the last generation PDE-5 inhibitor, Tadalafil. Evaluations of interactions between amino acid residues and the ligand by using a new computational tool to search a database of protein for the receptor with the ligand to serve as a parameter and then compared with new ligands were offered to corroborate the results obtained the study of docking. **Conclusions:** The MIA-QSAR model built was found to be highly predictive and, together with docking studies, was capable to predict the activities of novel PDE-5 inhibitors. These are potentially useful as new drugs, as supported by ADME-Tox calculations. [1] Freitas, M.P.; Brown, S.D.; Martins, J.A. *J. Mol. Struct.* 2005, *738*, 149. [2] Terret, N.K.; Bell, A.S.; Brown, D.; Ellis, P. *Bioorg. Med. Chem. Lett.* 1996, *6*, 1819. [3] Haning, H.; Niewöhner, U.; Schenke, T.; Es-Sayed, M.; Schmidt, G.; Lampe, T.; Bischoff, E. *Bioorg. Med. Chem. Lett.* 2002, *12*, 865. [4] Daugan, A.; Grondin, P.; Ruault, C.; de Gouville, A.C.M.; Coste, H.; Kirilovsky, J.; Hyafil, F.; Labaudinière, R. *J. Med. Chem.* 2003, *46*, 4525.

11.029

Eficácia da associação de mupirocina (2%) + dipropionato de beclometasona (0,025%) comparada à mupirocina (2%) pomada dermatológica em pacientes com foliculite. Juliato EG, Vilugron HDN, Silva LACV, Ferreira T, Bortolassi L, Frederico A LAL Clínica - Pesquisa e Desenvolvimento Ltda.

Introdução: A foliculite é uma infecção dos folículos pilosos causada por bactérias, geralmente do tipo estafilococos. A mupirocina é um agente antimicrobiano de largo-espectro, que atua inibindo a síntese protéica bacteriana. O dipropionato de beclometasona é um corticosteróide de ação tópica marcante que pode ser usado para tratar inflamações severas de pele. O objetivo do estudo foi avaliar a eficácia terapêutica da mupirocina (MUBE1) quando comparada à associação de mupirocina + beclometasona (MUBE2) através da contagem e classificações de lesões (pápulas e pústulas). **Métodos:** Estudo clínico randomizado, duplo-cego de fase II. Após aprovação do Comitê de Ética e a assinatura do termo de consentimento livre e esclarecido (TCLE), foram selecionados 38 indivíduos com idade entre 18 e 47 anos, de ambos os sexos e com quadro de foliculite. A avaliação do quadro clínico foi feita através da contagem e classificação das lesões (pápulas e pústulas), focando uma determinada região afetada, tendo como controle a região contra-lateral. Realizou-se um antibiograma para identificação da bactéria patogênica e de sua sensibilidade à mupirocina. Os indivíduos foram orientados a aplicar a pomada duas vezes ao dia (manhã e noite). O estudo teve a duração de até 28 dias, com avaliações clínicas no pré-tratamento (t0) e nos tempos 3, 7, 14, 21 e 28 dias, sendo que os indivíduos com evolução clínica de desaparecimento das lesões antes de 28º dia receberam alta clínica. A consulta pós-estudo foi realizada 7 dias após os término do estudo. **Resultados**: As relações entre lesões no ínicio e no final do tratamento foram estudadas através de teste não-paramétrico dos sinais, para dados pareados, e mostraram-se significantes dentro dos tratamentos (P<0,001). Observou-se melhora das contagens de pápulas e pústulas para ambos os tratamentos (MUBE1=69,23% e 89,74%, respectivamente; MUBE2=79,49% e 92,31%, respectivamente). Embora ambas as formulações tenham demonstrado redução do número de pápulas e pústulas ao final do tratamento (P<0,001) quando considerado diferença clinicamente significativa de 10%, o tratamento MUBE2 foi não inferior ao tratamento MUBE1 na contagem de pápulas. No caso de contagem de pústulas, o teste de não inferioridade foi não-significativo. **Discussão:** A associação MUBE2 foi superior à MUBE1 em relação à redução de pápulas, e não superior em relação à redução de pústulas. O uso de corticóide não apresentou superioridade na melhora do quadro de pústulas. **Auxílio Financeiro:** Glenmark Farmacêutica Ltda.

11.030

Toxicological reproductive study of *Aloe ferox* Miller on the pre-implantation and organogenesis in wistar rats. Maranhão HML, Leite VR, Vasconcelos CFB, Costa IMA, Lafayette SSL, Wanderley AG UFPE - Fisiologia e Farmacologia

Introduction: *Aloe ferox* (Af) is considered by the ethnomedicine as laxative and healing. Those activities have been well investigated, however, there are no studies that describe the security of its use during the period of pregnancy. **Methods:** The Af resin was supplied by Odaly Soares Laboratory, Ceará/Brazil. Wistar rats, with about 3 months of age and pregnant were randomly divided into 4 groups (n=9) and treated orally with water (Control, C) and Af 0.1 (T_1), 0.5 (T_2) and 2.5g/kg (T_3) during the pre-implantation and organogenesis periods (IPP- 1th to the 6th and OP – 7th to the 14th day of pregnancy, respectively). The gain in body mass mother was determined during the whole period of pregnancy and during the treatment. In the 20th day of pregnancy, the rats were sacrificed for evaluation of the reproductive indicators. The protocols were approved (n°007267/2009-96) by Ethics Committee of the UFPE. Statistical analysis: ANOVA + Tukey's test. **Results: (IPP)** - Body mass gain during the pregnancy (C=90.2±4.3; T_1=83.8±7.5; T_2=94.6±4.5; and T_3=87.9±6.7g) and in IPP (C=8.1±2.1; T_1=3.8±1.5; T_2=2.3±1.6 and T_3=3.1±2.0g). Number of live fetuses (C=101, T_1=90 and T_2=T_3=98) and dead (C=T_1=T_2=T_3=0), offspring/dam relationship (C=11±0.2; T_1=10±1.2; T_2=12±0.5 and T_3=11±1.0g), ovary mass (C=12.6±1.1; T_1=16.5±0.9; T_2=13.3±0.9 and T_3=14.5±1.6mg/100g), number of implantation sites (C=109; T_1=96 and T_2=T_3=102), resorptions sites (C=2, T_1=18, T_2=3 and T_3=12), viable implantations sites (C=107; T_1=78; T_2=99 and T_3=90), number of corpora lutea (C=12,3±0,4; T_1=12,7±1,3; T_2=13,1±0,6 and T_3=12,7±0,5) and mass of the placenta (C=0.49±0.01; T_1=0.48±0.01; T_2=0.48±0.01 and T_3=0.42±0.01*g). Mass of fetuses (C=2.47±0.02; T_1=2.43±0.06; T_2=2.40±0.02 and T_3=1.98±0.07*g), rate of implantation (C=T_2=100; T_1=92.9; and T_3=93.8%) and losses pre-implantation (C=8.3; T_1=16.7, T_2=7.4 and T_3=7.7%) and post-implantation (C=8.3; T_1= T_3 =0 and T_2=3.6%). **(OP)** - Body mass gain during the pregnancy (C=101.9±5.1; T_1=96.0±4.1; T_2=96.6±7.9; and T_3=81.7±4.8g) and in OP (C=23.9±1.6; T_1=21.6±2.2; T_2=18.6±3.3 and T_3=10.8±2.2*g). Number of live fetuses (C=97, T_1=101, T_2=90 and T_3=84) and dead (C=T_1=T_2=T_3=0), offspring/dam relationship (C=11±0.6; T_1=11±0.4; T_2=10±1.2 and T_3=11±0.7g), ovary mass (C=13.5±1.4; T_1=15.2±0.9; T_2=15.4±1.1 and T_3=12.7±1.4mg/100g), number of implantation sites (C=103; T_1=107, T_2=100 and T_3=94), resorptions sites (C=4, T_1=3, T_2=13 and T_3=1), viable implantations sites (C=99, T_1=104, T_2=87 and T_3=93), number of corpora lutea (C=11.7±0.8; T_1=12.2±0.3; T_2=12.6±0.7 and T_3=11.8±0.6) and mass of the placenta (C=0.413±0.003; T_1=0.407±0.006; T_2=0.447±0.009 and T_3=0.403±0.006g). Mass of fetuses (C=2.48±0.03; T_1=2.43±0.02; T_2=2.56±0.06 and T_3=2.38±0.02g), rate of implantation (C= T_1=T_2=T_3=100%) and losses pre-implantation (C=0; T_1=T_2=8.33 and T_3=4.2%) and post-implantation (C=T_1=0, T_2=6.7 and T_3=4.2%). **Conclusion**: The results show that there were not significant differences in the reproductive indicators. However, the largest dose (Af, 2.5g/Kg) that reduced the mass of the placentas and of the fetuses in the IPP and it decreased in the body mass gain of the mothers´ in the OP, suggesting a possible toxicity. Financial Support: CAPES

11.031

Randomized study comparing the effectiveness of two commercial loratadine plus pseudoephedrine preparations in patients with allergic rhinitis. Juliato EG[1], Vilugron HDN[2], Silva LACV[3], Ferreira T[4], Bortolassi L[5] - [1]LAL.Clínica- Pesquisa e Desenvolvimento Ltda.

Introduction: Allergic rhinitis is an inflammation of nasal membranes and is characterized by a complex of symptoms such as sneezing, nasal congestion, nasal itching and rhinorrhea. Other organs besides the nose may be involved as eyes, throat and ears. The inflammation of the mucous membranes is characterized by a complex interaction of inflammatory mediators, the most important immunoglobulin E (IgE) in response to an extrinsic protein stimulates the release of vasoactive substances such as histamine, which in turn activate various factors inflammatory, causing the disease. The treatment of rhinitis is based on the use of anti-histamines such as loratadine and pseudoepinefrine as a vasoconstritor. The objective was to compare the efficacy of both formulations by measurement of nasal flow and rhinorrhea. **Methods**: Clinical trial, randomized, double-blind and crossover. There were 23 patients (19 to 55 years) of both sexes, who signed the informed consent. We performed laboratory assay of IgE specific for mite (RAST), as one of the criteria for inclusion. The study was conducted in two periods, with washout of 7 days. In each period, there was an initial assessment (t 0) of the nasal mucosa. Then the patients were induced allergic process with a standardized solution of mite (*Dermatophagoides pteronyssinus*) in saline (1:10) sprayed in the nostrils. Fifteen minutes after induction, the drugs (T = test, R = reference) were administered. Evaluations of effectiveness occurred at 0, 0.25, 1, 2, 3, 4, 6, 8, 12 and 24 h. The assessment of nasal flow was performed by means of rhinomanometry and the parameters of rhinorrhea were classified in scores from 0 to 4 (absent to most intense). **Results**: The mean maximum inspiratory flow was 175.68% (t 8 am) and 194.02% (t 8.1 h), respectively. The mean maximum expiratory flow was 199.39 (t 8.26 h) and 209.89 (t 9.66 h) for R and T, respectively. The sum of scores of rhinorrhea was 0.25 in the t 17 and 13 to R and T, respectively. The most marked reduction of this parameter occurred in t 6h and was 1 for R and 1 for T. **Conclusion**: We have show that both drugs had the same clinical response in treatment of patients with allergic rhinitis following allergic sensitization. **Financial Support**: Ativus Pharmaceuticals Ltda.

11.032

Genotoxicity and citotoxicity of selenium compounds in human leucocytes *in vitro.* Meinerz DF[1], Rocha JBT[2], Pereira RP[1], Sudati JH[3], Santos DB[1], Barbosa NBV[1] UFSM - Química

Objective: Organoselenium compounds can exhibit important antioxidant and, consequently, they can exhibit potential pharmacological activities. However, at high levels of exposure, these compounds can be highly toxic to mammals. Here we have evaluated the potential genotoxic and cytotoxic effects of organoselenium compounds in human leucocytes *in vitro.* **Methods**: Heparinized venous blood was obtained from healthy volunteer donors from the Hospital of Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil (age 30±12) (n=4). The protocol of study was reviewed and approved by the appropriate institutional review board from Guidelines of the Committee of UFSM (0089.0.243.000-07). Leucocytes were isolated and incubated with organoselenium compounds (10, 40 or 100 mM): (*S*)-*tert*-butyl 1-diselenide-3-methylbutan-2-ylcarbamate **(1)** , (*S*)-*tert*-butyl 1-diselenide-3-phenylpropan-2-ylcarbamate **(2)**, (S)-2-amino-1-diselenide-3-methylpropanyl **(3)**, (S)-2-amino-1-diselenide-3-phenylpropanyl **(4)** or DMSO (vehicle) for 3h at 37°C (pre-incubation). Cell viability was determined with the Trypan blue exclusion test (cytotoxicity assay). DNA damage was determined by the Comet Assay (genotoxicity assay). One hundred randomly selected cells per sample were scored visually into five classes (from undamaged, 0, to maximum damaged, 4), according to tail intensity. The damage index (DI) was based on the length of migration and on the amount of DNA in the tail. **Results and discussion**: All compounds induced DNA damage that was not concentration-dependent (statistical analysis by One-way ANOVA followed by Duncan's multiple range test). Compound **(1)** and **(4)** were genotoxic to human leukocytes at 40 (DI=85.7±7.2 and 291.7±26.1, respectively) and 100 µM (340.7±29.3 and 384.2±14.9). Compound **(2)** and **(3)** were genotoxic at 10 (DI= 89.0±6.4 and 241.5±19.5) , 40 (344±23.9 and 365.7±14.5) and 100 µM (390.2±17.6 and 384.5±21.3) when compared with the control value (34.2±2.4). Compounds **(1)** and **(2)** decreased cell viability significantly at 10 (about 24 and 22%, respectively) and 40 µM (36 and 34%, respectively); whereas compounds **(3)** and **(4)** had no significant effects on cell viability (about 11%) when compared to control values (9%). These data indicate that organoselenium compounds can be cytotoxic and genotoxic to human leukocytes *in vitro*; however, these endpoints of cellular toxicity were not necessarily connected. These effects may be associated with the pro-oxidant activity exhibited by high concentrations of selenium compounds. However, additional studies are needed to elucidate the mechanism(s) by which the compounds display these toxic effects. **Supported by** CNPq, CAPES, FINEP, Fundação VITAE.

11.033

Genotoxicity and mutagenicity of a new telluroamino acid derivative of aspartic acid in mice after chronic exposure. Meinerz DF, Rocha JBT, Pereira RP, Sudati JH, Soares LC, Alberto EE, Braga AL, Santos DB, Barbosa NBV UFSM - Química

Introduction: Recently we have observed that a series of new telluroamino acid derivatives have remarkable Gluthathione Peroxidase- (GPx)-like activity, indicating their antioxidant potential. However, there is no study about their toxicity after *in vivo* exposure. Here we have investigated the mutagenic and genotoxic potential of a new telluroamino acid derivative of aspartic acid (TAAD), (S)-dimethyl 2-(3-phenyltellanyl) propanamido) succinate, which we have previously shown to exhibit the highest GPx-like activity from about 20 related derivatives (Braga et al., Org. Biomol. Chem.; 7:43; 2009). **Methods:** Swiss adult male mice weighing 40-60 g were used accordingly to guidelines of the Committee on Care and use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil (protocol- 0089.0.243.000-07). Mice were randomly divided in two experimental groups with 8 animals per group: (1) control group (which received DMSO s.c. for 21 days), and (2) treated group (which received daily 195 µmol/kg of the TAAD, s.c. for 21 days). One day after the last injection, mice were anesthetized to obtain total blood samples and were then euthanized. Peripheral blood samples from the sub-orbital sinus were collected to evaluate mutagenic effect using the micronucleous test and for evaluation of primary DNA lesions using the comet assay. Micronuclei presence was determined by three investigators that were blind to the treatment of the animals. For comet assay one hundred randomly selected cells per sample were scored visually according to tail intensity into five classes (from undamaged, 0, to maximum damaged, 4). The damage index (DI) was based on the length of migration and on the amount of DNA in the tail. **Results and discussion:** Statistical analysis by Man-Whitney U Test indicated that exposure to TAAD for 3 weeks caused a significant increase in the number of micronuclei in male mice ($p<0.003$)**.** Similarly, statistical analysis indicated that treatment of mice for three-weeks with TAAD caused a significant increase in the index of DNA damage in leucocytes ($p<0.01$)**.** In fact, treatment with TAAD caused a decrease in the frequency of damage 0 and increased the frequency of damage 3 and 4, when compared to control group. These results indicated that the compound tested was mutagenic in adult male mice (as judged by the increase in the frequency of micronuclei) and caused a significant genotoxic effects in mice leucocytes (DNA damage). These effects may be associated with the pro-oxidant activity exhibited by organotellurium compounds. However, additional studies are needed to elucidate the mechanism(s) by which this compound displays these toxic effects and, particularly, whether or not cellular thiol depletion occurs before the mutagenicity and/or genotoxicity of this telluroamino acid derivative. **Supported by** CNPq, CAPES, FINEP, Fundação VITAE.

11.034

Avaliação da citotoxicidade do *Ocimum gratissimum* (Og) e de seu constituinte, o ácido rosmarínico, em cultura de esplenócitos e medula óssea de camundongos Balb/c normais.

Figueirêdo CAV, Costa RS, Alcântara-Neves NM, Lima ATC, Velozo ES ICS-UFBA - Imunologia

Introdução: A ausência de droga eficaz e com reduzido efeito colateral para o tratamento da asma evidencia a necessidade do desenvolvimento de alternativas terapêuticas para essa patologia. Nesse sentido, inquérito popular realizado por nosso grupo na cidade de Salvador apontou o *Ocimum gratissimum L*. (Og), popularmente conhecido como manjericão-cheiroso, como uma das espécies mais utilizadas para o tratamento de asma e outras alergias respiratórias. O Og é membro da família *Lamiaceae* que inclui espécies já descritas por apresentar propriedade imunomoduladora atribuída ao fitoquímico polifenólico ácido rosmarínico (RosA). Estes dados nos estimularam a avaliar a imunomodulação do Og, sendo necessário a realização de ensaios preliminares avaliando a citotoxicidade a partir das frações hexânica (FHOg) e metanólica (FMOg), bem como do RosA. **Métodos:** Os extratos do Og foram preparados de acordo com técnica descrita previamente por Shetty et al. (2008). O ácido rosmarínico ((*R*)-*O*-(3,4-Dihydroxycinnamoyl)-3-(3,4- dihydroxyphenyl) lactic acid 3,4-Dihydroxycinnamic acid (R)-1-carboxy-2- (3,4-dihydroxyphenyl) ethyl Ester - $C_{18}H_{16}O_8$), foi obtido da Sigma-Aldrich. As amostras do RosA, da FHOg e da FMOg, nas concentrações variando de 1,95-1000microg/mL, foram testados em cultura de esplenócitos e de medula óssea de camundongos BALB/c normais (Protocolo experimental aprovado pela CEUA - Faculdade de Odontologia, UFBA, nº 0209). A avaliação da citotoxicidade foi realizada pelo método de redução do tetrazólio-MTT (Mosmann, J: J. Immunol. Meth., v.65; p.55, 1983), utilizando como controle negativo a cultura celular sem a adição de droga. Paralelamente foi realizado teste de exclusão com azul de Trypan. Todos ensaios foram realizados em duplicata. **Resultados:** As culturas estimuladas com diferentes concentrações da FHOg, e da FMOg bem como do RosA não apresentaram diferença estatisticamente significante quando comparado com o grupo controle negativo e portanto as drogas demonstraram não exercer atividade citotóxica, nas concentrações estudadas, sobre cultura de esplenócitos, tampouco na cultura de células da medula óssea. Estes resultados foram confirmados pelo método de exclusão com azul de Trypan. **Discussão:** Neste estudo foi observada a ausência de significativa atividade citotóxica *in vitro* do RosA e dos extratos hexânico e metanólico do Og tanto pelo método de MTT quanto pelo método de exclusão por Trypan demonstrando, preliminarmente, a segurança destes compostos. Tais resultados nos estimulam a investigar o potencial imunomodulador do Og e do RosA em modelo murino de alergia respiratória. **Apoio:** CAPES/CNPq

11.035

Plasmodium Berghei infection depresses liver monooxygenase activities and slows the clearance of diazepam and praziquantel in the mouse. De Carvalho RR, Hyssa JT, Gotardo MA, Oliveira ACAX de, Paumgartten FJR FIOCRUZ - Ciências Biológicas

Introduction. The effects of malaria infection on the expression and activity of drug metabolizing enzymes has been investigated at our laboratory. In previous published studies we have reported that *Plasmodium berghei* (ANKA) infection downmodulates the expression and activity of a number of cytochrome P450 isoforms in the mouse liver. In this study we evaluated the effects of *P.berghei* infection on the pharmacokinetics of diazepam (DZP) and praziquantel (PZQ) in the mouse. DZP is a widely used anxyolitic drug and a known substrate for CYP3A4 isoforms. PZQ, on the other hand, is an anthelmintic drug that is converted into its primary metabolite (PZQ-OH) by CYP2B (in mice, CYP2B9 and 2B10) and 3A isoforms. **Methods.** Female Swiss Webster mice **(**5-6 week old) were infected with *P.berghei* ANKA (10^6 parasitized red blood cells per mouse). Age-paired non-infected controls were injected with 0.2 ml of PBS solution alone. Control and infected mice (N= 5 mice per treatment group) were injected intraperitoneally with DZP (10 mg/kg body wt) or PZQ (200 mg/kg body wt), when parasitemia rose to levels > 25% pRBC (days 8-11 after infection). Blood was taken from the orbital sinus and drug plasma levels of DZP and PZQ were determined 15, 30, 60, 90 and 120 minutes, or 5, 15, 30, 60, 90 and 120 minutes after PZQ or DZP treatment, respectively. DZP and PZQ were determined by HPLC-UV after validation of the analytical methods at our laboratory. The study protocol was approved by the Ethical Committee on the Use of Animals of FIOCRUZ (CEUA, Nr P-0114-02). **Results.** Plasma levels (µg/mL) were as follows (Means ± SD): 1) DZP: Controls: 5min, 1.29±0.4, 15 min, 0.65±0.27, 30 min, 0.5±0.22, 60 min, 0.23±0.12, 90 min, 0.09±0.04, 120 min, 0.17±0.1; Infected mice: 5min, 3.4±2.0, 15 min, 2.47±0.58*, 30 min, 1.41±0.98*, 60 min, 0.41±0.35, 90 min, 0.2±0.29, 120 min, 0.21±0.24. 2) PZQ: Controls: 15 min, 28.1±11.4, 30 min, 43.8±9.9, 60 min, 24.9±2.7, 90 min, 6.3±3.3, 120 min, 3.5±3.0; Infected mice: 15 min, 56.5±19.6, 30 min, 35.9±12.9, 60 min, 78.8±24.7*, 90 min, 44.3±30.2*, 120 min, 60.0±25.9* (Infected levels different from those of respective controls are indicated by an asterisk, *P<0.05, Student's t test). **Discussion**. Results showed that plasma levels of DZP and PZQ in infected mice were higher than those in paired non-infected controls thereby indicating that malaria infection impaired the clearance of both drugs. Findings presented here are consistent with results from our previous studies demonstrating that *P.berghei* infection depressed activities and expression of CYP2B9, 2B10 and 3A11, CYP isoforms that mediate the conversion of PZQ and DZP into their primary metabolites. Financial support: CNPq.

11.036

Disposition of the enantiomers of mefloquine and its achiral metabolite carboxymefloquine in rats. Magalhães IRS, Aguiar FA, Fonseca P, Jabor VAP, Bonato PS FCFRP-USP - Física e Química

Introduction: Mefloquine (MQ) has been one of the most important drugs employed in the prophylaxis and treatment of malaria caused by multidrug-resistant strains of *Plasmodium falciparum*. Although there are controversies concerning differences in pharmacodynamic properties of these enantiomers, this drug has shown enantioselective disposition in humans. The main metabolite of MQ is carboxymefloquine (CMQ), which is achiral and lacks of antimalarial activity *in vitro*. Therefore, the aim of this study was to verify the disposition of the enantiomers of MQ and its achiral metabolite CMQ in rats. **Methods**: Aqueous suspension of racemic MQ hydrochloride was given orally to male Wistar rats (n = 3) in a single dose of 50 mg/kg and blood samples were collected over a period of 48 hours. The analytes were determined in plasma by a previously validated enantiospecific HPLC assay involving three-phase LPME as sample preparation technique and the experimental protocol was approved by the Ethics Committee on the Use of Animals (CEUA) of the University of São Paulo at Ribeirão Preto (process number 06.1.907.53.4). **Results and Discussion**: The peak plasma levels of (+)-(*RS*)-MQ (1391 ± 100.0 ng/mL) were significantly higher than those of (-)-(*SR*)-MQ (798 ± 151.0 ng/mL; Student´s *t* test, *p* value = 0.04). This result is consistent with a previously reported investigation concerning the enantioselective metabolism of MQ in rats, in which (−)-(*SR*)-MQ appeared to be preferentially metabolized *in vitro* [1]. On the other hand, peak plasma levels of the carboxy metabolite (494 ± 91.0 ng/mL) were lower than those commonly seen in human studies described in the literature, probably due to the formation of different metabolites in rats [2]. [1] Koch, M., *Arch. Pharm.*, 323, 749, 1990. [2] Fontaine, F., *Life Sci.*, 66, 2193, 2000. **Financial support**: CNPq, FAPESP

11.037

Postnatal development of the offspring of rats treated with meglumine antimoniate during pregnancy and lactation. Coelho DR, De Carvalho RR, Paumgartten FJR FIOCRUZ - Ciências Biológicas

Introduction. Meglumine antimoniate (MA), a pentavalent antimonial, was introduced in the market in the 1940s, and it is still the one of the first choice drugs for treatment of leishmaniasis. Since MA is an old drug for a neglected disease, gaps are found in its non-clinical safety data base (e.g., reproductive toxicity studies). In a previous study we found that Sb from MA is transferred to maternal milk and through it to suckling rat pups. In this study we investigated whether maternal treatment with MA during gestation and lactation impaired offspring growth and maturation. **Methods.** Wistar rats were treated with MA (0, 75, 150 and 300 mg Sb^{v}/kg body wt/day by sc route, N=6 per group) during pregnancy (from gestation day 1 onwards), parturition and lactation until weaning (postnatal day 21, PND21). The offspring of treated dams was evaluated regarding postnatal growth (body wt gain), somatic maturation (day on which developmental landmarks appeared) and neurobehavioural development (open field test on PND 25). The study protocol was approved by the Ethical Committee on the Use of Animals of FIOCRUZ (CEUA, L0016/08). **Results.** Dams treated with MA did not show body weight gain deficits or other signs of maternal toxicity. Duration of pregnancy, litter size and pup body weight at birth were not altered by maternal treatment with MA. Postnatal mortality and pup body weight gain in the offspring of mothers treated with MA did not differ from those recorded in the offspring of mothers treated with the vehicle only. Days of acquisition of landmarks of somatic maturation were as follows (Median, range - litter as the statistical unit of analysis): ear detachment, 0 (4, 2.5-4); 75 mg/kg (3, 2-4), 150 mg/kg (3.5, 3-4), 300 mg/kg (3, 2-3); fur, 0 (6, 6-7); 75 mg/kg (6, 6-6), 150 mg/kg (6,6-6), 300 mg/kg (6,6-6); incisors eruption, 0 (12, 11.5-13); 75 mg/kg (11, 10-12), 150 mg/kg (12,11.5-12), 300 mg/kg (11.5,10-12); eye opening, 0 (16, 15-17); 75 mg/kg (15.5, 14-17), 150 mg/kg (16,16-17), 300 mg/kg (15.75,15-17); preputial separation, 0 (38.25, 35-38.5); 75 mg/kg (37, 35-39), 150 mg/kg (39.25,37-41), 300 mg/kg (38.5, 36.5-42); vagina opening, 0 (35.5, 34-36); 75 mg/kg (34, 33-35), 150 mg/kg (36.5,34-39), 300 mg/kg (33.75, 31-36). No difference on the day of acquisition of somatic landmarks was found between control and MA-treated groups (Kruskal-Wallis test, $P>0.05$). Results of the open field test on PND25 were as follows (Mean±SD, litter as the statistical unit): Number of crossed squares, 0, 118±15; 75 mg/kg, 122±24; 150 mg/kg, 108±17; 300 mg/kg, 95,5±22,7 (ANOVA, $P>0.05$); rearing up, 0, 38.8±8.0; 75 mg/kg, 33.2±7.6; 150 mg/kg, 35±9; 300 mg/kg, 22.1±7.3* (ANOVA, Dunett's test, $*P<0.05$). **Discussion.** Results indicated that MA (up to 300mg Sb^{v}/kg body wt/day) given to rats during pregnancy and lactation did not produce maternal toxicity, did not increase pup mortality and did not impair postnatal growth and somatic maturation of their offspring. A reduction of number of rearing in the open field test on PND25, at the highest dose level, was the only adverse effect of MA noted in this study. Based on the foregoing findings, a study NOAEL (no-adverse-effect level) of 150 mgSb^{v}/mg body wt/day was set. **Financial support**: PAPES V; CNPq; FAPERJ.

11.038

Avaliação das interações medicamentosas em prescrições a pacientes hipertensos atendidos em Centro Médico da Região de Ribeirão Preto. Santos JC, Restini CA [1]UNAERP - Ciências Farmacêuticas, [2]USP - Física e Química

Introdução: A hipertensão arterial é uma disfunção crônica com alta prevalência entre a população brasileira e mundial. Há concretas estimativas de aumento significativo no número total de pessoas com essa doença para os próximos anos. Devido à característica assintomática durante o período inicial de sua instalação, cuja resposta terapêutica depende na maioria dos casos de politerapia, o tratamento medicamentoso da hipertensão propicia interações entre as drogas prescritas para este fim, bem como entre fármacos prescritos para outras doenças. Este evento resulta em sérios prejuízos aos pacientes, o que corrobora para o ciclo de interações. O objetivo deste trabalho é apresentar informações estatísticas sobre as interações medicamentosas presentes em prescrições médicas, no sentido de identificar os potenciais riscos e prejuízos à saúde dos pacientes atendidos em centro médico público. Paralelamente, traçar perspectivas sobre a conduta terapêutica desta doença com ênfase nos esquemas de tratamento disponíveis atualmente no serviço público de saúde do Estado de São Paulo. **Métodos:** Foram avaliados os prontuários e as prescrições médicas de 1073 pacientes atendidos na farmácia comunitária do Centro Médico Social Comunitário "Januário Theodoro de Souza", em Pradópolis-SP, entre fevereiro e março/2009. Deste total, foram inclusos na pesquisa 600 pacientes de ambos os sexos, os quais fazem uso de pelo menos um medicamento anti-hipertensivo. Após a coleta dos dados, foi analisada a literatura disponível e comparada com os resultados aqui obtidos. **Resultados:** Do total das prescrições analisadas, apenas 98 (16,3%) se basearam na monoterapia anti-hipertensiva. Ao todo, foram prescritos 1855 medicamentos, o que representa 3 medicamentos/paciente, não necessariamente fármacos hipotensores. Verificou-se a ocorrência de 1440 interações medicamentosas, sendo 563 (39,1%) para pacientes do grupo masculino e 877 (60,9%) do grupo feminino, com média de 2,4 interações/paciente. Das drogas não anti-hipertensivas, as mais prescritas foram: Ácido Acetilsalicílico e Dipirona, presentes em 24,5% e 14% das prescrições, respectivamente. **Discussão:** A maioria dos hipertensos é submetida à politerapia e estão freqüentemente expostos às interações medicamentosas, principalmente com AINEs, o que, dentre outros efeitos adversos, corroboram para o aumento da pressão arterial por interferirem em certos mecanismos, como os da fisiologia renal. Devido o número de sujeitos inclusos apresentar erro amostral reduzido (2%), juntamente com dados da literatura, o presente trabalho permite inferir que estes tipos de interações podem ser estendidas para outras populações e que geram prejuízos à terapêutica e à própria saúde dos pacientes, além de elevarem custos desnecessários à saúde pública. Para a melhora das perspectivas para os tratamentos farmacológicos envolvendo uso concomitante de vários medicamentos, destacam-se soluções simples como o entrosamento e trabalho conjunto entre os profissionais diretamente relacionados à terapêutica. Aprovação do Comitê de Ética em Pesquisa com Seres Humanos da UNAERP: 48/09. Todos os gastos para desenvolvimento desta pesquisa foram responsabilidade dos pesquisadores.

11.039

Influence of fludarabine on oral busulfan pharmacokinetics during conditioning therapy for hematopoietic stem cell transplantation. Castro FA, Lanchote VL, Voltarelli JC, Simões BP FMRP-USP - Clínica Médica, [2]FCFRP-USP

Bussulfan (BU) is an alkylating agent commonly administrated in combination with cyclophosphamide (CY) and/or fludarabine (FLU) on a pre-conditioning hematopoietic stem cell transplantation. BU has narrow therapeutic window and the recommended treatment (1mg/kg orally or 0.8 mg/kg iv, every 6 h for 4 days) is strictly related to serious adverse reactions, the decrease in survival for some patients and not reaches the desired effect. The BU is metabolized by conjugation with glutathione spontaneously or mediated by glutathione-S-transferase (GST). When BU is associated with CY, there is an enhance of immunosuppressor effects, probably related to GST expression, an enzyme involved in metabolism of two drugs, making the BUCY protocol even more toxic. The BU associated with FLU (30 mg/kg via iv) is a relatively new protocol and showed to be potentially less toxic then BUCY protocol. The pharmacokinetics of BU when combined with FLU is poorly known, especially when the association is made with oral BU. This study investigated the pharmacokinetics of oral BU when combined with FLU and CY. The study was approved by the Research Ethics Committee of the Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto(Case No. 8797/2006). Twenty-six patients with onco-haematological diseases presenting age on average of 32 years (range, 15-57 years) were included. Two different protocols were used, BU associated with CY and BU associated with FLU (BUCY and FLUBU). Eleven patients were included in FLUBU protocol and fifteen on BUCY. Serial blood samples were collected from 0-6h, during the 14th dose. The plasma samples were analyzed by HPLC-UV system in a reverse-phase column (C18). Pharmacokinetic analysis was performed using the WinNonlin 4.1 software. The steady-state concentrations (Css) obtained were associated with clinical response and BU pharmacokinetics was compared between two groups. Statistically significant variation between groups in BU concentrations was observed during therapeutic conditioning on both protocols. The Css of BU in FLUBU group was higher (1314.1 vs 954.8 ng/mL) than in BUCY group ($p \leq 0.05$, Mann-Whitney test). The clearance of BU was lower ($p \leq 0.05$, Mann-Whitney test) in FLUBU group when compared to BUCY (110.05 vs 157.4 L/h/Kg). The pharmacokinetic data suggest that BU metabolism is inhibited by FLU. Geddes, M. Biol. Blood Marrow Transplant**.** 14:220-228, 2008; Nath, E.C. Br. J. of Clin. Pharmacol. 66: 50-59, 2008; Shaw, P.J. Bone Marrow Transplant**.** 34: 197-205, 2004; McCUNE, S.J. Clin Pharmacokinet**.**39:155-165, 2000; **Financial Support:** CAPES

11.040

Disposition of ß-artemether and its main metabolite dihydroartemisinin in rats. Magalhães IRS, Vercesi JA, Aguiar FA, Jabor VAP, Bonato OS FCFRP-USP - Física e Química

Introduction: b-Artemether (ATM), a semi-synthetic drug originated from the naturally found agent artemisinin, has been a novel and efficient option in the treatment of malaria in the last years. In humans, ATM is promptly converted to the main metabolite dihydroartemisinin (DHA), which is intrinsically more active as an antimalarial agent than the parent drug. Thus, the goal of this research was to investigate the disposition of the ATM and its main metabolite DHA in rats. **Methods**: Male Wistar rats (n = 3) received a single oral dose of ATM (120 mg/kg) dissolved in soybean oil and blood samples were collected over a period of 8 hours. The analytes were assayed in plasma by a previously validated liquid chromatographic tandem mass spectrometric method involving three-phase LPME as sample preparation technique and the experimental protocol was approved by the Ethics Committee on the Use of Animals (CEUA) of the University of São Paulo at Ribeirão Preto (process number 06.1.907.53.4). **Results and Discussion**: The analytes attained maximum plasma concentrations within 1 hour after oral administration and were rapidly eliminated from this compartment. Furthermore, the peak plasma levels of DHA (213.1 ± 55.6 ng/mL) were significantly higher than those of its precursor (104.5 ± 31.0 ng/mL; Student´s *t* test, *p* value = 0.03). These results are in good agreement with the hypothesis that ATM undergoes extensive first-pass metabolism in rats, suggesting that this compound acts as a prodrug of DHA [1]. [1] Maggs, J. L., *Drug Metab. Dispos.*, 28, 209, 2000. Financial support: CNPq, FAPESP

11.041

Development and validation of bioanalytical HPLC method to the determination of ipriflavone in rat plasma. Albuquerque K[1], Almeida TM[2], Sales GS[2], Mosqueira VCF[3], Kano EK[3], Souza J[3], Grabe-Guimarães A[3] [1]CIPHARMA-UFOP, [2]EFAR-UFOP, [3]UFOP - Farmácia

Introduction: Ipriflavone, an isoflavone synthesized from daidzein (soy isoflavone) is commercialized as a supplement, without medical prescription in many countries. Although different methods have been described to the determination of its concentration in plasma, it is necessary to add confidence in pharmacokinetics studies, an easier, faster and inexpensive method. National regulatory issues describe parameters necessary to assure that the method is exact and precise. In order to approve the method, it is important to evaluate specificity, limit of quantification (LOQ), linearity, recovery and stability. **Objective:** To develop and validate a bioanalytical method to quantify ipriflavone in rat plasma using a high performance liquid chromatography (HPLC) system. **Methods:** The specificity of the method was evaluated through comparison of chromatograms from different drug-free rat plasma with standard solution. Linearity was evaluated by means of analyses of 10 different concentrations of ipriflavone in rat plasma, with 6 repetitions. Precision, accuracy, stability and recovery were evaluated using 3 different concentrations of ipriflavone, with 3 repetitions. To prepare plasma samples, different concentrations of ipriflavone (standard solution) were added to tubes containing 250 µl of rat plasma and methylparaben solution (internal standard, IS). The samples were vortexed for 30 s. After that, plasma proteins were precipitated with acetonitrile followed by filtration and evaporation at 25 °C under vacuum. Samples were reconstituted in mobile phase and injected onto HPLC column. Analyses were performed using a C18 column (150 x 4.6mm, 5µm) and detection wavelength at 254 nm. A linear gradient elution was used and the mobile phase constituted by two solutions: phase A containing water with 1% of acetic acid and phase B containing methanol. The flow rate was 1ml/min and column temperature was maintained at 40°C. All procedures used were in accordance with the guidelines of the Ethical Committee of UFOP (licence n. 2009/11). **Results**: Ipriflavone and IS peaks had a retention time of 8.9 min and 4.5 min, respectively. The LOQ was 40 ng/ml, and linearity was observed within the range of 40 ng/ml - 1000 ng/ml (R^2=99,54%). Precision and accuracy of this method was 7,16% and 99,98%, respectivelly. Recovery of ipriflavone and IS was around 80%. Standard solutions were stable at room temperature for at least 24 hours. **Conclusion:** The method tested displayed good recovery, precision and accuracy. The validation parameters evaluated characterize a reliable and reproducible HPLC method, with the advantages of being easy to execute and fast, which is adequate to pharmacokinetic studies of ipriflavone in rats. **Financial Suport***:* UFOP, FAPEMIG/REDE TOXIFAR, CAPES.

11.042

ENOs haplotypes affect the responsiveness to antihypertensive therapy in preeclampsia. Izidoro-Toledo TC[1], Sandrim VC[2], Palei ACT[3], Luizon MR[1], Cavalli RC[4], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]Santa Casa de Belo Horizonte, [3]FCM-UNICAMP - Farmacologia, [4]FMRP-USP - Ginecologia e Obstetrícia

Introduction: Genetic variants in eNOS gene have been associated with Preeclampsia (PE) and gestational hypertension (GH) development. However, it is unknown whether these polymorphisms are modulators to antihypertensive therapy responsiveness in these pregnant. **Material and Methods**: To answer this question, we enrolled 304 hypertensive pregnant (152 GH and 152 PE) and stratified them according a responsiveness criterion, which included clinical and laboratories parameters. We compared the frequencies of three eNOS genetic polymorphisms (T-786C, Glu298Asp and b/a intron 4) in PE and GH, responsive or non-responsive to antihypertensive therapy. Genotype and haplotype frequencies were evaluated and genetic variants were evaluated by TaqMan assay ® or RFLP. This study was approved by the local Ethics Review Board (CEP HCRP n° 4682/2006). **Results**: No difference were found in genotype or allele distribution between GH responsive or non-responsive and PE responsive or non-responsive (all $P > 0.05$). Conversely, the overall distribution of haplotype frequencies between PE responsive and PE non-responsive was very significant ($P=0.0003$). Specifically, the “C-Glu-a” and “T-Asp-a” haplotypes were associated with responsiveness and non-responsiveness to antihypertensive therapy in preeclampsia pregnant ($P<0.0062$). Regarding GH group, none difference was observed between responsive and non-responsive ($P>0.05$). **Conclusion**: Our findings suggest that genetic variants of eNOS may modulate the antihypertensive therapy responsiveness in preeclampsia woman. **Supported by**: FAPESP and CNPq

11.043

Different circulating metalloproteinases profiles in migraine with and without aura. Oliveira AM[1], Speciali, JG[2], Dach, F[2], Marcaccini AM[3], Gonçalves FM[4], Gerlach RF[3], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]FMRP - Neurologia, [3]FORP-USP - Morfologia, [4]UNICAMP - Farmacologia,

Introduction: Migraine is complex and disabling neurovascular disorder with higher prevalence in women than in men [1]. Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteases that degrade many components of the extracellular matrix [2]. These proteases (especially MMP-2 and MMP-9) are involved in the disruption of the blood-brain-barrier (BBB) and in the influx of inflammatory cells into the central nervous system [3]. However, while there is no clear evidence for an altered BBB during migraine attacks [4], experimentally induced cortical spreading depression (CSD) was shown to disrupt the BBB via an MMP-9-dependent mechanism [5], thus suggesting that MMPs activation could open the BBB and thus be a relevant mechanism in migraine**. Methods:** We studied 120 women divided into three groups: 40 healthy women without migraine (control group), 40 women with migraine without aura (MWA group), and 40 women with migraine with aura (MA group). This study was approved by the Ethics Committee at Faculty of Medicine of Ribeirao Preto (n.6120/2007), University of Sao Paulo, Brazil. Venous blood samples were collected and plasma was obtained. To determine MMPs (2 and 9) and TIMPs (1 and 2) levels were assayed by gelatin zymography and enzyme-linked immunosorbent assay. **Results**: We found increased plasma pro-MMP-2 levels and pro-MMP-2/TIMP-2 ratios in both groups of women (MA and MWA) compared with those found in controls ($p<0.05$). No significant differences in plasma TIMP-2 concentrations were found among the study groups ($p>0.05$). We found higher plasma MMP-9 levels in MWA patients, but not in MA patients, compared with controls ($p<0.05$). We found higher plasma concentrations TIMP-1 in MA patients, compared with controls ($p<0.05$). These findings were associated with significantly higher MMP-9/TIMP-1 ratios in MWA patients compared with controls ($p<0.05$) and significantly lower MMP-9/TIMP-1 ratios in MA patients compared with controls and with MWA patients ($P<0.01$ and $P<0.001$, respectively). **Discussion:** It is possible that the increased MMP-2 levels and net MMP-2 activity reported here may be involved in the cardiovascular alterations leading to increased risk of major cardiovascular diseases in migraine patients. Moreover, our findings provide evidence for increased net MMP-9 activity in migraine patients without aura. Therefore, these differences in circulating MMPs profiles between MWA and MA patients may reflect pathophysiological differences between these conditions. However, others studies will be need for explanation the results this study. **Supported by:** CAPES, CNPQ and FAPESP. **References:** [1] Pietrobon D, *Nat. Rev. Neurosci.,* 4: 386, 2003. [2] Hu J, *Nat. Rev. Drug. Discov.,* 6: 480, 2007. 3] Rosenberg GA. *Glia.,* 39: 279, 2002. [4] Edvinsson L, *Cephalalgia.,* 22: 22, 2008. [5] Gursoy-Ozdemir Y, *JCI*., 113: 1447, 2004.

11.044

Endothelial dysfunction and imbalance of angiogenic factors in patients with metabolic syndrome. Gomes VA[1], Sandrim VC[2], Palei ACT[2], Gonçalves FM[3], Casella-Filho A[4], Chagas AC[4], Tanus-Santos JE[2] [1]FCM-UNICAMP, [2]FMRP-USP - Farmacologia, [3]UNICAMP - Farmacologia, [4]InCoR-FMUSP

The metabolic syndrome (MetS) denotes a clustering of cardiovascular risk factors. Angiogenesis is a highly complex process during physiological and pathological conditions, such as tumor growth, rheumatoid arthritis, ischemic heart disease, diabetic retinopathy, etc. NOS is a regulator of cardiovascular homeostasis, therefore the measurement of nitric oxide (NO) bioavailability is of great clinical interest. Endoglin and VEGF a pivotal role by coordinating interaction between endothelial cells, extracellular matrix and the surrounding cells and its signaling also interacts with the NO pathway. Thus, we decided to compare the nitrite plasma concentrations, VEGF and endoglin in MetS patients with healthy controls and if exist relationship between these markers and concentrations of matrix metalloproteinases (MMPs). The study protocol was approved by USP – CAPPesq Ethics Committee (number 547/05). We studied 15 healthy subjects and 14 MetS patients. We measured plasma nitrite concentrations using an ozone-based chemiluminescence assay. The plasma concentrations of soluble endogline, VEGF, MMP-9 and MMP-8 were measured using enzyme immunoassays. Results: We found lower plasma nitrite and soluble endoglin (all $P<0.05$) concentrations in MetS patients compared with healthy controls. No difference in VEGF concentrations were found (all $P>0.05$). However, we found negative correlation between endoglin and MMP-9 (Spearman´s $r = -0.386$; $P<0.05$); and positive correlation between VEGF and MMP-8 (Spearman´s $r = 0.456$; $P<0.05$). Conclusions: Patients with MetS have reduced plasma nitrite levels and soluble endoglin compared with healthy controls. These results suggest that beyond endothelial dysfunction the angiogenesis seems impaired in MetS. Supported by: CNPq