ABSTRACTS

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05. Pain and Nociception Pharmacology

05.001 Related mechanisms of C5a/C5aR during neuropathic pain. Quadros AU[1], Fonseca MMD[2], Ferreira MD[3], Sagar DR[4], Cunha FQ[2], Chapman V[4], Cunha TM[2] [1]FM-USP – Farmacologia, [2]FMRP-USP – Farmacologia, [3]FMRP-USP – Bioquímica e Imunologia, [4]University of Nottingham – School of Life Sciences

Introduction: Emerging data indicate that C5a and its receptor, C5aR, participate in acute and chronic pain, although the related mechanisms are largely unknown. The present study aimed to further address the peripheral and spinal mechanisms by which C5a/C5aR signalling mediates neuropathic pain development. **Methods:** Neuropathic pain was induced by PSNL (peripheral sciatic nerve ligation). Nociceptive behaviour was evaluated by von Frey filaments (mechanical), Hargreaves (heat) and acetone test (cold). Spinal WDR neurons response was evaluated by in vivo extracellular single cell electrophysiology. Molecular analyses were done by qRT-PCR, ELISA/Miliplex, FACS and Immunofluorescence. **Results:** Firstly, intrathecal (i.t.) injection of C5a recombinant reduced mechanical threshold in a dose-dependent manner from 1 up to 24 hours. Moreover, directly spinal administration of C5a recombinant promoted facilitation of WDR neurons response after paw mechanical stimulation with both noxious and mainly innocuous filaments. More important, the absence of C5aR in male and female mice submitted to PSNL resulted in less development and maintenance of mechanical, cold and heat nociceptive responses. Besides that, systemic and i.t. treatment with DF2593A, a C5aR antagonist, reduced mechanical and cold allodynia. Also, spinal administration of PMX-53, a C5aR antagonist, reduced the excitability and the mechanical evoked response of spinal WDR neurons to noxious and mainly innocuous filaments, as C5a recombinant. Likewise, sciatic nerve, DRG and spinal cord express C5aR in baseline conditions, decreasing in this order. As well, there is an increase in C5aR mRNA expression and in C5a protein released in sciatic nerve in the first hours after lesion, between 3 and 7 days in the DRG and after 10 and 14 days in spinal cord. This result is corroborated by the very similar time profile of CD45+ cells in these tissues. In both, sciatic nerve and DRG, the most present cells are neutrophils and macrophages and those are also the main cells of C5aR expression, showed by Immunofluorescence. These cells, active by C5a, are signaling to release of cytokines and chemokines. Indeed, in the absence of C5aR the release of these mediators is significantly reduced. **Conclusion:** Taken together, these results indicate that C5a/C5aR are clearly involved in both, genesis and maintenance of neuropathic pain, participating in response to polimodal stimulus. These effects seem to occur in peripheral and spinal sites, by among others, modulation in cytokines and chemokines release by neutrophils and macrophages in an ascending communication and sensitization of pain pathways. **Financial Support**: FAPESP All mice experiments followed the rules of IASP and Animal Ethics Committee from USP (120/2014); and rats experiments followed the Project License 40/3647 from ASPA, United Kingdom.

05.002 Antinociceptive effect of aripiprazole by PI3K/AKT/NO/cGMP/KATP pathway activation. Ferreira RCM, Pelaez JMN, Capettini LSA, Duarte IDG, Aguiar DC, Moreira FA, Romero TRL ICB-UFMG - Farmacologia

Introduction: Aripiprazole is an antipsychotic drug used to treat schizophrenia and bipolar disorder. Recently, it was evaluated its peripheral analgesic component, however, the mechanism involved in this effect is not fully established. Therefore, the aim of the study was to obtain pharmacological evidence for the involvement of nitric oxide system in the peripheral antinociceptive effect induced by aripiprazole. **Methods:** To induce hyperalgesia, mice paws were treated with intraplantar prostaglandin E_2 (PGE_2, 2 µg) injection. Nociceptive thresholds were measured using the mice paw pressure test. Nitrate/nitrite as indicative of NO production into the paw was made using Griess test. To analyze the expression of iNOS enzyme into the paw, it was used the western blot technique. All drugs were injected locally (20µl) into the right hind paw of Swiss male mice with n=4 animals per group for the pharmacological test and n=5 animals per group for NO_x^- dosage and western blot technique. **Results:** Aripiprazole induced antinociceptive effect in a dose-dependent manner (12, 25, 50 and 100 µg/paw). To analyse the PI3K/Akt/mTOR pathway in this process, it was used the inhibitors of PI3K, AS605240 (90 µg/paw) and mTOR kinase, Rapamycin (25 µg/paw). AS605240, but not Rapamycin, reverted the antinociceptive effect induced by Aripiprazole (100 µg/paw). To evaluate the participation of NO pathway, the antinociceptive effect was antagonized by the non-selective inhibitor of nitric oxide synthase, L-NOARG (12, 24 and 48 µg/paw). The same response was observed when injected L-NIL, a selective inhibitor of inducible nitric oxide synthase, iNOS (24 µg/paw), but not, when was given a selective inhibitor of neuronal nitric oxide synthase (nNOS), L-NPA (24 µg/paw). The injection of a selective guanylyl cyclase inhibitor, ODQ (50, 100 and 200 µg/paw), the ATP-sensitive K(+) channel blocker, Glibenclamide (40, 80 and 160 µg/paw) and the non-selective K(+) channel blocker, Tetraethylammonium (30, 60 and 120 µg/paw) were able to reverse the antinociceptive effect of aripiprazole. However, the injection of Ca(2+)-activated K(+) channel blockers Dequalinium (50 µg/paw) and Paxilline (20 µg/paw) did not reverse this effect. The administration of Zaprinast, a cGMP-specific phosphodiesterase type 5 (24 µg/paw), potentiated the antinociceptive effect induced by aripiprazole (25 µg/paw). By Griess test, the group PGE_2+Aripiprazole (64.30 $\pm$14.05) did not alter the levels of NO_x^- when compared to the control group PGE_2+Sal/Tween80 5% (57.35 $\pm$13.49), however, the group Ethanol 2%+Aripiprazole (90.50 $\pm$ 19.91) increased the levels of NO_x^- when compared to the control group PGE_2+Sal/Tween80 5% (57.35 $\pm$13.49). Using the western blot technique, it was observed that the group PGE_2+Aripiprazole had increased levels of iNOS when compared to the control group PGE_2+Sal/Tween80 5%. **Conclusion:** The results provide evidence that aripiprazole induces peripheral antinociceptive effects via PI3K/AKT/NO/cGMP/KATP pathway activation. **Financial support and acknowledgments:** CNPq (Nº 448283/2014-0), FAPEMIG and CAPES. Research approval by the Committee for Ethics in Animal Experimentation (CEUA, Brazil) under the protocol number 109/2011.

05.003 Arthritis-induced hyperalgesia in the TMJ of rats activates microglial cells from the trigeminal subnucleus caudalis. Bonfante R[1], Rocha-Neto LM[1], Abdalla HB[1], Macedo CG[2], Napimoga HN[2], Clemente-Napimoga JT[2] [1]FOP-UNICAMP – Ciências Fisiológicas, [2]São Leopoldo Mandic – Imunologia e Biologia Molecular
Introduction: This study assessed the expression and activity of microglial cells of the trigeminal subnucleus caudalis (Vc) in the development of persistent inflammatory hyperalgesia induced by arthritis in the temporomandibular joint (TMJ) of rats. **Methods:** Male Wistar rats were sensitized with a subcutaneous emulsion injection containing methylated bovine albumin (mBSA, 500µg), phosphate buffered saline (PBS) and complete/incomplete Freund's adjuvant. The emulsion was injected at different locations on the back of animals 7 and 14 days after the first immunization. 21 days after the initial immunization, arthritis was induced in the immunized animals by intra-articular injection (Challenge) of mBSA (10mg / TMJ / week) diluted in PBS through two protocols: Acute phase - Intra-articular challenge and after 24 hours sample collection; Persistent phase - Weekly intra-articular challenge for 3 weeks and sample collection after 7 and 14 days of the last challenge. After treatment, animals were terminally anesthetized and Vc was collected to analyze the release of fractalkine (FKN) and cathepsin S (CatS) by ELISA assay and the expression of the microglial marker CD11b / c, p38 MAPK, P2X7 receptor and the FKN receptor CX3CR1 by Western Blot method. **Results:** The persistent protocol induced significantly greater release of CatS on day 14 when compared to the control group (26.6%±5.35%; p=0.0093: ANOVA, Bonferroni's test). The release of FKN was significantly higher on days 7 and 14, compared to the control group (23.4%±4.21 and 45.6%±2.99%, respectively; p=0.0003: ANOVA, Bonferroni's test). P2X7 receptor expression was significantly higher on days 7 and 14, compared to the control group (23.36%±2.6% and 18.42%±2.77% p=0.0061: ANOVA, Bonferroni's test). It was not possible to observe a statistically significant difference between the treated and control groups for CD11b / c, p38 MAPK and the CX3CR1 receptor. For the acute protocol, it was possible to observe a significant reduction of FKN release 24 h after intra-articular challenge, compared to the control group (14,27%±2,90%; p=0.008: T test). **Conclusion:** Peripheral injuries result of albumin-induced arthritis in the TMJ can promote changes in microglia cells VC sustained by persistent release of Cathepsin S and Fractalkine associated with the increase of the expression of P2X7 receptor.
Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo #2014/09975-7 Ethics Comitee/UNICAMP: 3413-1

05.004 Diabetes inhibits Na+/K+/ATPase in the peripheral neurons of trigeminal system. Rocha-Neto LM[1], Furtado FF[1], Bonfante R[1], Abdalla HB[1], Macedo CG[2], Clemente-Napimoga JT[2,1] [1]Unicamp – Fisiologia, [2]Faculdade São Leopoldo Mandic – Fisiologia

Introduction: Diabetes in early phase in known to result a variety of painful conditions induced by a modification in the neuronal activation and transmission. Objectives:The aim of this study was to investigate the mechanism involved in the diabetes-induced hyponociception in the TMJ of rats. **Methods:** Wistar rats (± 150 g, n=4-6/group) were treated with an intraperitoneal injection of vehicle (normoglycemic – NG group) or Streptozotocin 75 mg/kg (diabetic – DB group). Diabetes-induced hypernociception was assessed by the animals' nociceptive behavior induced by an intra-articular injection of capsaicin 7, 14, 21, 28, 35 and 42 days after the diabetic induction. After behavioral assays, animals were euthanized and their trigeminal subnucleus caudalis were removed to analyze the release of protein level of the CD11b (microglial cells), p38MAPK and CX3CR1 (Fraktalkine receptor) by Western Blot analyses; and the release of Diacylglicerol (DAG), Na+/K+ATPase, Fraktalkine (FKN) and Catepsin S (CatS) by ELISA. **Results:** Early phase of diabetes induced hyponociception into TMJ of rats 7, 14, 21, 28, 35 and 42 days after the diabetic induction ($P<0.05$: Two-way ANOVA, Bonferroni's test). Western Blot analysis demonstrated no statistical difference in the expression of CD11b/c, p38MAPK and CX3CR1 among DB rats and NG rats ($P>0.05$: Two-way ANOVA, Bonferroni's test). ELISA analysis demonstrated no statistical difference in the release of FKN or CatS among DB rats and NG rats ($P>0.05$: Two-way ANOVA, Bonferroni's test). Diabetes significantly reduced the release of DAG and Na+/K+ATPase ($P>0.05$: Two-way ANOVA, Bonferroni's test). **Conclusion:** The results suggest that diabetes-induced hyponociception in the TMJ of rats is a result of a damage in the peripheral neurons of the trigeminal system mediated by the inhibition of the Na+/K+ATPase. Financial support: CNPq CEUA/UNICAMP: 3415-1

05.005 Role of TRPA1 receptor in the nociception induced by irritants compounds in mice. Oliveira JRJM[1], Norões MM[2], Gonçalves MC[3], Ferreira J[3], André E[1] [1]UFPR – Farmacologia, [2]UFRN – Biofísica e Farmacologia, [3]UFSC – Farmacologia
Introduction: Toluene, xylene and formalin are volatile organic compounds widely used in several commercial products and common environmental contaminants. In humans, their contact with the skin can elicit irritant contact dermatitis and pain. The mechanisms underlying these effects remain unknown. Many agonists of ion channel known as transient receptor potential ankyrin 1 (TRPA1), a molecular integrator for various noxious stimuli, are pungent compounds that causes irritation when in contact with the skin. In light of these similarities, we hypothesized that the topic instillation of toluene, xylene and formalin with mice skin could to induces nociceptive and edematogenic response by modulating TRPA1 channels. **Methods**: Using experimental models of nociception, male Swiss mice received by topic instillation 20µl of toluene (30% – 100%), xylene (30% – 100%), formalin (37%) or vehicle (acetone) in the right paw and the nociceptive response was observed. In other experiment, the nociceptive response was evaluated in mice that were previously treated with TRPA1 antagonist HC030031 (100 mg/kg, orally) or desensitized with resiniferatoxin (50 µg/kg, subcutaneously) prior to the chemical irritants challenge. In addition, the nociceptive response and edema were also evaluated in genetic deletion of TRPA1 mice. **Results**: We observed that the instillation of toluene and xylene, but not formalin, in the paw of mice elicits nociceptive responses (53.00±12.21 seconds; 48.00±4.91 seconds, respectively). However, all chemical compounds induced edematogenic responses (0.04±0.01 mm; 0.06±0.01 mm; 0.04±0.01 mm; respectively) in mice. The nociceptive response induced by toluene (35.00±3.47 seconds) and xylene (60.5±4.94 seconds) was inhibited significantly by the oral treatment of HC030031 (25.00±2.80 seconds; 9.10±0.85 seconds; respectively), while resiniferatoxin degeneration abolished it. The nociceptive response induced by toluene (17.40±2.30 seconds) and xylene (32.00±5.10 seconds) were also prevent in genetic deletion of TRPA1 mice (2.4±0.8 seconds; 0.3±0.1 seconds; respectively). Furthermore, the ear edema induced by formalin (0.04±0.01 mm), toluene (0.04±0.01 mm), and xylene (0.06±0.01 mm) were almost completely abolished in genetic deletion of TRPA1 mice (0.00±0.00 mm; 0.01±0.00 mm; 0.02±0.01 mm; respectively). **Conclusion**: The present findings suggest that TRPA1 could be involved in some of the symptoms of irritant contact dermatitis, such as pain and neurogenic inflammation. **Financial support**: This study was supported by Araucária Foundation and CNPq. **Animal research ethical committee**: The protocols were approved by Ethics Committee of UFPR (process number 698).

05.006 Interaction between hydrogen sulfide (H2S) and the different redox species of nitric oxide (NO) – Effects on nociception and inflammation in the rat temporomandibular joint (TMJ). Oliveira MF, Sandy MV, Teixeira SA, Costa SKP, Muscará MN ICB-USP – Farmacologia

Introduction: We have recently observed that the slow release H_2S donor GYY-4137 has both anti-nociceptive and anti-inflammatory effects on the rat temporomandibular joint (TMJ) synovitis induced by the intra-articular (i.art) injection of carrageenan, and these effects were blunted by the nitric oxide (NO) donor S-nitroso N-acetyl penicillamine (SNAP). Controversial results can be found in the literature regarding the effects of NO on pain and inflammation, although previous results from our laboratory demonstrated the antinociceptive effects of nNOS-derived NO (1). Based on these observations, we decided to study the interaction between H_2S and NO (at different redox status) on inflammation and nociception in the rat TMJ. **Methods**: Under anesthesia with inhalatory isofluorane (3% in O2), male Wistar rats (4-6 wk. old) received intra-articular (i.art.) injections of either SNAP or the nitroxyl (NO^-) donor Angeli's salt (AS; 1 nmol). Four hours later, mechanical allodynia was evaluated by measuring the force threshold necessary for head withdrawal with the aid of an electronic analgesimeter based on the Von Frey filaments principle. SNAP effects on mechanical allodynia were evaluated both in terms of dose- and time-dependent responses. AS (1 nmol) was also injected and the effects of H_2S were analysed by co-injecting NaHS (1 nmol). Myeloperoxidase (MPO) activity was measured in the TMJ capsule tissue as a marker of neutrophil infiltration. The results were analysed by ANOVA followed by the Dunnett's test or unpaired Student t-test. **Results:** In comparison with saline (control group), the intra-articular injection of SNAP into the rat TMJ (0.01-1 nmol) evoked mechanical allodynia in a dose-dependent manner (maximal response: 25.0±4.7 vs. 7.4±1.3 g, $P<0.001$), and the time-course curve obtained after the injection of 0.03 nmol evidenced a fast and long-lasting effect (16 h after injection: 30.0±5.7 vs. 19,5±9,0 g; $P<0.05$). No significant differences were observed between saline and SNAP in terms of MPO activity. The administration of 1 nmol NaHS alone neither causes any effect on mechanical allodynia nor affected that induced by 1 nmol NaHS (17.1±6.8 vs. 15.4±2.9 g). AS (1 nmol) also resulted in increased mechanical allodynia (13.3±3.2 vs. 4.2±3.5g; $P<0.001$); however, the intensity of this response was approximately 10-fold lower than that caused by the same dose of SNAP. **Conclusion:** These data suggest a pro-nociceptive effect of NO independent of leukocyte migration to the TMJ cavity. The administration of SNAP and NaHS did not change the effect caused only by SNAP-derived NO. Although the anionic form of this molecule (NO^-) also increased mechanical allodynia, this effect was lower than that caused by NO and the effects of H_2S on this response remain to be investigated. **Financial Support** and acknowledgments: FAPESP (grant 2016/18123-0), CNPq and CAPES. Research approval by the local Ethics Committee for Animal Experimentation (CEUA-ICB nº36/2017). References: 1. Tesser-Viscaíno et al. Brain Res, 2009; 1302:85.

05.007 N-Acylhydrazone derivative (LASSBio-1027) ameliorates hypernociception in acute and chronic pain murine model. Rezende B[1], Montes GC, Fraga CAM, Barreiro EJ, Zapata-Sudo G, Sudo RT ICB-UFRJ

Introduction: Pain is the main symptom reported by patients in rheumatologic medical care. Non-steroidal anti-inflammatory drugs are first-line drugs for treatment of pain, however, are associated with side effects. Activation of adenosine receptors have beneficial effects such as antinociceptive and anti-inflammatory actions. Molecular docking studies and binding assays demonstrated that 3,4-methylenedioxybenzoyl-2-thienylhydrazone (LASSBio-1027) is a ligand to both A_{2A} and A_3 receptors. The aims of this study was to investigate the beneficial effects of LASSBio-1027, in acute and chronic pain murine model. **Methods:** Protocols were approved by the Animal Care and Use Committee at Universidade Federal Rio de Janeiro (113/14). Formalin injection (20µL i.pl.) in male *Swiss* mice (25-30 g), was used to induce acute pain in order to promote the nociception in two typical phases. The antinociceptive effect was evaluated after oral gavage administration of vehicle (DMSO), LASSBio-1027 (25, 50 and 100 mg/kg), morphine (30 mg/kg) and acetyl salicylic acid (ASA, 300 mg/kg). Monoarthritis was used as a model of chronic pain, which was induced by subcutaneous injection of complete Freund adjuvant (CFA) around the tibio-tarsal joint in male *Swiss* mice under 2% sevoflurane anesthesia. After thermal and mechanical hyperalgesia and, also paw edema were observed, the animals were treated by oral gavage with vehicle (DMSO), LASSBio-1027 (25, 50 and 100 mg/kg), thalidomide (100 mg/kg) and ASA (300 mg/kg). Expression of TNF-α, iNOS, p-38 were evaluated using western blotting assays of paws and spinal cord of the animals with monoarthritis treated or not with the derivative. The tibiotarsal joint were stained with toluidine blue *O* to examine the histological alterations. **Results:** Reactivity of the animals after formalin injection was reduced from 39.7 ± 5.1 s to 18.8 ± 4.1 and 18.4 ± 1.6 s following treatment with LASSBio-1027 at doses of 50 mg/kg and 100 mg/kg, respectively. In the second phase, the time of liking/biting decreased from 218.8 ± 42.8 s (vehicle group) to 117.2 ± 17.9 s after treatment with 100 mg/kg of LASSBio-1027. Antinociceptive activity of LASSBio-1027 was reverted with pre-treatment of MRE 3008F20, an adenosine A_3 antagonist but not after pre-treatment with ZM 241385, an adenosine A_{2A} antagonist. Thermal and mechanical hyperalgesia and paw edema were reduced after treatment with LASSBio-1027 (100 mg/kg). TNF-α, iNOS and phosphorylated p-38 was increased in paws and spinal from animal with monoarthritis and was recovered after treatment with LASSBio-1027. Extensive inflammatory infiltrate and partial loss of proteoglycans were observed in the tibial and ankle joints from vehicle-treated mice which were reduced in animals treated with LASSBio-1027. **Conclusions:** Activation of adenosine receptor A_3 by LASSBio-1027 could produce antinociceptive effects in acute and chronic pain model indicating a new alternative for the treatment of pain. **Financial Support**: CNPq, Capes, FAPERJ, INCT-INOFAR. CEUA 113/14

05.008 Mechanisms involved in the antinociceptive and anti-inflammatory effects of [4",5"] dihydro-obovatin on a pre-clinical model of temporomandibular joint pain. Gomes FIF[1], Do Val DR[2], Santos RS[1], Arriaga AMC[3], Bezerra MM[1], Chaves HV[1]
[1]UFC – Farmacologia, [2]UFPE – Biotecnologia, [3]UFC – Química Orgânica
Introduction: Temporomandibular joint (TMJ) disorders are clinical conditions that cause pain in the TMJ region. We designed and patented [4",5"] dihydro-obovatin (BR1020130287938) based on its naturally occurring counterpart, which possesses antinociceptive effects. A growing body of evidence suggests that the heme oxygenase-1 (HO-1) and NO/cGMP/PKG/ATP-dependent potassium channels pathways are involved in peripheral antinociception mediated by drugs and natural products. Additionally, inflammatory stimuli trigger the release of characteristic cytokines, which ultimately promotes the release of final mediators responsible for inflammatory pain. Here, we aim to determine whether the [4",5"] dihydro-obovatin mechanism of action depends on the HO-1 and NO/cGMP/PKG/K+ATP pathways as well as on cytokine, ICAM-1, and CD55 expression. **Methods:** Male Wistar rats (160–220 g) received [4",5"] dihydro-obovatin (0.1, 1.0, or 10 µg/kg; v.o.) 60 min before formalin injection (1.5%; i.art). Sham groups and positive controls received saline solution (i.art.) and indomethacin (5mg/kg; v.o.), respectively. The ensuing nociceptive response was quantified for 45 min. To investigate the involvement of the HO-1 and NO/GMPc/PKG/K+ATP pathways, animals were pre-treated (s.c.) 30 min before formalin injection with the respective inhibitors: ZnPP IX (3 mg/kg), aminoguanidine (30 mg/kg), ODQ (5 mg/kg), KT5823 (4 µg/mL), and glibenclamide (10 mg/kg). [4 ", 5"] dihydro-obovatin (10 µg/kg; v.o.) was then administered and the nociceptive behaviour assessed as previously mentioned. Periarticular tissues were excised for quantification of TNF-α, IL-1β, IL-8, IL-10 levels (ELISA) and ICAM-1 and CD55 (Western blot). **Results:** [4",5"] dihydro-obovatin antinociceptive effects did not depend on the HO-1 integrity nor on the cGMP/PKG activation. It, however, occurred via NO and ATP-dependent potassium channels. It reduced TNF-α, IL-1β, IL-8 levels, promoted the rise of IL-10 levels, and reduced ICAM-1/CD55 expression in the periarticular tissues. **Conclusion:** [4",5"] dihydro-obovatin reduced nociceptive and inflammatory parameters in a rodent model of TMJ pain, being an interesting novel therapeutic in the field of orofacial pain research to be further investigated. **Financial Support:** This work was supported by Brazilian grants from Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Instituto de Biomedicina do Semiárido Brasileiro (INCT-IBSAB). **Research Approval by the Local Ethical Committee:** number 13/15

05.009 Beneficial effect of lodenafil and sildenafil in reducing neuropathic pain signs in rats submitted to spinal nerve ligation. Silva CFB, Montes GC, Zapata-Sudo G, Sudo RT ICB-UFRJ – Pesquisa em Desenvolvimento de Fármacos

Introduction: Treatment of neuropathic pain is still an unsolved problem in medicine. Currently available drugs partially reduce symptoms of neuropathic pain in about 50% of patients with several limitations due to side effects. Lee et al. (2014) demonstrated antinociceptive effect caused by intrathecal injection of of sildenafil, an inhibitor of enzyme phosphodiesterase 5 (PDE-5). The aim of this study was to investigate the beneficial effects of carbonate of lodenafil (PDE-5 inhibitor) a compound designed by association of two sildenafil molecules in spinal nerve ligation-induced thermal and mechanical hyperalgesia in rats. **Methods:** Protocols were approved by the Animal Care and Use Committee at Universidade Federal Rio de Janeiro (DFBC 40/16). Male Wistar Rats (220 -280 g) were used to evaluate the thermal (Ugo Basile 37370, Italy) and mechanical (Insight EFF 301, Brazil) stimulations response. The animals were randomly divided in 4 groups (n= 6 per group), in which 3 groups were submitted to L5 spinal nerve ligation (SNL) surgery and the remaining 1 group submitted to SHAM surgery, under ketamine/xylazine anesthesia. The thermal and mechanical responses were measured in the control (before SNL), 7 d after surgery and then, 3, 7, 10 and 14 d after daily treatment (oral route by gavage) with DMSO (vehicle), sildenafil (30 µmol/kg) or lodenafil (30 µmol/kg). Data were expressed as mean ± SEM and the statistical analysis were performed by two-way ANOVA following by Bonferroni test (multiple comparisons) using GraphPad Prism® 6.0 software. **Results:** Thermal and mechanical hyperalgesia was reduced after SNL surgery. The latency for thermal stimulation of group vehicle decreased from 11.67 ± 0.1 to 9.6 ± 0.2 s ($p<0.05$), which was not changed upon treatment. For the sildenafil group SNL reduced thermal latency from 11.7 ± 0.1 to 9.2 ± 0.1 s ($p<0.05$). The latency after 7 d treatment increased to 11.1 ± 0.2 s ($p<0.05$) with no additional changes during treatment. For Lodenafil group thermal latency decreased from 11.5 ± 0.2 s to 8.9 ± 0.2 s ($p<0.05$); 7 d after treatment enhanced to 11.1 ± 0.2 s ($p<0.05$) with no additional changes during treatment. The threshold mechanical allodynia of the group vehicle decreased from 39.73 ± 0.2 g to 27.82 ± 0.7 g ($p<0.05$) which was not changed upon treatment. Sildenafil group decreased from 39.6 ± 0.2 g to 27.8 ± 0.8 g ($p<0.05$), 7 d after of treatment enhanced to 36.5 ± 0.3 g ($p<0.05$) with no additional changes during treatment. Lodenafil group decreased from 39.7 ± 0.2 g to 27.8 ± 0.8 g ($p<0.05$), 7 d after of treatment increased to 33.8 ± 0.5 g ($p<0.05$) with no additional changes during treatment. SHAM group did not show significant changes to thermal hyperalgesia and mechanical allodynia evaluations. **Conclusions:** The data presented in this study suggest that lodenafil, a substance with PDE-5 inhibitor property and formed by linkage of two molecules of sildenafil, may be recommended to revert signs and symptoms of neuropathic pain. **Reference.** Lee, HG et al., Neurosci Lett., 480, 182-5, 2010. **Financial Support.** CNPq; CAPES; FAPERJ; INCT/INOFAR **CEUA:** DFBC 40/16

05.010 Angiotensin II Type 2 receptor activation is involved in an acute gouty attack in rodents. Vieira TN[1], Ferreira J[2], Silva CR[1] [1]UFU – Genética e Bioquímica, [2]UFSC – Farmacologia

Introduction: Individuals with gout arthritis frequently experience a range of comorbidities where hypertension has been one of the most common, affecting 60-80% of gouty patients. Clinical studies previously demonstrated that angiotensin I-converting enzyme inhibitors (ACEi) increase the risk of acute gouty attack and, recently, Silva CR and coworkers demonstrated that it was mediated in part by kinin B_1 receptor stimulation. However, ACEi can also modulate the renin-angiotensin system resulting in Angiotensin II type 2 Receptor (AT2R) activation and it could be the accessory mechanism targeting the inflammatory responses induced by MSU. The AT2R are expressed and co-localized with the TRPV1 receptors in rodents and human sensory neurons and AT2R inhibitors were shown to reduce pain signaling in animal models in vivo and in rodent and human sensory neurons in vitro. However, there are just a few studies investigating the effects of AT2R on acute pain, and no one in gouty arthritis. Thus, the aim of this study is assess the contribution of angiotensin AT2 receptors activation on the nociceptive and inflammatory responses induced by intra-articular injection of MSU in mice. **Methods:** Adult male Wild type C57BL/6 mice (20-25 g) were used and all procedures were approved by our Institutional Ethics Committee (process number 080/16). Firstly, MSU crystals (0.01-100 µg/articulation) or vehicle (PBS), were injected intra-articular (IA) into the medial side of the left tibio-tarsal joint (ankle) of mice. Another group of animals were treated with the AT2R antagonist, PD123319 (10 mg/kg v.o.), administered 0.5 hours before IA MSU (30 µg/articulation) injection. In addition, a third group of animals were IA injected with the AT2R agonist (10-100 µg/articulation). All groups were analyzed for mechanical allodynia, thermal hyperalgesia and overt pain-like behaviors. **Results:** MSU (10, 30 and 100 µg/articulation) was able to induce mechanical allodynia (2-72 hours after IA injection) and overt pain-like behaviors (1-24 hours after IA injection). Lower doses (0.01 µg/articulation) were not able to induce mechanical allodynia or overt pain-like behaviors; and any MSU concentration was able to induce thermal hyperalgesia. At the tested concentration, oral PD123319 treatment was active in prevent mechanical allodynia (44% of prevention) and overt pain-like behaviors (56% of prevention) from 1 up to 6 h after IA MSU injection. Additionally, animals treated with the AT2R agonist (30 ug/ articulation) developed overt pain-like behaviors, from 1 up to 6 h after AT2R agonist IA injections. Conclusions: Our findings suggest that AT2R activation is involved on pain resulting from gouty acute attack development in rodents. However, more studies need to be done to confirm and clarify this event, including the investigation of the role of AT2R on acute gouty attacks precipitated by ACEi. Research support: This study was supported by Conselho Nacional de Desenvolvimento Científico (CNPq).

05.011 Structural analogue of eugenol exhibits antinociceptive activity in mice. Aragão Neto HC, Fonsêca DV, Braga RM, Almeida RN UFPB – Psicofarmacologia
Introduction: Eugenol is a phenylpropanoid present in many medicinal plants. It acts in the inflammatory process and reduce pain perception. It has been shown that some eugenol analogues have similar activity. Ortho-eugenol, a synthetic isomer of eugenol, has no reports in the scientific literature as to its ability to reduce pain. Based on the constant research for new drugs with therapeutic efficacy and fewer side effects, we were interested in studying the antinociceptive properties of ortho-eugenol. **Methods:** In the acetic acid-induced writhing test, Swiss male mice were divided into five groups (n=8) and pretreated with vehicle, ortho-eugenol (50, 75 and 100mg/kg, i.p.), or morphine (6 mg/kg, i.p.), and the substance's capacity to decrease the number of contortions was evaluated as indication of analgesic effect. In the glutamate test the same division occurred, except for the standard group, which was treated with MK-801 (0.03mg/kg, i.p.), and the paw licking time was evaluated as indication of nociception. **Results:** In the acetic acid-induced writhing test, ortho-eugenol reduced the number of writhes in a dose-dependent manner (4.438 ± 2.129; 1.875 ± 2.432; 1.429 ± 2.512), compared to the control group (18.43 ± 4.6). The intraperitoneal administration of acetic acid stimulates the release of several inflammatory mediators, suggesting that the tested substance might have reduced the pain perception by decreasing this release. In the glutamate test, only the ortho-eugenol experimental dose of 100 mg/kg (68.1 ± 12.8) was capable to reduce licking time when compared to control group (136.4 ± 21.2). Glutamate is the major excitatory neuro-transmitter involved in nociceptive transmission. The release of glutamate is increased due tissue damage, suggesting that the nociception reduction caused by ortho-eugenol might be associated to a possible interaction with the glutamatergic system. **Conclusion:** Based on these results, ortho-eugenol demonstrated characteristics of a drug with analgesic activity, and its mechanism of action most likely is related to reduction in the release of inflammatory mediators. Keywords: Ortho-eugenol, Isomer, Analgesic. References: Diogo Vilar da Fonsêca, International Immunopharmacology, Volume 38, Page 402, 2016. **Financial Support** and acknowledgements: CNPq and Federal University of Paraíba. All experimental procedures were previously approved by CEPA - the Ethics Committee for Animal Research UFPB - under the certificate in 0201/2013.

05.012 Evaluation of the toxicological activity and anotinociceptive potential of rose oxide in rodents. Leite LCTF, Piauilino CA, Lopes EM, Lima MPD, Aguiar LCT, Sousa DP, Almeida FRC UFPI – Farmacologia

Introduction: Pain is a response to stimuli with actual or potential tissue damage and represents an essential adaptive response of the organism. Rose oxide (RO) is a monoterpene present in several plant species and has a structure similar to other monoterpenes that have sedative, antinociceptive and antidepressant activities already described in the literature. The aim of this study was to evaluate the toxicological and antinociceptive activities of RO. **Methods:** During the acute toxicity evaluation the animals (female Swiss mice, 25-30 g) received the RO (2000mg/kg, p.o.) or vehicle (2% Tween 80 in saline 0.9%, p.o.) and were observed in a period of 14 days. The animals (n = 6-8) were treated with RO (6.25, 12.5, 25 or 50 mg/kg, p.o.)(capsaicin test) and with RO (12.5, 25 or 50 mg/kg, p.o.)(glutamate test), vehicle or morphine (5 mg/kg, s.c.), and after 30 or 60 minutes were subjected to the stimuli, capsaicin (2 µg/20 µL/paw) or glutamate (2 µmol/paw), injected into the right hind paw. Nociception was evaluated by quantifying the paw licking time after capsaicin (5 min) and glutamate (15 min). To investigate the possible mechanisms of action in the glutamate test, animals (n=6-8) were treated intraperitoneally with naloxone (2 mg/kg-NAL), L-arginine (600 mg/kg-L-ARG) or bicuculline (1 mg/kg-BIC) 20 or 15 min before RO administration (25 mg/kg). In the open field and rota rod tests, animals were treated with RO (50 mg/kg, p.o.), diazepam (4 mg/kg, i.p.) or vehicle to evaluate their motor performance. All protocols were approved by the Ethics Committee on the Use of Animals (ECUA /UFPI N°148/2016). Statistical analyzes were performed using one-way ANOVA followed by the Tukey test, $p < 0.05$. **Results and Discussion:** During the 14 days observation, there were no deaths or behavioral changes, so it was not possible to calculate the LD50, as well as, there were no significant differences between the groups, regarding body mass, body weight and biochemical parameters. In the assessment of antinociceptive activity, RO was able to reduce paw licking time at 12.5 (20.78 ± 11.67), 25 (24.52 ± 7.84) and 50 mg/kg (14.54 ± 7.34) in the capsaicin test when compared to vehicle (43.04 ± 8.96)($p<0.05$). In glutamate-test the doses 25 (64.94 ± 12.48) and 50 mg/kg (45.96 ± 12.48) reduced paw licking time when compared to the control group (111.70 ± 7.34). Pretreatment with NAL (139.79 ± 28.70) did not reverse the RO antinociception (43.43 ± 19.60, RO + NAL -52.67 ± 20.73), as well as L-ARG (97.03 ± 5.01, RO = 54.38 ± 10.50, L-ARG + RO -67.52 ± 4.14). However, BIC (73.34 ± 13.56) reversed the antinociception of the RO (RO 43.43 ± 9.67; RO + BIC - 90.60 ± 21.84)($p<0.05$). In the open field test, animals RO treated (72.80 ± 8.2) did not reduce the number of invasions when compared to the diazepam group (6.20 ± 2.53), being similar to the vehicle (62.57 ± 6.96). In the Rota-rod, the RO and vehicle animals did not alter the motor performance in the rotating bar, different from those treated with diazepam (14 ± 5.49). In conclusion, these results suggest an acute antinociceptive effect of rose oxide in mice, involving GABAergic system, however without altering the exploratory and locomotor capacity of the animals. **Financial support:** UFPI/ FAPEPI/CAPES

05.013 Further investigation of antinociceptive and pronociceptive mechanisms of *Acmella oleracea* in mice. Dallazen JL[1,2], Maria-Ferreira D[1], Luz BB[1], Nascimento AM[1], Cirpriani TR[1], Souza LM[3], Werner MF[1] [1]UFPR, [2]Farmacologia, [3]Instituto de Pesquisa Pelé Pequeno Príncipe

Introduction: *Acmella oleracea* is a flowering herb popularly known as jambu and very used in Amazon culinary and folk medicine to treat toothache, elicit unique sensations of pungency, tingling and numbness. Our previous studies have demonstrated that the intraplantar injection of hexanic fraction (HF) rich in alkylamides (spilanthol) from jambu flowers promotes both antinociceptive and pronociceptive effects at 0.1 and 30 µg/20µL, respectively, both mediated by TRP receptors (Dallazen et al., 48º SBFTE, 2016). Thus, our aim was to investigate additional mechanisms involved in the antinociceptive and pronociceptive effects of HF. **Methods:** Phytochemical analysis (GC–MS) of HF confirmed the presence of alkylamides, including spilanthol as majority. Male Swiss mice (CEUA/BIO-UFPR: 970) were intraplantally injected (20µL) with 0.1 µg or 30 µg of HF, and a synthetic isobutylalkenyl amide (IBA) was used as control at same doses. To investigate the role of opioid system, mice received naloxone (1 mg/kg, i.p.) or vehicle (saline 0.9%, i.p.), followed by morphine (1 mg/kg, s.c.), HF or IBA (0.1 µg/20µL, i.pl.) or vehicle (20µL) in the nociception induced by glutamate (20 µmol/20µL, i.pl.). In parallel, the same opioidergic treatments were employed to evaluate the HF and IBA (30 µg/20µL, i.pl.)-evoked licking and guardian behaviors. The involvement of histaminergic system was evaluated by pretreatment with ketotifen (5 mg/kg, i.p.) or HF (0.1 µg/20µL, i.pl.) in the nociception induced by compound 48/80 (C48/80, 10 µg/20µL, i.pl.). Similarly, mice were pretreated with ketotifen (5 mg/kg, i.p.) to evaluate if the histamine releasing was involved in the HF (30 µg/20µL, i.pl.)-induced nociceptive behaviors. At the end, the skins of hindpaws were excised to histologic mast cell degranulation analysis through toluidine blue staining (% of mast cell degranulated in 10 fields/section). **Results:** Morphine, HF and IBA (0.1 µg) reduced the nociception induced by glutamate (C: 170.5±11.8 s) in 72%, 67% and 74%, respectively, and naloxone reverted only the antinociception promoted by morphine. The licking behavior evoked by HF (C: 48.2±5.1 s) or IBA (53.8±4.8 s) at 30 µg were reduced by morphine in 82% and 91%, respectively, and naloxone reversed all nociceptive-evoked responses. However, the guardian behavior induced by HF 30 µg was unchanged by morphine (45.5±4.9 s). C48/80 evoked licking behavior that was reduced by both ketotifen and HF 0.1 µg in 43% and 77%, respectively (C: 73.6±6.9 s), whereas the guardian behavior was reduced in 75% and 96% by ketotifen and HF, respectively (C: 83.4±11.2 s). Both licking (60.2±5.4 s) and guardian (93.2±23.0 s) behaviors induced by HF 30 µg were reduced by ketotifen in 35% and 88%, respectively. Histological analysis revealed an intense mast cell degranulation induced by HF 30 µg (88%) and C48/80 (90%), when compared to the vehicle control group (16%). Ketotifen prevent the mast cell degranulation induced by C48/80 and FH 30 µg in 61% and 52%, respectively. However, FH 0.1 µg was ineffective in prevent the mast cell degranulation. **Conclusion:** In addition to our previous results, these data reveals that HF at high doses induce nociception modulated by opioid system and histamine release. Meanwhile, the HF and IBA antinociceptive effects at lower doses did not involve opioid and histaminergic mechanisms, suggesting an anesthetic effect. **Financial support:** CAPES.

05.013 Further investigation of antinociceptive and pronociceptive mechanisms of *Acmella oleracea* in mice. Dallazen JL[1,2], Maria-Ferreira D[1], Luz BB[1], Nascimento AM[1], Cirpriani TR[1], Souza LM[3], Werner MF[1] [1]UFPR, [2]Farmacologia, [3]Instituto de Pesquisa Pelé Pequeno Príncipe

Introduction: Since many neuropathic pain patients do not receive appropriate treatment due to reduced effective analgesic drugs, the identification of new targets to indicate novel analgesic drugs is urgent. The role of alpha1-subunit 2.3 of voltage-gated calcium channels (that generates R-type currents) in neuropathic pain is widely unknown. Secondary hyperalgesia-induced by capsaicin in health individuals is a human pain model used to produce proof-of-concept for novel analgesic drugs useful to treat neuropathic pain. Similar to neuropathic pain, subcutaneous capsaicin injection cause secondary hyperalgesia maintained by a spinal cord sensitization of the pain pathways. The aim of the present study was to investigate the effect of Cav2.3 knockdown, especially at the spinal cord, on the spontaneous nociception and the primary or secondary hyperalgesia caused by capsaicin in mice. **Methods:** Female C57Bl/6-UFSC mice (N=9-10, 20-25 g) were used and the experiments followed the ARRIVE guideline. To knockdown Cav2.3, animals were treated with an oligonucleotide antisense against $Ca_v2.3$ (2.5 nmol/site, intrathecal, twice a day for 3 days – AS) or with a mismatch oligonucleotide (MM). Mice were evaluated by von Frey's and Hargreaves' tests (to detect paw mechanical thresholds or heat latency, respectively) before and after the treatments with AS/MM and capsaicin. Twelve hours after the last AS/MM injection, mice were injected with capsaicin (20 nmol/site, intradermal into the proximal part of the right hind paw). The time spending licking and lifting the injected paw (spontaneous nociception) was evaluated in the first 5 minutes after capsaicin injection. Significative reduction at mechanical thresholds (in the distal part of right hind paw, i.e. secondary mechanical hyperalgesia) or at heat latencies (in the proximal part of the paw, i.e. primary heat hyperalgesia) where detected from 0.5 to 8 hours after capsaicin treatment. **Results:** The paw withdrawal threshold before (0.9±0.1 and 1.0±0,1 g, for MM or AS, respectively) were not different from after treatment with the oligonucleotides (1.0±0.1 and 0.8±0.1 g for MM or AS, respectively). Similarly, the latency to withdrawal after heat stimulus were also similar before (12±1.4 and 10±1.0 s, for MM or AS, respectively) and after treatment (time of response 11±1.4 and 9±0.9 s, for MM or AS, respectively). Moreover, spontaneous nociception (11±2 and 10±3 s, for MM or AS, respectively) or primary thermal hyperalgesia (7.0±1.2 and 4.2±0.4 s, for MM or AS, respectively) induced by capsaicin were similar in AS and MM treated animals. On the other hand, the secondary mechanical hyperalgesia detected 0.5 to 8 hours after capsaicin injection was largely reduced by AS treatment (inhibition of 78±13% at the 2 hours hyperalgesia peak). **Conclusion:** The knockdown of $Ca_v2.3$ specifically prevented the development of capsaicin-trigged secondary hyperalgesia, without altering primary hyperalgesia or nociceptive pain. Thus, Cav2.3 is a potential target to the treatment of neuropathic pain. Acknowledgments: **Financial Support** by CNPq, CAPES and INCT-Inovamed. The project was approved by the Ethics Committee on Animal Use of UFSC (PP00872).

05.015 Antihyperalgesic and anti-inflammatory activity of the hexanic and hydroalcoholic fractions extracted from *Piper glabratum* in mice. Leitão MM[1], Santos JA[1], Mota J[2], Kassuya CAL[1] [1]UFGD – Ciências da Saúde, [2]UEMS – Química
Introduction: *Piper glabratum*, popularly known as pariparoba or false Jaborandi, is present in tropical and subtropical regions, is widely used in folk medicine to treat wounds and bruises, suggesting potential anti-inflammatory activity. The aims of the present study were to evaluate the anti-inflammatory and anti-hyperalgesic activities of hexane (HEF) and hydroalcoholic (HAF) fractions of *P. glabratum*, in models of carrageenan-induced paw edema and pleurisy. **Methods:** In paw edema model, the mechanical (electronic von Frey analysis) and cold (acetone test) hyperalgesia and oedema (plethysmometer measurement) was analyzed in four groups of *Swiss* mice (n=6) after 100 µL of carrageenan 1% was administered in the right hind paw. 1h before the experimental groups were treated orally with of the HEF (19,5 mg/kg) and HAF (83,37 mg/kg), the control group received saline (0.9 %) and a positive control dexamethasone (1 mg/kg, s.c.). In pleurisy test, female *Swiss* mice (n=6) were divided in 5 groups: Naive, negative control (saline, p.o.), experimental groups (HEF and HAF 100 mg/kg, p.o.) and positive control group (Dexamethasone 1 mg/kg, s.c.). After 1 hour from their respective treatments, in intrapleural injection of carrageenan 1 % (100 µL) was made while the naive group received only sterile saline injection (100 µL). After 4h, the animals were euthanized and the thoracic cavity was washed with 1 mL of phosphate-buffered saline (PBS). The total number of leukocytes was determined by a KX-21N unit of Sysmex. **Results:** Oral administration of HAF significantly reduced the mechanical hyperalgesia ($P < 0.05$) 3h after of the injection of carragenan, a decrease of 100 % of mechanical hyperalgesia. In paw edema, the HEF significantly reduced the volume on 1h ($P < 0.001$), 2h ($P < 0.001$) and 4h ($P < 0.05$), with inhibition of 83 ± 3%, 78 ± 2% and 44 ± 6% respectively. HAF significantly reduced ($P < 0.05$) only after 2h of the carrageenan administration, with inhibition of 39 ± 11%. In the cold sensitivity, the HEF significantly decrease the thermal sensitivity in 55 ± 11% ($P < 0.001$) and 48 ± 13% ($P < 0.05$) at 3h and 4h, respectively, compared to the control group. The HAF significantly decrease in 65 ± 7% ($P < 0.001$) and 52 ± 13% ($P < 0.05$) at 3h and 4h, respectively. In pleurisy induced by carrageenan, the fractions did not reduce leukocyte migration compared to the control group. **Conclusion:** Based on the results obtained in this study, the fractions have antidematogenic and antihyperalgesic activity, but do not inhibit the migration of leukocytes in the doses and models tested. In this case, further studies may be performed to elucidate the mechanisms of action. **Financial support:** CAPES, CNPq and FUNDECT. **Number of research approval by the Animal Research Ethical Committee:** 026/2016 - Ethics Committee for Animal Use - CEUA - Federal University of Grande Dourados – UFGD.

05.016 Antinociceptive activity of LQFM 096, **a new triazolic derivative**. Cardoso CS[1], Silva DPB[1], Silva DM[1], Florentino IF[1], Vasconcelos JP[2], Leão LM[3], Menegatti M[2], Costa EA[1] [1]UFG – Farmacologia, [2]UFG – Farmácia, [3]UFG – Química

Introduction Aiming the development of new effective analgesic prototypes with less capacity to induce adverse effects, the compound LQFM 096, a new triazolic derivative, was designed and synthesized in the Laboratory of Medicinal Pharmaceutical Chemistry of the UFG, through the structural modifications based on biososterism of rings. This study proposed to evaluate the antinociceptive activity of compound LQFM 096, as well as to investigate the mechanisms of action associated with this effect. **Methods** Adult -male Swiss albino mice weighing 30 - 40g were used, provided by the central animal house of Federal University of Goiás (CEUA/UFG nº 017/13). To evaluate the antinociceptive activity of compound LQFM-096, the tests of abdominal writhing induced by acetic acid, pain induced by formalin, hot plate and tail flick were performed. Participation of opioid receptors was assessed by pretreatment with naloxone, an antagonist of this receptor. In addition, the involvement of ionic channels ASICs was evaluated in the acid saline test. The differences between the groups were detected by Student's t-test and between three or more groups by one-way ANOVA, followed by the Newman-keuls test. **Results** In order to evaluate antinociceptive activity, we initially used the acetic acid-induced abdominal writhing test, in which treatments with LQFM 096 at doses of 10, 20 and 40 mg/kg significantly reduced the number of abdominal writhes induced by acetic acid in 30.80 % ($P < 0.001$), 42.29 % ($P < 0.001$) and 51.29 % ($P < 0.001$) respectively, compared to the control group (95.14 ± 4.97). To confirm and characterize this effect was performed the formalin test. The compound LQFM 096 (20 mg/kg, p.o.) reduced the lick time in the first and second phases of the formalin test by 56.5% ($P < 0.01$) and 43.1% ($P < 0.01$), respectively, when compared to the control group (67.63 ± 4.67s in the 1st phase and 221.4 ± 19.81s in the 2nd phase). Although the compound LQFM 096 showed antinociceeptive effect in 1st phase of the formalin test, the hot plate and tail flick tests discarded a possible central action, since the compound did not present an increase in latency time in these two tests. The antinoceptive effect in the first phase of formalin was antagonized by pre-treatment with naloxone (3 mg/kg, s.c), suggesting the involvement of opioid receptors in this effect. In addition, LQFM 096 inhibited pain induced by acid saline reduced the licking time in 51.5 %, compared to the control group (229 ± 9.36s). **Conclusion** Thus the results shown that the triazole compound LQFM 096 possess antinociceptive activity in models of pain induced by different chemical agents. However, the thermal pain tests suggest that LQFM 096 has only peripheral action. In addition, with the analgesic effect reversed by pre-treatment with naloxone, we suggest the involvement of the opioid receptors in the antinociceptive activity this triazolic derivative. **Financial support:** CNPq, CAPES, PEC-PG

05.017 Involvement of cytokines in paclitaxel-induced acute pain syndrome in mice. Oliveira FFB[1], Fonseca MDM[2], Lopes AHP[2], Cunha TM[2], Vale ML[1] [1]UFC – Fisiologia e Farmacologia, [2]FMRP-USP – Farmacologia

Introduction: Paclitaxel is a first-line chemotherapeutic agent used to treat various solid tumors types. However this drug causes pathological pain, includes pain that occurs immediately after treatment (paclitaxel-associated acute pain syndrome) occurring within days after each dose and usually abating within days and pain that persists for weeks to years after cessation of paclitaxel treatment. Mechanisms underlying paclitaxel associated acute pain syndrome remain unknown. In this study, has evaluated the involvement of cytokines on paclitaxel-associated acute pain syndrome. **Methods:** The study was approved by the Ethics Committee on Animal Research of the School of Medicine of Ribeirão Preto (nº 42/2015) and the Ethics Committee on Animal Research of the Federal University of Ceara (nº 17/2014). Male wild-type mice (C57BL/6) and knockout mice (IL-6, TNFR1/R2; IL-1R, CCR2) were used. Paclitaxel (4mg/kg) or vehicles were injected into mice via intravenous. Mechanical sensitivity was assessed by von Frey filaments, where a series of monofilaments were tested in ascending order to generate response for each animal. Each von Frey filament was applied mid-plantar area of right hind paw from beneath for about 3 seconds or until a withdrawal response occurred. Cold sensitivity was assessed with a stimulus (acetone) applied to the ventral surface of right hind paw. Mice were observed for 1 minute and the number of licks and the duration of lifting of the paw were recorded. This value was later averaged across all animals in each group to yield the group response threshold and withdrawal the paw latency. Homogenates of dorsal root ganglia, spinal cord and sciatic nerve tissue were collected for quantitative real-time polymerase chain reaction were prepared for mRNA of cytokines quantification were performed in all groups of mice at baseline (naïve) and 2, 4, 6 and 24 hours after paclitaxel injection. The quantitative determination of cytokines release too was performed through by enzyme-linked immunosorbent assays in the dorsal root ganglia, spinal cord, sciatic nerve and blood in all groups of mice at baseline (naïve) and 2, 4, 6 and 24 hours after paclitaxel injection. **Results:** Paclitaxel induced mechanical and cold hypersensitivity within 2 hrs and peaked between 4 and 6 hrs but resolved within 24 hrs after a single injection of paclitaxel. Meanwhile, thresholds of hind paw withdrawal responses to mechanical and cold stimulus in mice receiving vehicle (control group) remained unchanged. The paclitaxel-induced acute mechanical and cold hypersensitivity were inhibited in mice IL-6, TNFR1/R2, IL-1R and CCR2 knockout. Paclitaxel has also induced increased expression of IL-6, TNF-α, IL-1β, MCP-1 and KC in dorsal root ganglia, spinal cord, sciatic nerve and blood in wild-type mice. **Conclusion:** Our results suggest that expression IL-6, TNF-α, IL-1β, MCP-1 and KC contribute to the paclitaxel associated acute pain syndrome.

05.018 Analgesic effects of intranasal ketamine in rats models of orofacial pain. Araya EI[1], Claudino RF[1], Nones CFM[1], Chichorro JG[1] [1]UFPR – Farmacologia
Introduction: There is growing evidence that intrasanal administration of ketamine is able to induce analgesia in patients with chronic pain, promoting rapid pain relief with a small number of side effects (Kulbe, Home Healthc Nurse, 16:367, 1998; Carr et al., Pain, 108:17, 2004; Huge et al., Eur J Pain, 14:387, 2010). The bioavailability of intranasal ketamine has been described as approximately 45%, which is slightly greater in comparison with sublingual administration, rectal administration, and especially oral administration (Malinovsky et al., Br J Anaesth. 77:203, 1996; Yanagihara et al., Biopharm Drug Dispos, 24:37, 2003). The intranasal route also provides a non-invasive method of bypassing the blood-brain barrier to rapidly deliver therapeutic agents to the brain, spinal cord and other cerebral structures, but also, it allows drug delivery to orofacial structures innervated by the trigeminal nerve (Johnson et al., Mol Pharm, 7:884, 2010). Thus, herein it was investigated the analgesic effect of intranasal administration of S-ketamine in rats in different models of orofacial pain. **Methods:** Nociceptive responses induced by formalin injected into the upper lip and facial heat hyperalgesia induced by capsaicin and carrageenan were used to assess the effect of intranasal ketamine on acute orofacial pain models in rats. The effect of intranasal ketamine was also evaluated on heat and mechanical hyperalgesia induced by infraorbital nerve constriction (CION). In addition, the locomotor activity was assessed after intranasal ketamine administration in the open-field test. All the procedures were previously approved by UFPR´s institutional Committee for the Ethical Use of Animals (authorization #980). **Results:** Intranasal ketamine (0.5 mg/kg) failed to modify the first phase of the orofacial formalin test, but reduced about 40% the second phase. Intranasal ketamine also reduced the facial heat hyperalgesia induced by capsaicin and by carrageenan, both injected into the upper lip of rats. In the CION model, intranasal ketamine at 0.5 mg/kg reversed the heat hyperalgesia and at 1 mg/kg attenuated the mechanical hyperalgesia 4 and 14 days after the surgery, respectively. Repeated intrasanal ketamine (0.5 mg/kg, once a day over 4 consecutive days) reduced the heat hyperalgesia on days 4-7 on CION rats, suggesting no tolerance to the analgesic effect. The open field test did not reveal locomotor deficits in rats treated with intranasal ketamine. **Conclusion:** Our data demonstrated that intranasal ketamine produces analgesic effects in inflammatory and neuropathic orofacial pain models and may represent an adjuvant in the treatment of such conditions, especially when rapid pain relief is needed. **Acknowledgments:** EIA and CFMN are recipients of CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) doctoral scholarship, RFC is recipient of CAPES post-doctoral scholarship.

05.019 Acute treatment with adenosine receptor A3 agonist alters hiperalgesic response in rats with chronic inflammatory pain. Cioato SG[2,1,3], Lopes B[4,1,3], Salvi AA[1,3], Medeiros LF[5,1,3], Torres ILS[4,2,5,1,3] [1]UFRGS – Farmacologia da Dor e Neuromodulação: Modelos Animais, [2]UFRGS – Farmacologia e Terapêutica, [3]Hospital de Clínicas de Porto Alegre – Experimentação Animal, [4]UFRGS – Fisiologia, [5]UFRGS – Medicina

Introduction: Previous studies have demonstrated the antinociceptive effects of adenosine and its analogues, supporting clinical potential use for painful conditions. The therapeutic use of A1R and A2A adenosine receptor agonists is limited because of adverse effects, however the role of A3 receptor (A3R) agonists, as analgesic and anti-inflammatory, needs to be clarified. In this context, there is great interest in development of A3 agonists, for example IB-MECA (A3N6-(3-iodobenzyl) adenosine-5'-N-methyluronamide), for management of different painful conditions, with potential pain relief and few adverse effects. The aim is to evaluate the analgesic effect of acute administration of IB-MECA upon hyperalgesic response induced by inflammatory pain model in Wistar rats. This study was approved by Ethical Committee of Animal Use of Clinics Hospital of Porto Alegre (GPPG: 150530) and all procedures were performed according to Guide for the Care and Use of Laboratory Animals 8th ed. **Methods:** 48 male Wistar rats (60-70 days) were divided into inflammatory pain (Complete Freund Adjuvant 1mg/mL, intradermic injection of 100µl in left hindpaw); sham (100µl of saline in left hindpaw) or control (without manipulation). After 14 days, the three groups above were subdivided into: 0.5 µmol/kg of IB-MECA) in DMSO 3%, DMSO 3% in saline solution, or morphine 5mg/kg (all intraperitoneal adminsitration). Randall Selitto test and von Frey tests were performed at baseline, 10 and 14 after pain induction and 30 min after treatment, while Hot Plate was performed at baseline, 10 and 14 after pain induction and 30, 60, 90 and 120 min after treatment. For statistical analysis, repeated-measures or one-way ANOVA/SNK was performed. **Results:** At days 10 and 14, the rats of inflammatory pain group exhibited decreased thermal and mechanical response threshold (repeated-measures ANOVA; $P<0.05$). IB-MECA increased the mechanical response threshold at 30 min (one-way ANOVA; $P<0.05$) and thermal response threshold until 120min after IB-MECA treatment (repeated-measures ANOVA; $p<0.05$), interesting to note that IB-MECA did not present effect in control and sham groups. **Conclusion:** The inflammatory pain model induced by CFA injection promotes an increase in the hyperalgesic response assessed 14 days after administration. The mechanical and thermal hyperalgesic behavior was reversed by IB-MECA treatment in chronic inflammatory pain model. Biochemical analyses are necessary to investigate the signaling mechanism involved to analgesic effect of IB-MECA. **Financial Support:** CAPES, CNPq, FIPE-HCPA.

05.020 HUF-101 prevents the development of mechanical allodynia induced by paclitaxel in mice. Silva NR[1], Fonseca MDM[1], Mechoulam R[2], Cunha TM[1], Guimarães FS[1] [1]FMRP-USP – Pharmacology, [2]Hebrew University of Jerusalem – Medicinal Chemistry and Natural Products

Introduction: Paclitaxel is the frontline chemotherapeutic agent used to treat many of the most common solid tumors. Chemotherapy-induced peripheral neuropathy (CIPN) is the main dose-limiting adverse effect of paclitaxel and can lead to dose reduction or even discontinuation of therapy, thus affecting survival. Overall, approximately 68% of the patients receiving chemotherapy develop CIPN within the first month of treatment, which is related to both the single as well as the cumulative drug administration. This pain is often resistant to standard analgesics and so far there is no clear preventive treatment and the underlying mechanisms remain poorly defined. Cannabidiol (CBD) is a phytocannabinoid with multiple pharmacological effects and has already shown effects in CIPN in preclinical studies. Our group showed that the fluorinated-derived of the CBD molecule, HUF-101, exhibited antinociceptive properties in a lower dose compared to CBD in acute pain models induced in mice. These effects involved the activation of the CB1 and CB2 receptors, without causing the tetrad of cannabinoid-induced effects. **Methods:** First protocol for prevention of CIPN: C57BL/6 mice (20-25-g) received intraperitoneal (i.p.) injection of HUF-101 (3, 10, or 30 mg/kg) or vehicle and 30min after received injections of PCX (8mg/kg) or saline (10mL/Kg) in days 0, 2, 4 and 6. Second protocol for prevention of CIPN: The animals received intraperitoneal (i.p.) injection of HUF-101 (3, 10, or 30 mg/kg) or vehicle for 14 days (0-14). PCX (8mg/kg) or saline (10mL/Kg) were administered in days 0, 2, 4 and 6, 30min after HUF-101. Protocol for reversion of CIPN: The animals received intraperitoneal (i.p.) injection of PCX (8mg/kg) or saline (10mL/Kg) in days 0, 2, 4 and 6. HUF-101 (3, 10, or 30 mg/kg) or vehicle were administered from the 7th to the 20th day. In order to evaluate the mechanical allodynia induced by PCX, Von Frey filaments were applied to the right plantar surface of the animal until the flinch or licking response of the stimulated paw. The baseline responses were determined on day 0. Subsequently, the tests were performed on days 1, 3, 5, 7, 10, 14 and 21. **Results:** The treatment with PCX (8 mg/kg) was able to induce mechanical allodynia in all protocols. This effect was prevented by treatment with HUF-101 (3, 10 e 30 mg/kg) for 14 days. HUF-101 (3, 10 e 30 mg/kg) administered on days 0, 2, 4 and 6 prevented allodynia until day 5. However, these same doses were not effective to reverse the already installed allodynia induced by PCX. **Conclusion:** These results indicate that HUF-101 prevents, but not reverses, the effects of allodynia caused by PCX. Thus, this new compound could be a therapeutical alternative for the treatment of CIPN. **Financial support:** CAPES, CNPq, FAPESP, and FAEPA. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 100/2016).

05.021 Nicorandil inhibits mechanical allodynia in the model of neuropathic pain induced by paclitaxel by activating opioidergic and serotoninergic mechanisms.
Morais MI[1], Braga AV[1], Rodrigues FF[1], Melo ISF[1], Fátima A[2], Coelho MM[1], Machado RR[1] [1]UFMG – Ciências Farmacêuticas, [2]UFMG – Química
Introduction: Neuropathic pain is a chronic disorder usually associated with central or peripheral nervous system lesions or diseases. A myriad of neurochemical mechanisms may contribute to establishment of neuropathic pain, thus contributing to the refractoriness to the traditional analgesic therapies. As low as 25% of the patients exhibiting neuropathic pain get a relief greater than 50% after using the available analgesic medicines. Nicorandil, a drug that releases nitric oxide (NO) and opens ATP-sensitive potassium channels, has been approved in some countries to treat patients with angina pectoris. The activity of nicorandil in models of nociceptive and inflammatory pain has been recently demonstrated, thus justifying additional investigations in models of neuropathic pain. **Methods:** The effect induced by nicorandil (50, 100 or 150 mg/kg, *per os*-*p.o*.) on the mechanical allodynia induced by paclitaxel (2 mg/kg, 2 mL/kg, intraperitoneal-*i.p*.) in male Swiss mice (25-30 g) was evaluated. To investigate putative mechanisms mediating the antinociceptive activity of nicorandil in the model of neuropathic pain induced by paclitaxel, opioidergic (naltrexone 5 or 10 mg/kg, *i.p*.) and serotonergic (cyproheptadine 5 or 10 mg/kg, *i.p.*) antagonists and an ATP-dependent potassium channel blocker (glibenclamide, 20 or 40 mg/kg, *p.o.*) were used. Nicorandil was administered twice (8 mL/kg, *p.o*.), within a two hour interval. **Results**: Nicorandil inhibited the mechanical allodynia induced by paclitaxel when administered once or twice in the seventh or fourteenth day after first injection of paclitaxel. A greater antinociceptive effect was observed when nicorandil was administered twice within two hours interval. Naltrexone and cyproheptadine, but not glibenclamide, attenuated the antinociceptive effect induced by nicorandil. **Conclusion:** The results demonstrate that nicorandil exhibits antinociceptive activity in the model of neuropathic pain induced by paclitaxel. This activity may be mediated by activation of opioidergic and serotonergic receptors, but not ATP-sensitive potassium channels. The results indicate that nicorandil may represent a pharmacotherapeutic strategy in the treatment of patients with neuropathic pain and justify additional preclinical and clinical assays aiming to evaluate its potential use as an analgesic drug.
Financial support: CNPq, FAPEMIG **Approval protocol number:** 339/2015
References: DUTRA, M.M.G.B. European Journal of Pharmacology, v. 768, p. 160, 2015. DUTRA, M.M.G.B. Pharmacology, Biochemistry and Behavior, v. 106, p. 85, 2013. JENSEN, T.S. Pain, v. 152, p. 2204, 2011.

05.022 Involvement of dorsal root ganglia NMDA receptors on acute pain in rats. Mendes DR[1], Lemes JBP, Silva MV, Fagundes RGM, Lotufo CM UFU – Fisiologia
Introduction: It was recently shown that NMDA receptors expressed at satellite cells at the dorsal root ganglia are important for the maintenance of inflammatory hyperalgesia (ref). Since this process occur mainly through C fiber sensitization, we decided to test whether NMDA receptors at the dorsal root ganglia participates in nociceptive transmission involving C fibers. **General objective:** To study the involvement of NMDA receptors in the dorsal root ganglion in C fiber dependent processing of acute pain and hyperalgesia. **Methodology:** Experiments performed in male Wistar rats (200 g). The effects of the intraganglionic (i.gl.) administration of the NMDA antagonist, AP-5 (9µ in 5 µl) or vehicle (NaCl 0,9%, 5 µl) was evaluated in nociception and mechanical sensitization. The antagonist or vehicle was administered 30 minutes before capsaicin (intraplantar, 10 µg in 50 µl), Prostaglandin E2 (intraplantar, 1 µg, 50 µl) or formalin (intraplantar 2,5%, 50 µl). Capsaicin and formalin-induced nociception was evaluated through counting flinching and licking of the paw for 5 minutes after capsaicin injection or 60 minutes after formalin injection. The mechanical sensibility was evaluated through Radall-Selitto test before and after 3 hours of Prostaglandin E2 administration in control animals and animals that had C fibers destructed, 7 days before, by intrathecal capsaicin injection (10 µg, 10 µl). Results shown as mean and E.P.M. of 5 animals per group. Data was analyzed by ANOVA followed by Tukey test ($p<0,05$). (CEUA protocol/UFU 008/14) **Results:** Intraganglioc injection of AP-5 attenuated nociceptive behavior induced by capsaicin, (35g ± 4g) in relation to control animals (76g ± 10g). In the formalin test, the treated animals with AP-5 by intraganglionic injection shown a similar behavior to the control animals (61g ± 4g) at the phase I, noted in the *first* 10 minutes. At phase II, which is the nociceptive phase (355g ± 46g) compared to control (551g ± 24g). Inflammatory hypernociception induced by PGE2 was attenuated in AP-5 treated animals by intraganglionic injection (6,1g ± 0,2g) in relation to the controls (2,0g ±0,0g). The AP-5 treatment by intraganglionic pathway or C fibers destruction by intrathecal capsaicin didn't changed animals mechanical sensibility (6,0g ± 0,1g), *being that* AP-5 (i.gl.) or preview capsaicin administration (i.t.) attenuated PGE2 induced sensibility (2,0g ± 0,0g). Intrathecal capsaicin injection destroy C fibers 24h later your administration. By that way, rats treated previously with capsaicin (i.t.) didn't answer to intraplantar capsaicin test. **Conclusion:** The results supports that NMDA glutamatergic receptors in the dorsal root ganglia is involved not only in inflammatory hypernociception, but in acute hypernociception too. Through that, the results shown that this process occurs in situations dependent of C fibers nociceptive activation. **Financial support:** Fapemig.

05.023 Antioxidant and antinociceptive effect of 2-PHE in the sciatic nerve partial ligation model França ARS[1], Piauilino CA[1], Lopes EM[1], Gomes LS[1], Sousa DP[2], Almeida FRC[1] [1]UFPI – Medicinal Plants, [2]UFPB – Pharmaceutical Sciences
Introduction: Neuropathic pain is a chronic condition that results of damage in the somatosensory system. Studies have shown that several natural compounds isolated from plants are effective in the treatment of neuropathic pain. 2-Phenylethanol (2-PHE), an aromatic alcohol with rose-like odor, can be found in the essential oil of many plants and the similarity of its chemical structure to other compounds found in essential oils with antinociceptive activity suggested another potential effect of 2-PHE. The objective of this study was to investigate the antinociceptive and antioxidant activities of 2-PHE in a neuropathic animal model. **Methods:** Neuropathic pain was induced by partial sciatic nerve ligation (PSNL) in female Swiss mice (n=6-8, 25-30g). The mechanical nociceptive threshold was measured using von Frey filaments, what was performed from 24 h after surgery to the 8th day of treatment, on days 2, 4, 6 and 8. The animals were treated with 2-PHE (50, 100 or 200 mg/kg, p.o.); Pregabalin (10 mg/kg, p.o.;PG-10) or vehicle (1% tween 80 in saline 0.9%, p.o.). In the thermal sensitivity test to cold, we used acetone test. To evaluate the antioxidant activity, we measured the levels of reduced glutathione (GSH), reactive species to thiobarbituric acid (TBARS) and superoxide dismutase (SOD) in the blood serum of animals treated and untreated with 2-PHE. The results were statistically evaluated using ANOVA one-way followed by Tukey or Bonferroni test, $p<0.05$. All the protocols were approved by Ethics Committee on the Use of Animals (ECUA /UFPI n° 82/2014). **Results:** 2-PHE-100 on days 6 and 8 (1.63±0.17; 1.63±0.17) and 2-PHE-50 mg/kg on day 8 (1.60±0.10) and PG-10 after 24 h and until for 8th day (1.83±0.48; 4.33±0.62; 6.67±0.42; 7.67±0.33), were statistically different from the vehicle group (7,67±0.33; 0,40±0.00; 0,31±0.07; 0.16±0.00; 0.20±0.04), increasing the paw withdrawal threshold measured using Von Frey test. The oral treatment with 2-PHE-50 (1.00±0.24; 1.56±0.24; 1.11±0.26), 2-PHE-100 (1.17±0.40; 1.33±0.33; 1.67±0.42) or 2-PHE-200 mg/kg from the 4th day (1.83±0.40; 1.50±0.22; 1.17±0.17) and PG-10 from the 2th day and until the 8th day (1.17±0.40; 0.67±0.21; 0.50±0.22; 0.17±0.17), significantly reduced acetone-induced cold allodynia, compared to the vehicle (0.00±0.00; 2.83±0.17; 2.83±0.17; 2.83±0.17; 2.83±0.17). Furthermore, 2-PHE-50 (3.07±0.15), 100 (11.94±0.90) and 200 mg/kg (4.02±0.40) and PG-10 (2.71±0.29) were effective in reducing the levels of reactive species to thiobarbituric acid when compared to the vehicle (18.58±1.16). In addition, 2-PHE-50 (5.45±0.24), 100 (5.71±0.23) or 200 (5.45±0.30) mg/kg and PG-10 (6.48±0.33) also significantly increased GSH levels, when compared to the vehicle group (4.26±0.17). In line with these data, 2-PHE-50 mg/kg (0.45±0.05) and PG-10 (0.23±0.00) were also effective in increasing SOD levels, when compared to the vehicle group (0.25±0.02). **Conclusion:** Taken together, our findings suggest that 2-PHE has potential antinociceptive effect and its mechanism of action is probably due to its antioxidant activity. **Financial support:** UFPI/FAPEPI-CAPES

05.024 The role of CCR2+ and CX3CR1+ cells in genesis and maintenance of neuropathic pain Guimarães RM[1], Ferreira MD[1], Fonseca MDM[2], Kusuda R[2], Cunha TM [1]FMRP-USP – Imunologia, [2]FMRP-USP – Farmacologia

Neuropathic pain is a type of chronic pain, which can be generated by injury to the peripheral nerve, being characterized by changes in the activity of the neural system and in the interactions between immune system and glial cells. The involvement of myeloid cells at the site of the peripheral nerve injury is well described and many studies have suggested that these circulating monocytes may also infiltrate the spinal cord and dorsal root ganglion (DRG). However, the functional role of these cells in nerve tissues is still unclear. The aim of this study was to investigate the role of myeloid cells, specifically CCR2+ and CX3CR1+ cells in the spinal cord and DRG after induction of neuropathy, as well as to investigate the source of these cells, the possible mechanisms involved in their recruitment and how they may contribute to the induction and maintenance of neuropathy. For this, wild-type and genetically modified animals (CX3CR1+/GFP/CCR2+/RFP) underwent a neuropathy model known as SNI (spared nerve injury) and after 3, 7, 10 and 14 days of surgery their spinal cord and DRG were collected, which were used for qRT-PCR, immunofluorescence and flow cytometry assays. In spinal cord samples, it was possible to observe an increase in CX3CR1+ and CD45intermediate cells at all-time points evaluated, representing microglial activation. On the other hand, no increase in CCR2+ inflammatory monocytes was observed. In the DRG, it was noticed an expressive increase of CX3CR1+, CCR2+ and CD11b+CD45+ cells on the seventh day after neuropathy. In addition, Ly6G-CD11b+ cells isolated by FACSAria from DRGs collected 7 days after the induction of neuropathy showed increased expression of IL-6, IL-1β and TNF-α cytokines. Finally, DRGs collected from CCR2 knockout animals after 7 days of neuropathy induction displayed a reduction in IL-1β and TNF-α cytokine expression. These data demonstrate that, there are no inflammatory monocytes in the spinal cord after neuropathy, unlike GRD. In addition, both CCR2+ and CX3CR1+ cells appear to play important roles in neuropathy and CCR2+ cells are likely to be responsible for the production of inflammatory cytokines involved in pain. **Financial Support**: CAPES, CNPq, FAPESP and CRID Animal research ethical committee: CEUA - FMRP 002/2017

05.025 Hypernociceptive effect of the lectin isolated from *Platypodium elegans*

Laranjeira EPP[1], Almeida LM[1], Silva IB[2], Nascimento KS[2], Cavada BS[2], Assreuy AMS[2], Pires AF[1] [1]UECE, [2]UFC

Introduction: Lectins are proteins that bind specifically and reversibly to mono- and/or oligosaccharides, without altering their covalent structure, via carbohydrate-recognition domain (CRD) (Peumans; Van Damme, Plant Physiol, 109, 347, 1995). The lectin isolated from seeds of *Platypodium elegans* demonstrated high efficiency in the capture of glycoproteins in solution and induced acute inflammation via lectin domain and macrophage activation (Araripe, Int J Biol Macromol, 102, 323, 2017), but nothing has been shown in respect to its role in inflammatory pain. **Methods:** The lectin from *Platypodium elegans* seeds (PELa) was purified by affinity chromatography in a mannose-agarose column (Araripe, Int J Biol Macromol, 102, 323, 2017) Swiss mice (25-30 g; n=8) were maintained according to the experimental protocols were approved by the Ethics Committee of UECE (CEUA Nº 10130208-8/40). Hypernociception was measured by the frequency of paw withdrawal in response to 6 applications of the flexible Von Frey filament (0.8 g) before and after (0.5 - 72 h) subcutaneous (s.c.) intraplantar injection of PELa (3, 30 and 300 µg/paw) or sterile saline (NaCl 0.9%). Involvement of the CRD in lectin activity was evaluated by s.c. administration of PELa previously incubated (60 min at 37 ºC) with 100 mM α-methyl-D-mannoside (α-CH_3). The participation of prostaglandins was also investigated by the s.c. administration of indomethacin (5 mg/kg) one hour before lectin injection. Results are presented as Mean ± S.E.M., and differences were analyzed by ANOVA followed by Bonferroni's test. $p < 0.05$ was considered significant. **Results:** PELa increased animals paw withdrawal in response to mechanical stimulation with von Frey filaments in 2.1x (32.47 ± 3.8) at 3 µg, 2.3x (35.41 ± 2.6) at 30 µg and 4.2x (64.99 ± 6.3) at 300 µg, compared to saline (15.53 ± 1.4). The hypernociceptive effect (300 µg/paw) started at 0.5 h, and attained maximum effect at the fourth hour (85.41 ± 4.26 % *vs*. saline: 16.66 ± 9.96 %). PELa hypernociceptive effect (64.25 ± 10.02) was completely abolish by its association with α-CH_3 (24.25 ± 5.6%) and by pretreatment with indomethacin (30.92 ± 5.33). **Conclusion:** PELa is the first lectin of the Dalbergieae tribe to present hypernociceptive activity via CRD and cyclooxygenase pathway. **Keywords:** *Platypodium elegans*; lectin; Hypernociception **Financial Agencies:** CAPES, FUNCAP, CNPq. **Approval of Animal Research Ethical Committee:** CEUA UECE Nº 10130208-8/40.

05.026 Anti-hyperalgesic action of N-type calcium channel blockers and TRPA1 antagonist in mice models of HIV-related pain. Lückemeyer DD, Prudente AS, Ferreira M A, Tonello R, Ferreira J UFSC – Farmacologia

Introduction: Pain arising from HIV-related sensory neuropathy (HIV-SN) is very difficult to manage as drugs commonly used for neuropathic pain are usually not effective. The aim of this study was investigate the possible antinociceptive effect of intrathecal (i.t.) ziconotide and CTK01512-2, two a N-type voltage-gated calcium channel (VGCC) blockers, and HC-030031, an antagonist of TRPA1, in mice models of HIV-SN. Method: HIV-SN was induced by the injections of gp120 (100 ng/site, i.t, on days 0, 3 and 6) and/or stavudine (50 mg/Kg, intravenously, on days 0 and 4) in both female and male C57Bl/6 mice. Before and after treatments, von Frey test was performed and adverse effects were investigated. Animals were also i.t. treated with CTK01512-2, ziconotide (100 pmol/site) and HC-030031 (30 nmol/site) 13/14 days after HIV-SN induction. N-type VGCC and TRPA1 mRNA was detected by qPCR in dorsal root ganglion and spinal cord. **Results:** The treatment with HIV-gp120, stavudine and HIV-gp120+stavudine, but not boiled HIV-gp120 and/or saline, produced hyperalgesia both in female and male mice. At 13^{th}/14^{th} day after induction, we detected the hyperalgesia peak and increased levels of TRPA1 and N-type VGCC mRNA splicing variants e37a and b. I.t. CTK01512-2 or ziconotide, but not HC-030031, was able to fully reverse HIV-gp120-, stavudine- or HIV-gp120+stavudine-induced hyperalgesia. Ziconotide, but not CTK01512-2 and HC-030031, produced motor and sensorial adverse effects at the tested dose. **Conclusion:** I.t. N-type VGCC blockers produced a marked antinociceptive effect in mice models of HIV-related pain. **Financial Support**: CNPq, CAPES, INCT-Inovamed The project was approved by the Ethics Committee on Animal Use of UFSC (PP00872).

05.027 Participation of TNF-α and astrocytes in Ehrlich tumor-induced pain in mice. Domiciano TP, Zarpelon AC, Ferrari LS, Campos CC, Fattori V, Borghi S, Filho JCA, Cunha FQ, Cunha TM, Verri WAV UEM, FMRP-USP

TNF-α was the first cytokine described to mediate inflammatory pain. Its role in neuropathic pain is also well-established. Astrocytes are important cells in the mechanism of central sensitization. This glial cell population is related to the development and maintenance of neural plasticity and chronic pain in neuropathies. However, although the role of TNF-α and glial cells in the physiopathology of cancer pain has been explored in a wide variety cancer pain models, the mechanisms involving TNF-α signaling and spinal cord astrocytes activity needs to be further addressed. In this sense, the present study has the proposal to evaluate the participation of TNF-α and astroglia in the physiopathology of Ehrlich tumor-induced pain in mice. **Methods:** Male C57BL/6 and TNFR1$^{-/-}$ mice were used in this study. Mice were inoculated with 1x10^{6} cells/25µl of Ehrlich tumor cell suspension the right hind paw. TNF-α protein levels were determined in paw tissue (ELISA) and its mRNA expression together protein levels in spinal cord (ELISA and RT-qPCR, respectively). TNFR1$^{-/-}$ mice or wild type mice treated with vehicle (saline) or etanercept (10 mg/Kg, intraperitoneally, daily) were used for the evaluation of mechanical and thermal hyperalgesia (digital analgesimeter and hot plate test respectively) and paw edema (caliper) prior to inoculation and after every 2 days, until the day 12. On day 12, immunofluorescence and western blot analyses were performed for the evaluation of astrcoytes activity (through the detection of glial fibrillary acidic protein, GFAP). All data were analyzed using Prism 5.0 statistical program (GraphPad software, Inc.). Were used one-way and two-way ANOVA with Tukey's post hoc test for analysis with three or greater groups. A *p* value equal or less than 0.05 was considered statistically significant. **Results:** We demonstrated that the levels of TNF-α increased gradually in paw tissue, being significant between 8-10 days when compared to control animals. In spinal cord, increased mRNA expression and protein levels of TNF-α were detected in day 12 post-tumor inoculation. Tumor inoculation induces mechanical hyperalgesia, which were significantly inhibited in TNFR1 deficient and etanercept-treated mice on day 2 and from 6-12 and from 2-10 days post-tumor inoculation, respectively. However, thermal hyperalgesia and paw edema was not affected in TNFR1$^{-/-}$ mice or etanercept treatment. Furthermore, tumor induced increased mRNA expression of GFAP in spinal cord between 8-10 days after inoculation. Astrocytes activation in ipsilateral dorsal horn of the spinal cord was confirmed through the increased detection of GFAP protein levels in tumor-inoculated animals. **Conclusions:** The present data highlights TNF-α and astrocytes as potential targets for the control of Ehrlich tumor-induced mechanical hyperalgesia and neuroinflammation. **Acknowledgements and financial support:** This work received **Financial Support** from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Ministério da Ciência, Tecnologia e Inovação (MCTI), Secretaria da Ciência, Tecnologia e Ensino Superior (SETI), Fundação Araucária and Governo do Estado do Paraná. **Ethical approval:** Animals' care and handling procedures were in accordance with the International Association for Study of Pain (IASP) guidelines and with the approval of the Ethics Committee for Animal Use (CEUA) of the Universidade Estadual de Londrina, process number 14543.2013.03.

05.028 Infection by *Plasmodium berghei* strain ANKA prevents the development of inflammatory hyperalgesia, but not of mechanical and thermal, induced by chloroquine in mice. Aguida WR[1], Neves AB[2], Aguiar MFS[2], Feitosa IB[3], Teles CBG[1], Dias QM[1] [1]Fiocruz RO – Neuro e Imunofarmacologia, [2]UNIR, [3]IBCCF-UFRJ

Introduction: Malaria is a serious infectious disease caused by the unicellular protozoan, Plasmodium. Pharmacological treatment of malaria has been shown to be effective in controlling and healing the disease, despite recurrent reports of therapeutic failure, especially in non-adherence. Among the several causes, non-adherence to treatment may be associated with adverse reactions produced by antimalarial drugs. The chloroquine is linked to the development of myopathies, pure sensory neuropathies and mixed sensorimotor neuropathies. The algias induced by antimalarial in combination with the algias commonly triggered during Malaria could have negative consequences on the quality of life of the patient. In this sense, the present study had as proposal to evaluate the effect of the treatment with chloroquine in mice infected by Plasmodium berghei on the nociceptive threshold in models of mechanical, thermal and chemical nociception. **Methods**: The study used male Balb/c mice (25-30g) provided by FIOCRUZ RO facility. The study started with the infection of the mice by Plasmodium berghei strain ANKA (10^7 infected red blood cells / animal injected by intraperitoneal via). The parasitemia of infected animals was quantified on the 5th and 8th day after infection by counting blood cells in blood smears stained with Panoptic dye. On the 5th day post-infection the treatment with chloroquine was started. The chloroquine was administrated by oral via during three days with doses of 8.6 mg / kg (1st day) and 6.45 mg / kg (2nd and 3rd day). One day after last treatment with chloroquine, the nociceptive responses were evaluated with frequency response in 10 application of von Frey filament (4g), the response threshold (in seconds) to the thermal stimulus in the hot plate test and the number of behaviors in the formalin 1% test. **Results**: The results show that chloroquine treatment significantly abolished Plasmodium berghei infection. In the behavioral tests, the frequency of response to von Frey filament application was significantly higher in the group treated with chloroquine + uninfected (T + UI) and in the group treated with chloroquine + infected (T + I) when compared to groups untreated with chloroquine + infected (UT + I) and untreated + uninfected (UT + UI). In the hot plate test, all groups had similar reduction of the nociceptive thermal latency response when compared to the UT + UI control group. Finally, the results show that the number of nociceptive responses in both phases of formalin test was significantly higher in the T + UI groups when compared to other groups. **Conclusion**: The results show that chloroquine treatment significantly reduces the nociceptive threshold in all models tested. In addition, the study shows that, in animals untreated with chloroquine, Plasmodium berghei infection increases the response to thermal stimuli, but not to chemical or mechanical. On the other hand, *Plasmodium berghei* infection prevents the increase of nociceptive responses induced by chloroquine in inflammatory nociception, but not in mechanical or thermal. This effect may result from the consequent morpho functional changes of the nervous system due to neuroinflammation in cerebral malaria. Key words: Chloroquine; Plasmodium berghei; Nociception. **Financial Support**: CNPq Research approval by Animal Research Ethical Committee (Protocol 2015/17)

05.029 Prophylactic endocannabinoid hydrolysis inhibition alleviates end stage osteoarthritis pain and neuropathy in mice. McDougall JJ, Muley M, Reid A, Krustev E Dalhousie University – Pharmacology

Introduction: Although osteoarthritis (OA) is primarily a degenerative disorder, some patients exhibit episodic acute inflammatory events. We hypothesize that these acute inflammatory flares drive the development of joint disease and chronic OA pain. Previous studies have demonstrated that endocannabinoids are anti-inflammatory in joints and can reduce OA pain [1, 2]. Accumulation of these endocannabinoids is limited in joints by tissue enzymes such as fatty acid amide hydrolase (FAAH). The present study investigated whether blocking FAAH activity during the onset of OA inflammation could reduce joint pain during the chronic phase of the disease. **Methods:** Male C57Bl/6 mice (20-42g) received an intra-articular injection of sodium monoiodoacetate (MIA; 0.3mg). On days 1 and 14, joint inflammation was assessed by measuring joint diameter (oedema), intravital microscopy (leukocyte trafficking) and laser speckle contrast analysis (synovial blood flow). Joint secondary allodynia was determined by applying von Frey hairs to the ipsilateral hindpaw. In acute experiments, animals were treated with a single dose of URB597 (0.3mg/kg; topical over the exposed knee joint). For chronic studies, mice received 4 injections of URB597 (0.3mg/kg i.p.; days 0-3) and underwent pain assessment on day 14. Drug treatment was compared to vehicle-injected control animals. **Results:** Twenty-four hours after intra-articular injection, MIA produced a local inflammatory reaction (joint oedema, joint hyperaemia and leukocyte adherence) compared to saline-injected sham animals ($P<0.05$; $n=9$-27). By day 14, these inflammatory effects had resolved ($P>0.05$; n=5-6). Acute administration of URB597 significantly reduced MIA-induced leukocyte adherence and hyperaemia on day 1 ($P<0.05$; $n=6$-8), suggesting that endocannabinoids are anti-inflammatory in this model. Prophylactic treatment of OA mice with repeated injection of URB597 (days 0-3) prevented the development of secondary allodynia on day 14 ($P<0.05$; $n=8$-10). **Conclusions:** Acute synovitis associated with early onset OA was inhibited by blocking endocannabinoid breakdown in the joint. Early treatment of OA knees with URB597 attenuated mechanonociception at a later time point. These findings suggest that promoting articular endocannabinoids during acute inflammatory flares could be protective against the development of end stage OA pain. **References:** [1] E. Krustev, et al. Arthritis Res. Ther. 16 (2014) 437. [2] N. Schuelert et al. Pain 152 (2011) 975. Funding was provided by The Arthritis Society and Nova Scotia Health Research Foundation. Ethics was approved for the experimental use of animals (Protocol # 15-117)