05. **Pain and Nociception**

05.001 **Neuropathic pain following spinal cord injury**: **A possible role of endothelin ET_A and ET_B receptors**. Forner S, Martini AC, Rae GA UFSC – Farmacologia

Introduction: Traumatic spinal cord injury (SCI) is a devastating neurologic disorder that compromises major motor, sensory, autonomic and reflex functions. Individuals with SCI often develop chronic neuropathic pain, which may have a major impact on their quality of life. This condition is due to functional and structural plastic changes that occur centrally following injury, and include changes in receptor function to increase neuronal excitability. Nevertheless, neuropathic pain is present in 40% to 50% of SCI patients and usually develops within the first year and tends to become chronic. Endothelins are a family of peptides that exert their biological effects via distinct endothelin A (ET_AR) and endothelin B (ET_BR) receptors and contribute to sensory changes in inflammatory and neuropathic pain. However, their role in nociception following SCI still remains to be elucidated. **Methods:** SCI was induced in adult male Wistar rats by inflating an embolectomy catheter at T_{10} level. Motor behaviour was assessed in an open-field arena using the Basso, Beattie and Bresnahan locomotor rating scale. All SCI animals developed complete paraplegia, followed by modest motor improvements over the first 3 weeks post-SCI. We evaluated the sensitivity of the animals to mechanical (von Frey monofilaments) and thermal (Hargreaves test) stimulation of the paws on days 2, 7, 14, 21 and 28 after SCI. Through real-time PCR, spinal cord and dorsal root ganglion mRNA levels for ET_AR and ET_BR were evaluated in all periods post surgery, as well as protein expression of both receptors of spinal cord through western blot analysis. We assessed changes in mechanical sensitivity of hindpaws (von Frey monofilament) 21 days post-surgery from 30 min up to 270 min after the administration of BQ-123 (ET_AR antagonist, 20nM, i.t.). All procedures were approved by UFSC's Ethics Committee (#PP00680). **Results:** The frequency of responses to mechanical stimulation of forepaws was unchanged at any time point, but that of hind paws was increased at 14, 21 and 28 days. A significant reduction in withdrawal latency to heat stimulation of forepaws was detected 7 and 14 days post-SCI and of hind paws at days 14 and 21. An upregulation of ET_AR expression was detected in spinal cord at 21 days post injury when compared to sham-operated group, but ET_BR expression was not altered. ET_AR mRNA level was increased in the spinal cord on days 7, 14, 21 and 28 days and on DRG on the 7^{th} day post-SCI. ET_BR mRNA levels were increased in the spinal cord on days 2 and 7 post-surgery, but those in DRG were unchanged at all periods analyzed. Treatment with BQ-123 (20nM, i.t.) 21 days after SCI reduced mechanical sensitivity of hindpaws measured 150 and 210 min after administration when compared to its vehicle group. **Conclusion:** These findings indicate that SCI induces the activation of ET_AR and ET_BR, especially ET_AR leading to significant development of mechanical and thermal sensitivity. Moreover, the results suggest that blocking ET_AR activation with BQ-123 may influence the sensory changes seen after SCI, which may hold therapeutic potential for treating neuropathic pain from SCI. **Suppported by:** CNPq, CAPES, FAPESC, PRONEX.

05.002 TRPA1 receptor agonist sensitizes peripheral nociceptors from rats with painful peripheral mononeuropahty to mechanical stimuli. Scarante FF, Schreiber AK, Jesus CHA, Justa HC, Cunha JM UFPR – Pharmacology

Introduction: Neuropathic pain is a consequence related to damage or dysfunction of central or peripheral nervous tissue, which may arise from diverse etiologies, as trauma, herpes zoster infection and diabetes. In general, it is a difficult to treat condition, presenting resistance to the main analgesic treatments, leading to impaired quality of life and secondary complications. Once mechanical is a common symptom reported by patients with neuropathic pain, this study aims to investigate the role of TRPA1 on this parameter in CCI (chronic constriction injury) model, an animal model of neuropathy. **Methods**: Male Wistar rats (180-200 g, n= 8-10/group) provided by central biotherium of Federal University of Paraná were used. Peripheral neuropathy was induced by left sciatic nerve constriction surgery, as previously described by Bennett and Xie (1988). Briefly, 4 ligations were made with 1mm spacing between them on left sciatic nerve of animals under deep anesthesia (CCI group), while sham group only had the nerve exposed. Behavioral tests were conducted before and on different days after surgery to evaluate sensorial changes on both groups. To evaluate influence of TRPA1 receptors on neuropathy, after 19 days of surgery animals were placed in individual cages to accommodate to the environment for 15 min, then received the TRPA1 agonist mustard oil (MO - 0.1 or 0.5%, i.pl.). The stereotyped behavior of flinching on the injected paw was observed during 20 min. Right after, mechanical threshold was evaluated with an electronic analgesimeter. All procedures were approved by the local Ethics Committee (CEUA/BIO-UFPR; #649). **Results**: CCI animals exhibited a significant decrease on mechanical threshold in the ipsilateral paw since the first week after surgery, while sham animals showed no alterations on this parameter. CCI group also presented a higher response (number of flinches) to increasing concentrations (0.1 and 0.5%) of the MO injection when compared to sham group in the nineteenth day. Furthermore, it was observed that MO (at higher dose, 0.5%) also induced a significant decrease on mechanical threshold in both CCI and sham groups (reduction of 58% and 50%, respectively). **Discussion**: Corroborating with the fact of previous researches have already observed that there is an increase of TRPA1 expression after CCI surgery in DRG (dorsal root ganglion), our study observed an increase in the nociceptive response (flinching behavior) to the TRPA1 agonist MO. A possible peripheral sensitization to mechanical stimuli could be present at this model, dependent of TRPA1 expression, once CCI rats demonstrated higher responses than sham group to mechanical stimuli. Correlating these data with what was found on diabetic neuropathy in our group (unpublished data), it is interesting to note that despite of being different etiologies of neuropathy, both of that show a TRPA1 importance on development of sensorial disorders. Further studies still need to be conducted (using a specific TRPA1 antagonist) to confirm the role of TRPA1 receptors on the development of mechanical allodynia in neuropathic pain states. **Financial Support**: This work had financial support of CNPq.

05.003 Inflammatory mechanisms by which the sustained isometric contraction induces hyperalgesia in rats. Melo B[1], Santos DF[1], Jorge CO[1], Garcia J[1], Parada CA[2], Oliveira-Fusaro MCG[1] [1]FCA-Unicamp, [2]IB-Unicamp

Introduction: Muscle pain, especially the one induced by sustained isometric contraction has an important socioeconomic impact. However, the mechanisms by which the sustained isometric contraction induces hyperalgesia are unknown. We have recently developed a new model of mechanical hyperalgesia induced by sustained isometric contraction of the gastrocnemius muscle of rats. Therefore, the aim of this study was to analyze the inflammatory mechanisms by which the sustained isometric contraction of the gastrocnemius muscle of rats induces mechanical hyperalgesia. **Methods**: Male Wistar rats (200 - 250g) from the CEMIB-UNICAMP were used. The sustained isometric contraction was performed through an electrical stimulator (Grass, SX88R). Two stimulating needle electrodes were inserted into the gastrocnemius muscle and an electrical stimulation with amplitude of 2 V and a pulse-width of 19 ms for 1 hour was adopted. The mechanical hyperalgesia was quantified by the pressure analgesimeter Randal Sellito applied to the belly of the gastrocnemius muscle. In order to investigate whether the mechanical hyperalgesia induced by sustained isometric contraction was mediated by bradykinin, sympathetic amines or prostaglandins, the following drugs were used respectively: the B_1 or B_2 bradykinin receptor antagonist des-Arg9-[Leu8]-Bradykinin (DALBK) and bradyzide; the non selective β adrenoceptor antagonist atenolol as well as the selective β_2 adrenoceptor antagonist ICI 118,551; and the cyclooxygenase inhibitor indomethacin. All drugs were administered (i.m.) before sustained isometric contraction and the total volume administered was 50µL. All experimental procedures were previously approved by the Ethics Committee in Animal Research of the State University of Campinas (2448-1). **Results**: Pre-treatment with DALBK (30µg), bradyzide (15µg), atenolol (6.0µg), ICI 118,551 (1.5µg) or indomethacin (100µg) in the ipsilateral, but not in the contralateral gastrocnemius muscle, prevented ($p>0.05$, Tukey, $n=5$) the mechanical hyperalgesia induced by sustained isometric contraction. **Discussion**: These data have demonstrated the involvement of bradykinin, sympathetic amines and prostaglandins in mechanical hyperalgesia induced by sustained isometric contraction of the gastrocnemius muscle of rats. Considering that this study has demonstrated the involvement of important inflammatory mediators in the model of mechanical muscle hyperalgesia induced by sustained isometric contraction, it is possible to suggest that this new model has potential to be important in the study of muscle hyperalgesia in rats. Financial Support: FAPESP (2011/11064-4; 2012/10402-6).

05.004 TRPA1 receptor stimulation by hydrogen peroxide is critical to trigger pain and inflammation during acute gout attack. Trevisan G[1], Hoffmeister C[1], Rossato MF[1], Oliveira SM[1], Silva MA[1], Silva CS[1], Nassini R[2], Materazzi S[2], Fusi C[2], Petri GP[3], Geppetti P[2], Ferreira J[4]
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Introduction: Gout is the principal cause of inflammatory arthritis in men and postmenopausal women. However, the efficacy of the current treatments is still limited. Acute gout attacks are produced by articular deposition of monosodium urate (MSU) crystals and cause severe joint pain and inflammation, associated with oxidative stress. The transient potential receptor ankyrin 1 (TRPA1) is a sensor for oxidative substances (such as hydrogen peroxide - H_2O_2) found in peptidergic sensory fibers associated to inflammatory pain, but its role in gout is unknown. The goal of this study was to explore the TRPA1 participation in an experimental model of acute gout attack in rodents.
Methods: Experiments were performed using male Wistar rats (200-250 g, N=5-8) bred in our animal house, and wild-type (*Trpa1*$^{+/+}$) or TRPA1 deficient mice (*Trpa1*$^{-/-}$) (25-30 g, N=7-10) (Jackson Laboratories, Italy). Protocols were approved by the Ethics Committee of the Federal University of Santa Maria (process number 108/2011(2)) or by the University of Florence (research permit number 204/2012-B). TRPA1 role in MSU intra-articular (i.a., ankle) injection-mediated inflammatory responses were evaluated using TRPA1 antagonists, defunctionalization of TRPA1 expressing fibers, and also TRPA1 genetic ablation of the TRPA1. TRPA1 expression, nociception, edema, plasma extravasation, neutrophil infiltration, interleukin 1β (IL-1β), H_2O_2 production or calcitonin gene-related peptide (CGRP) release were investigated after MSU i.a. injection. The possible activation of TRPA1 by H_2O_2 during the gout attack was also evaluated mimicking it with H_2O_2 or preventing it using H_2O_2-detoxifying enzyme catalase and the reducing agent dithiothreitol (DTT). **Results:** TRPA1 antagonism, gene ablation or sensory fiber defunctionalization largely reduced nociception, edema, neutrophil infiltration, plasma extravasation and IL-1β increase caused by MSU i.a. injection. We have also observed that i.a. injection of MSU not only increased TRPA1 expression, but also CGRP release (an index of TRPA1 function) in MSU injected tissue. Besides inflammation, MSU also increased the level of H_2O_2 in the synovial tissue, but this effect was prevented by catalase and DTT. Finally, the signs of gout attacks were mimicked by i.a. injection of H_2O_2, and these effects were prevented by TRPA1 antagonism, gene ablation or sensory fiber defunctionalization. **Discussion:** Our results suggested that MSU i.a. injection increases tissue H_2O_2 thereby stimulating TRPA1 on sensory nerve endings to produce inflammation and nociception. Thus, the blockage of TRPA1 seems to be a useful target in acute gout attacks management. **Financial agencies:** This study was supported by Conselho Nacional de Desenvolvimento Científico (CNPq), Coordenação de Aperfeiçoamento de Pessoal deNível Superior (CAPES) to J.F. and in part by Ente Cassa di Risparmio di Firenze (Italy) to S.M. **Acknowledgements:** Fellowships from CNPq and CAPES are acknowledged.

05.005 Muscle hyperalgesia is mediated by neutrophils and P2X3 receptors. Jorge CO[1], Melo B[1], Santos DF[1], Parada CA[2], Oliveira-Fusaro MCG[1] [1]FCA-Unicamp, [2]IB-Unicamp

Introduction: Among the kinds of pain that affect people throughout their lives, muscle pain, specially the one induced by sustained isometric contraction, is one of the most prevalent and has an important socioeconomic impact. Despite its clinical relevance, the molecular mechanisms involved in the development of muscle pain induced by sustained isometric contraction are poorly understood. Recently, we have developed a new model of muscle hyperalgesia and we have demonstrated the involvement of bradykinin, sympathetic amines and prostaglandins in this process. Considering the well-described role of neutrophils and endogenous ATP via activation of P2X3 receptors on inflammatory pain, the aim of this study was to verify whether the mechanical hyperalgesia induced by sustained isometric contraction is mediated by neutrophils and peripheral P2X3 receptors. **Methods**: Male Wistar rats (200 - 250g), from CEMIB-UNICAMP were used and all experimental procedures were previously approved by the Ethics Committee in animal research of the State University of Campinas (2448-1). The sustained isometric contraction was performed by electrical stimulator (Grass, SX88R) with intensity of 2V, pulses of 19-ms duration, for 1 hour, directly to the belly of the gastrocnemius muscle of rats. The mechanical hyperalgesia was quantified by the pressure analgesimeter (Randal Sellito) applied directly on the muscle belly. To investigate whether the mechanical hyperalgesia was mediated by neutrophils or endogenous ATP via activation of P2X3 receptors, fucoidan, an inhibitor of neutrophil migration, and the selective P2X3 receptor antagonist A317491 were administered before sustained isometric contraction. The total volume administered was 50µL. **Results**: Pre-treatment with fucoidan (25mg/kg, s.c., 20 min., n=5) or with A317491 (60µg, i.m., 5 min, n=5) prevented ($p>0.05$, Tukey, n=5) the mechanical hyperalgesia induced by sustained isometric contraction. Administration of A317491 (60µg, i.m., 5 min) in the contralateral gastrocnemius muscle did not affect ($p<0.05$, t test) the hyperalgesic response. **Discussion**: The data of the present study have demonstrated that neutrophils and endogenous ATP via peripheral P2X3 receptors play an important role in the development of muscle hyperalgesia. This study suggests that neutrophils and P2X3 receptors are important targets to control muscle inflammatory pain. Support: FAPESP (2011/11064-4).

05.006 Development of a new model of muscle hyperalgesia. Santos DFS[1], Melo B[1], Jorge CO[1], Garcia J[1], Parada CA[2], Oliveira-Fusaro MCG[1] [1]FCA-Unicamp, [2]IB-Unicamp

Introduction: Muscle pain due to sustained isometric contraction is one of the most prevalent kinds of pain and has an important socioeconomic impact. However, despite its clinical relevance, the molecular mechanisms involved in the development of muscle pain induced by sustained isometric contraction are unknown. This is mainly due to the absence of a more realistic experimental model that has a good degree of prediction of pharmacological control of pain. Therefore, the aim of this work was to develop a new model of muscle hyperalgesia induced by sustained isometric contraction. **Methods**: Male Wistar rats (200 - 250g), from CEMIB-UNICAMP, were used and all experimental procedures were previously approved by the Ethics Committee in animal research of the State University of Campinas (2448-1). The sustained isometric contraction was performed by electrical stimulator (Grass, SX88R) with intensity of 0.5, 1.0 or 2.0V, pulses of 19-ms duration, for 15 min., 30 min., or 1 hour, directly to the belly of the gastrocnemius muscle of rats. The mechanical hyperalgesia was quantified by the pressure analgesimeter (Randal Sellito) applied directly on the muscle belly. To investigate the hyperalgesic character of the behavioral responses, QX-314, a lidocaine N-ethyl bromide quaternary salt, were used. To investigate whether the mechanical hyperalgesia induced by sustained isometric contraction was mediated by inflammatory mediators, dexamethasone was used. **Results**: The sustained isometric contraction induced by electrical stimulation with 2V, for 1 hour, induced mechanical hyperalgesia ½ and 1 hour post sustained isometric contraction significantly greater than that induced by stimulations of 2V for 15 and 30min ($p<0.05$, Two Way ANOVA, Bonferroni post test), of 0.5 and 1.0V for 1 hour ($p<0.05$, Two Way ANOVA, Bonferroni post test) and sham ($p<0.05$, Two Way ANOVA, Bonferroni post test). Intramuscular but not subcutaneous administration of QX134 (2%) 5 min before sustained isometric contraction or pretreatment with dexamethasone (1mg/kg, s.c.) prevented the mechanical hyperalgesia ($p<0.05$, Tukey test, n=5). Administration of QX134 (2%) in the contralateral gastrocnemius muscle did not affect ($p<0.05$, t test) the hyperalgesic response. **Discussion**: This data demonstrated that the sustained isometric contraction of gastrocnemius muscle of rats induced mechanical hyperalgesia mediated, at least in part, by inflammatory mediators. This study points out that this new model has potential to be an important model to study muscle hyperalgesia.

05.007 Involvement of TRPV-1 and TRPA-1 channels in the antinociceptive and antiedematogenic effects of hydroalcoholic extract from *Machaerium hirtum* (Vell.)Stellfeld (Barks). Lopes JA[1], Nishijima CM[1], de Souza Maria NCV[2], Sannomiya M[2], Rocha LRM[1], Hiruma-Lima CA[1] [1]IBB-Unesp-Botucatu – Fisiologia, [2]EACH-USP

Introduction: *Machaerium hirtum* (Vell.) Stellfeld, popularly known as "Jacarandá-de-Espinho" or "bico-de-andorinha" belongs to the Fabaceae family, and is of common occurrence at the Brazilian Cerrado. Its barks have been used in folk medicine against cough, diarrhea, cancer and gastric ulcer. The phytochemical profile of the hydroalcoholic extract from the barks (HEM) revealed a mixture of triterpenes (α and β-amyrin) and many C-glycosylated flavones (apigenin). Studies have reported that these compounds have antinociceptive and anti-inflammatory effects. **Methods**: The capsaicin, cinnamaldehyde or menthol-induced nociception tests were performed to investigate the antinociceptive effect of HEM according to Sakurada *et al.,* 1992; Andrade *et al.,* 2008; Baggio *et al.,* 2011. Swiss male mice (25-30g; n=7-10) were treated orally with vehicle (saline 10mL/kg) or HEM (62.5; 125; 250mg/kg) 1h before intraplantar injection of 20 µl of capsaicin (2µg/paw), cinnamaldehyde (40nmol/paw) or menthol (2µg/paw) and were observed for 6min (for capsaicin or cinnamaldehyde) and 20min (for menthol).The time (s) mice spent licking the injected paw was registered and considered as indicative of nociception. The antiedematogenic effect of HEM against xylene-induced ear edema was evaluated according to Swingle *et al.,* 1981. Fasting mice (2hours) were treated with vehicle or HEM (62,5;125;250mg/kg, p.o) 1hour before topical application of xylene (20 µl) to the anterior and posterior surfaces of the right ear. Dexamethasone (5mg/kg, i.p) was used as positive control 2 hours before xylene topical application. Statistical significance was determined by ANOVA followed by Dunnett´s test; levels of $P<0.05$ were considered to be statistically significant. Animal Ethics Committee Protocol Number: 367/2011 UNESP. **Results** and **Discussion**: The present study demonstrated that administration of HEM caused a significant inhibition of nociception induced in the hind paw by capsaicin (29.6%; 42% at the doses of 125 and 250mg/kg, respectively) and cinnamaldehyde (44%; 38.6%; 35%), which are highly selective agonists of TRPV1 and TRPA1 channels, but not against menthol, a TRPM-8 agonist. The results also show that HEM caused an antiedematogenic effect against ear edema induced by xylene (39%; 47% - 125 and 250mg/kg, respectively), an irritant aromatic agent responsible for neurogenic inflammation through activation of TRPA-1 channel. Financial Support: Biota FAPESP/ CAPES

05.008 New muscarinic agonist reversed thermal hyperalgesia and mechanical allodynia signs in model of morphine-induced tolerance in rats. Monteiro CES[1], Nascimento-Júnior N[2], Zapata-Sudo G[1], Fraga CAM[2], Barreiro EJ[2], Sudo RT[1] [1]ICB-UFRJ – Desenvolvimento de Fármacos, [2]FF-UFRJ

Introduction: Tolerance is observed after prolonged use of morphine. The present work describes the effectiveness of a new muscarinic receptor agonist pyrazolo [3,4-b] pyrrolo [3,4-d] pyridine (LASSBio-981) to reverse the morphine-induced tolerance for the analgesic effect in rats with spinal nerve ligation (SNL). **Methods:** The protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro (Protocol DFBCICB 065). Thermal hyperalgesia and mechanical allodynia were induced by SNL (L5) in rats. Seven days after surgery with sustained pain symptoms, morphine was infused by osmotic mini-pumps (2.5 mg/kg/day i.p.) during 30 days. LASSBio-981 (10 mg/kg p.o.) was administered orally after 16 days of morphine infusion and establishment of tolerance for 14 days. **Results**: Withdrawal to thermal stimulation was significantly reduced from 11.4 ± 0.2 s to 7.5 ± 0.3 s (n= 4) after surgery (on 7^{th} day after surgery) and increased ($P<0.05$) by morphine (2.5 mg/kg/day i.p., n= 4) to 12.0 ± 1.4 s, on day 8 after surgery. During 16 days of treatment with morphine, the withdrawal to thermal stimulation was significantly decreased, no significant difference of the post surgery control. Oral administration of DMSO to SNL animals and tolerance to morphine did not induce a significant change in the parameter. The withdrawal to thermal stimulation was significantly reduced from 11.3 ± 0.6 s to 7.1 ± 0.6 s (n= 4) after surgery and increased ($P<0.05$) by morphine to 14.3 ± 0.7 s, on day 8 after surgery. After 16 days of treatment with morphine, the withdrawal to thermal stimulation was significantly decreased, no significant difference of the post surgery control. The administration of LASSBio-981 (10 $mg.kg^{-1}$ p.o) significantly increased the withdrawal to thermal stimulation from 7.7 ± 0.2 s to 12.6 ± 1.4 s, 12.2 ± 0.5 s, 13.0 ± 0.7 s, on days 24, 26 and 30, respectively after surgery. Mechanical withdrawal threshold was significantly reduced from 39.8 ± 0.5 g to 17.6 ± 1.3 g (n= 4) after surgery and increased ($P<0.05$) by morphine (2.5 mg/kg day^{-1} i.p., n= 4) to 33.7 ± 0.8 g, on day 8 after surgery. During 16 days of treatment with morphine, the mechanical withdrawal threshold was significantly decreased, no significant difference of the post surgery control. LASSBio-981 (10 mg/kg p.o) significantly increased the withdrawal threshold from 17.9 ± 1.4 g (n= 4) to 35.2 ± 1.0 g. The administration of methoctramine (10 µg i.t) reduced time-dependent the analgesic effect of LASSBio-981. **Discussions**: We previously described the effectiveness of compound LASSBio-981, M2 muscarinic receptor agonist in model of neuropathic pain [1]. A previous study [2] has reported that spinal endogenous acetylcholine plays an important role in mediating the analgesic effect of systemic morphine through both muscarinic and nicotinic receptors. Our study provides the first evidence that M2 muscarinic receptor prevent and reverses the morphine-induced tolerance in rats with neuropathic pain. **References**: [1] Mendes et al. Clin and Exp Pharm & Physiology, 2013. In Press: Antyhiperalgesic affects of a novel muscarinic agonist (LASSBio-873) in spinal nerve ligation; [2] Chen and Pan. Anesthesiology 95: 525, 2001. **Financial Support:** INCT/INOFAR, CNPq, CRISTÁLIA, FAPERJ

05.009 Role of interleukin-33/ST2 receptor signaling in chronic constriction injury-induced neuropathic pain in mice. Zarpelon AC[1], Rodrigues FC[1], Carvalho TT[1], Souza GR[2], Ferreira SH[2], Alves-Filho JC[2], Liew FY[3], Cunha TM[2], Cunha FQ[2], Verri Junior WA[1] [1]UEL – Patologia, [2]FMRP-USP – Farmacologia, [3]University of Glasgow – Immunology, Infection and Inflammation

Introduction and aims: IL-33 is a member of IL-1 family of cytokines that signal through ST2 receptor. We have reported that IL-33/ST2 signaling mediates mechanical hyperalgesia in innate and Th1/Th17 inflammation. In the present study we addressed the role of IL-33/ST2 signaling in chronic constriction injury (CCI)-induced neuropathic pain in mice. **Methods:** Sex matched wild type (WT - Balb/C background) and $ST2^{-/-}$ mice from the University of São Paulo were used. Mechanical hyperalgesia was evaluated by an electronic version of von Frey filaments, cytokine levels were determined by ELISA, proteins activation were determined by western blot and specific targets were inhibited by pharmacological tools. CCI was induced by one ligation of the sciatic nerve. Statistical differences were considered significant for $P<0.05$. Procedures were approved by the Ethics Committee of Londrina State University (Of.Circ.47/2010).

Results and discussion: CCI induced significant IL-33 production in the spinal cord (L4-L6), and hyperalgesia during 20 days starting 3 days after surgery, which was inhibited in $ST2^{-/-}$ mice. IL-33 intrathecal (i.t.) injection induced dose-dependent hyperalgesia, which was inhibited in $ST2^{-/-}$ and $TNFR1^{-/-}$ mice and by IL-1ra as well as induced spinal production of TNFα and IL-1β. The hyperalgesia induced by IL-33 i.t. injection was reduced by inhibitors of PI3K, mTOR, MAP kinases (p38, ERK and JNK) and NFκB. In agreement, CCI-induced spinal activation of PI3K, AKT, mTOR, MAP kinases and NFκB were reduced in $ST2^{-/-}$ mice compared to WT mice as determined by western blot. The treatment with minocycline and fluorocitrate inhibited IL-33-induced hyperalgesia, which lined up with diminished activation of spinal GFAP and Iba-1 in CCI $ST2^{-/-}$ mice compared to CCI WT mice. Therefore, IL-33/ST2 signaling pathway mediates chronic constriction injury-induced neuropathic pain by activating spinal mechanisms and glia.

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05.010 Thalidomide reduces delayed-onset muscle soreness (DOMS) induced by intense acute swimming in mice. Borghi SM[1], Pinho-Ribeiro FA[1], Zarpelon AC[1], Cardoso RDR[1], Casagrande R[2], Verri Junior WA[1] [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas

Introduction: Thalidomide is a synthetic glutamic acid derivative also named as α-N-phthalimidoglutarimide, known to inhibit TNF-α production by enhancing the degradation of its messenger RNA suggesting an important immunomodulatory effect and its possible use in the treatment of inflammatory painful conditions like DOMS. Thus, the antihyperalgesic, anti-inflammatory and antioxidant effects of thalidomide were investigated in a mouse model of intense acute swimming-DOMS. **Methods**: Animals' care and handling procedures were in accordance with the International Association for Study of Pain (IASP) guidelines and with the approval of the Institutional Ethics Committee for Animal Research of the Universidade Estadual de Londrina, process number 2066.2011. Sham animals swam for just 30 s. DOMS was induced by one exercise session of intense acute swimming during 120 min, without additional stimulus. Muscle mechanical hyperalgesia was evaluated between 6-48 h after the swimming session by electronic von Frey anesthesiometer. TNF-α, IL-1β and IL-10 production were evaluated in the soleus and gastrocnemius muscles and spinal cord (L4-L6) by ELISA. Edema (2-48 h) was measured by distal hindlimb circumference using a caliper, and by weighting of the soleus and gastrocnemius muscles. Leukocyte recruitment and oxidative stress were analyzed in the soleus and gastrocnemius muscles by MPO activity and skeletal muscle GSH levels, respectively. Histological analyses of the soleus muscle were performed to assess tissue injuries. Thalidomide (5-45 mg/kg) was given intraperitoneally to animals 30 min before plus reinforcement at 12 h after the intense acute swimming, depending on the experiment. **Results**: Thalidomide inhibited in a dose- and time-dependent manner the exercise-induced increased of nociceptive response between 12 and 36 h (82% at 24 h). Additionally, thalidomide treatment significantly reduced the increased levels of cytokine (TNF-α, IL-1β and IL-10) induced by intense acute swimming in the soleus muscle (35%, 39% and 24%, respectively) and spinal cord (90%, 88% and 57%, respectively) and reduced inflammatory parameters such as edema (59% at 24 h) and leukocyte recruitment in the soleus muscle (67%) of exercised animals. Moreover, the peripheral oxidative stress observed by the reduced glutathione (GSH) depletion in the soleus muscle after intense acute swimming was reversed by thalidomide pre-treatment (93%). In the gastrocnemius muscle no significantly changes were observed in the same experiments. **Discussion**: These results suggest that thalidomide could be considered a new pharmacological tool, capable of reducing muscle pain, frequently observed after intense acute physical exercise in which the body is unaccustomed. Therefore, thalidomide reduced DOMS-induced muscle mechanical hyperalgesia, edema, oxidative stress and cytokine production. Financial support and acknowledgements: We appreciated the technical support of Pedro S. R. Dionísio Filho. This work received financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), SETI / Fundação Araucária and Governo do Estado do Paraná.

05.011 Depot dexamethasone delays allodynia development and inhibits dorsal root ganglion NF-kappa B translocation in sciatic nerve-injured rats. Bastos LFS[1], Vago JP[2], Caux TR[2], Costa BL[3], Godin AM[4], Menezes RR[4], Pena RR[1], Machado RR[4], Fialho SL[3], Sousa LP[2], Moraes MFD[1], Coelho MM[4] [1]UFMG – Fisiologia e Biofísica, [2]UFMG – Análises Clínicas e Toxicológicas, [3]FUNED – Desenvolvimento Farmacotécnico, [4]UFMG – Produtos Farmacêuticos

Introduction: Neuropathic pain is an unmet clinical need worldwide, with prevalence as high as 7-8% in industrialized countries (Bouhassira et al, 2008; Torrance et al, 2006). Although neuroimmune interactions associated with the development of pain sensitization in models of neuropathic pain have been widely studied, some aspects require further investigation. We thus aimed to examine whether a prolonged treatment with dexamethasone – an anti-inflammatory and immunossupressant drug – delays the development of allodynia, and whether such an effect is associated with peripheral anti-neuroinflammatory effect. **Methods** Chronic constriction injury (CCI) of the sciatic nerve was performed in Wistar rats under anesthesia induced by ketamine (90 mg/kg) and xylazine (9 mg/kg) hydrochlorides. Four ligatures with a flexible nylon thread were tied loosely around the sciatic nerve at mid-thigh level. Sham-operated rats underwent the same surgical procedure, save that the sciatic was not ligated (Bastos et al, 2012; Bennett & Xie, 1988). Electronic von Frey test was applied to assess allodynia (Bastos et al, 2012; Vivancos et al, 2004). A modified release implant containing dexamethasone acetate (2.4 mg in PLGA) was placed perineurally at the moment of CCI or at day 12 after this surgery. Dorsal root ganglia (DRG's; L4-L5) were harvested and nuclear extracts were assayed by Western blot in order to detect NF-kappa B p65/Rel*A* translocation (Martins et al, 2011). ImageJ was used to semi-quantitatively analyze the immunoblots. This study was approved by the local ethics committee from UFMG (protocol # 168/2009). **Results:** The dexamethasone implant delayed the development of allodynia for approximately three weeks in CCI rats compared with vehicle-treated animals when the implantation was performed at day 0, but allodynia was not reversed when the implantation was performed at day 12 after surgery – a time point at which the allodynia was fully developed. Dexamethasone treatment prevented rat body mass gain, and this catabolic effect indicates that the drug was distributed systemically after placement of the implants, but not only around the site of implantation. NF-kappa B was translocated in DRG's in CCI rats compared with naïve or sham animals (day 15) and dexamethasone implant inhibited p65/Rel*A* translocation in CCI rats compared with vehicle-treated animals. **Discussion:** This study shows the delay of CCI-induced allodynia development and peripheral neuroinflammation induced by a steroidal anti-inflammatory drug and reinforces the evidence that immune/inflammatory response is essential for triggering the development of CCI-induced allodynia, preliminarily to further investigation of the association between peripheral neuroinflammation and the development of experimental neuropathic pain. **Acknowledgements:** We thank CAPES, (Brazil), CNPq (Brazil), FAPEMIG (Minas Gerais, Brazil) and Pró-Reitoria de Pesquisa (PRPq, UFMG, Minas Gerais, Brazil) for constant support. **References:** 1. Bastos LFS et al. Neurosci Lett 510: p. 20, 2012; 2. Bennett GJ & Xie. Pain 33: p. 87, 1988; 3. Bouhassira D et al. Pain 136: p. 380, 2008; 4. Martins FS et al. Int J Med Microbiol 301: p. 359, 2011; 5. Torrance N et al. J Pain 7: p. 281, 2006; 6. Vivancos GG et al. Braz J Med Biol Res 37: p. 391, 2004

05.012 Evaluation of the antinociceptive effect of Y-terpinene and possible mechanisms of action in mice. Freitas FFBP[1], Souza RDS[1], Reis Filho AC[1], Sousa SEL[1], Sousa DP[2], Almeida FRC[1] [1]NPPM-UFPI, [2]UFS – Pharmacy

Introduction: The pain is often associated with many diseases. Current therapies are usually insufficient due to severe side effects and limited effectiveness. So, the search for new molecules is continuous and necessary. The essential oils are products with great pharmacologic potential, used in aromatherapy, agricultural and food industries. The monoterpene γ-terpinene (γ-TPN) is a chemical constituent of the essential oil of many plant species that exhibit various pharmacological activities, such as analgesic and anti-inflammatory. The aim of this study was to investigate the antinociceptive effect of oral γ-TPN, in chemical nociception models, as well as possible mechanisms involved. **Methods:** Male Swiss mice (n = 6-9, 20-30 g) were treated with γ-TPN (0.78-50 mg/kg, po) 60 min before the intraplantar injection of formalin (2 %), capsaicin (2 µg) or glutamate (10 µmol). Control animals received vehicle (C- 2 % Tween 80 in saline), MK 801 (0.03 mg/kg ip) or morphine - MOR (5 mg/kg sc). Nociception was evaluated by quantifying paw licking time after formalin (5 min (first phase) and 15–30 min (second phase), capsaicin (5 min) or glutamate (15 min). To determine the mechanism of action in the glutamate test, the animals were pretreated (20 min before γ-TPN) with naloxone - NAL (opioid antagonist, 2 mg/kg ip) and glibenclamide - GLIB (ATP- sensitive K+ channels inhibitor, 3 mg/kg ip). The locomotor activity was evaluated in the open field test. The protocols were approved by the Animal Ethics Committee/UFPI (n°. 008/12) and were carried out in accordance with the current ethical guidelines for investigation of experimental pain in conscious animals. **Results:** γ-TPN (12.5 and 25 mg/kg) significantly reduced the formalin response in both phases of the test when compared with vehicle. 1st phase (C: 77.09 ± 9.04; γ-TPN-12.5: 14.62 ± 9.11; γ-TPN-25: 16.94 ± 4.48; MOR: 18.19 ± 3.10) and 2nd phase (C: 66.79 ± 8.20; γ-TPN-12.5: 25.89 ± 4.90; γ-TPN-25: 26.94 ± 9.66; MOR: 17.32 ± 5.46) (*$p<0.05$). A significant reduction in time length spent on licking the paw was observed with γ-TPN (25 and 50 mg/kg) in the capsaicin test (C: 40.31 ± 2.46; γ-TPN-25: 22.70 ± 3.37; γ-TPN-50: 20.97 ± 4.83; MOR: 4.63 ± 1.93) (*$p<0.05$) and in the glutamate-induced nociception (C: 104.30 ± 6.2; γ-TPN-3.125: 43.53 ± 12.34; γ-TPN-6.25: 52.29 ± 12.58; MK-801: 40.77 ± 7.84)(*$p<0.05$). The γ-TPN antinociceptive effect was reversed by NAL (C: 106.88 ± 6.22; MOR: 11.36 ± 1.36; NAL+MOR: 84.99 ± 10.66; γ-TPN-3.125: 43.53 ± 12.34; NAL+γ-TPN-3.125: 133.83 ± 11.48) and GLIB (C: 112.72 ± 5.50; γ-TPN-3.125: 41.77 ± 10.83; GLIB+γ-TPN-3.125: 105.65 ± 10.69; GLIB: 88.24 ± 8.53). In the open field test, γ-TPN (12.5 and 25 mg/kg po) did not change the frequency of crossings. **Discussion:** These results provided for the first time, convincing evidence that oral administration of the monoterpene γ-TPN exerted pronounced antinociception when assessed in chemical-induced nociception models in mice. The antinociceptive action of γ-TPN possibly involves the participation of opioid system via potassium channels. It is unlikely that the observed effects are due to central depressant activity since γ-TPN was unable to modify the animals' motor behavior in the open field test. **Financial Support:** UFPI, CAPES/Brazil.

05.013 Antinociceptive activity of the ethanolic extract from the flowers of *Acmella oleracea* (L.) R.K. Jansen in mice. Corso CR[1], Nomura EO[1], Hocayen PAS[1], Nascimento AM[2], Cipriani TR[2], Baggio CH[1], Werner MFP[1] [1]UFPR – Farmacologia, [2]UFPR – Bioquímica

Introduction: *Acmella oleracea* (L.) R.K. Jansen is commonly used as an analgesic by some communities from Amazon region, mainly to treat toothache. This study evaluated the antinociceptive effect of the ethanolic extract obtained from flowers of *A. oleracea* (EEAO).

Methods: Male mice (~30 g) were treated by intraperitoneal route (i.p.) with EEAO (10, 30 or 100 mg/kg) before the induction of orofacial nociceptive response by 20 µl of formalin (2.5%), capsaicin (5.2 nmol) and cinnamaldehyde (100 nmol), hindpaw thermal heat hyperalgesia (hot plate test) and mechanical allodynia (traumatic sciatic nerve injury) (CEUA/BIO-UFPR, 544). **Results**: EEAO (10, 30 and 100 mg/kg) reduced both neurogenic and inflammatory phases of the formalin- and capsaicin- and cinnamaldehyde-induced orofacial nociception. Interestingly, EEAO at 100 mg/kg (i.p.) also reversed capsaicin-induced heat hyperalgesia assessed as the latency to paw withdrawal in the hot plate test at 15 and 30 min (capsaicin: 5.0 ± 0.6 s and 5.4 ± 0.7 s, EEAO: 17.7 ± 3.5 s and 19.2 ± 1.9 s, respectively). Also in the hot plate test, paw withdrawal latency was increased by EEAO (100 mg/kg) and this response was partialy reversed by naloxone at 30 min. Furthermore, EEAO (100 mg/kg) also reduced mechanical allodynia caused by partial sciatic nerve ligation in 58 ± 14% at 3 h. The EEAO did not affect the locomotor activity of mice in the open field test, and the estimated LD50 value of EEAO was 889.14 mg/kg. **Discussion**: Taken together, EEAO exhibited a significant antinociceptive activity in all experimental pain models used in this study. Moreover, the phytochemical and biochemical analysis of the EEAO found the presence of alkamides like spilanthol, which is responsible for the local anesthetic for toothache. Thus, based on these data, it is possible to suggest that the potent effects promoted by EEAO are related to their anesthetic properties. However, further studies are necessary for the elucidation of mechanisms underlying this action. **Financial Agencies**: CNPq (476653/2010-0)

05.014 Antinociceptive effect of dipyrone and its metabolites on hyperalgesia induced by carrageen and prostaglandin E_2. Assis DCR[1], Malvar DC[1], Vaz ALL[2], Melo MCC[1], Rae GA[3], Clososki GC[2], Souza GEP[1] [1]FCFRP-USP – Física e Química, [2]FCFRP-USP – Química Organic, [3]UFSC – Farmacologia

Introduction: Dipyrone is a pro-drug with potent analgesic and antipyretic effects. After its administration, dipyrone is rapidly hydrolyzed to 4-methylaminoantipiryne (4-MAA), which is further metabolized to 4-formylaminoantipiryne (4-FAA), 4-aminoantpiryne (4-AA) and 4-acetylaminoantipiryne (4-AAA) (Cohen, *Eur. J. Clin. Pharmacol*., 54:549, 1998). Differently from non steroidal anti-inflammatory drugs, dipyrone's antinociceptive effect is not related only to inhibition of prostaglandin E_2 (PGE_2) synthesis, but also involves other incompletely understood mechanisms (Sachs, *PNAS*, 10:10, 2004). Aiming to better understand its antinociceptive effects, the present study compared the analgesic effect of dipyrone and its metabolites on hypernociception induced by carrageenan and prostaglandin E_2.**Methods:** Male Wistar rats weighing 180 to 200 g were habituated to the test room for at least 1 h prior to experiments. Mechanical hyperalgesia was assessed by electronic von Frey apparatus according to the protocol described by Vivancos, *Braz J Med Biol Res* 37: 391, 2004. Dipyrone (60-120 mg/kg) and its metabolites 4-MAA (60 and 120 mg/kg), 4-AAA (180 mg/kg), 4-AA (120 mg/kg) and saline (control group) were given intraperitoneally 2 h after PGE_2 (100 ng/paw), carrageenan (200 ug/paw) or saline injection. Hypernociception was evaluated 3 hours after injecting the nociceptive stimuli. Data are expressed as mean ± S.E.M. and were statistically evaluated using ANOVA followed by Tukey test, $p < 0.05$. This study was approved by the Ethic Committee of FMRP/USP (process n° 019/2012). **Results:** Dipyrone (DIP) and its metabolites 4-MAA and AA showed analgesic effects against carrageenan (Cg)-induced hyperalgesia. The intensities of hyperalgesia (Δ withdrawal threshold, in g (D g)) were: saline + saline: 0.72 ± 0.47; saline + Cg: 12.5 ± 0.27; DIP (120mg/kg) + Cg: 6.9 ± 0.91; 4-AA (120 mg/kg) + Cg: 3.25 ± 0.77; 4-MAA (120 mg/kg) + Cg: 2.85 ± 1.13. DIP and the metabolite 4-MAA reduced the PGE_2-induced hyperalgesia, whereas 4-AA did not: D g, saline + saline: 0.53 ± 0.37; saline + PGE_2: 10.1 ± 0.69; DIP (120 mg/kg) + PGE_2: 3.0 ± 1.54; 4-AA + PGE_2: (120 mg/kg): 10.7 ± 1.48; (240 mg/kg): 9.96 ± 0.99; 4-MAA (60 mg/kg): 1.28 ± 0.65; (90 mg/kg): 0.98 ± 0.98). **Discussion:** These results show for the first time that, like dipyrone, its metabolite 4-MAA reduced PGE_2-induced hyperalgesia, suggesting that this metabolite is responsible for dipyrone antinociceptive effect independent on PGE_2 synthesis inhibition. Both 4-MAA and AA are effective against CG-induced hyperalgesia. **Financial support:** CAPES, CNPq and FAPESP.

05.015 Actions of Phα1β peptide purified from the Brazilian spider *Phoneutria nigriventer* venom on the adverse effects caused by morphine in mice. Tonello R[1], Rigo F[2], Gewehr C[2], Gomez MV[2], Ferreira J[1] [1]UFSM, [2]Santa Casa BH

Introduction: Opioids, used alone or with adjuvant analgesics, are the standard therapy for the management of several kinds of pain, however their use is limited by adverse effects. Voltage-dependent calcium channel (VDCC) is involved in analgesia, but its role on opioid-induced side effects is little known. Thus, the goal of this study was to evaluate the possible actions of the peptide Phα1β, a VDCC blocker, on the antinociceptive and the adverse effects produced by single or repeated administration of morphine in mice. **Methods:** Male adult C57/Bl6 mice (N=6-8, 20-30 g) were used in this study (Ethics Committee: 23081.005024/2010-88). We firstly assessed the effects of a single administration of morphine (1-10 mg/kg, subcutaneous-s.c.) or saline on heat or mechanical nociception and on gastrointestinal transit (GIT). Vehicle or Phα1β (0.1 or 30 pmol/site, intrathecal-i.t.) were injected 5 min before morphine. Next, we evaluated the effect of Phα1β on mechanical and heat hyperalgesia, tolerance, constipation and withdrawal syndrome induced by repeated administration of morphine in mice (increasing doses 3 times a day for 3 consecutive days). In the 4th day, the mice received a morphine (10 mg/kg, s.c.) or saline challenge and were treated with Phα1β (0.01-30 pmol/site, i.t) or vehicle. **Results:** A single s.c. administration of morphine reduced heat, but not mechanical, nociception (maximal inhibition-Imax=100% and effective dose 50-ED50=3.1 (2.9-3.4) mg/kg) as well as decreased GIT (reduction of 70 ± 7%) 60 minute after treatment. The treatment with Phα1β (30 pmol/site, i.t.) did not alter neither nociception nor GIT administered alone, but slightly increased (20 ± 7%) the effect of a single dose of morphine on thermal nociception and GIT (45 ± 6%). Repeated treatment with morphine caused not only tolerance to its antinociceptive effect, but also paradoxically increased heat and mechanical nociception (hyperalgesia) in treated mice. Phα1β (0.1-30 pmol/site) was able to reverse both mechanical (Imax=95 ± 15% and ED50=2.5 (1.7-3.8) pmol/site) and heat (Imax=100% and ED50=2.4 (0.1-3.3) pmol/site) hyperalgesia induced by repeated morphine. Furthermore, a sub-threshold dose of Phα1β (0.1 pmol/site, i.t.) also largely reversed (83 ± 1%) the antinociceptive tolerance produced by repeated morphine treatment. Finally, repeated treatment with morphine also induced constipation and withdrawal syndrome, both effects reversed (23 ± 4 and 100%, respectively) by the treatment with Phα1β (30 pmol/site, i.t.). **Discussion:** Strategies used on opioid treatment include the concomitant administration of non-opioid adjuvant analgesics, which may increase opioid-induced analgesia and reduce opioid-related side effects. We observed that the injection of Phα1β was effective in potentiate the analgesic effect caused by a single dose of morphine as well as reduce hyperalgesia, tolerance, constipation and withdrawal syndrome induced by repeated administration of morphine in mice. Thus, Phα1β, possibly by blocking VDCCs, could be a useful adjuvant drug to be used together opioids. **Sources of research support:** CAPES/Toxinologia, CNPq and PPGBTox/UFSM.

05.016 Involvement of circulating platelets and neutrophils in the hyperalgesia induced by platelet releasate. Carrilho JM, Rosa JG, Santoro LM, Giorgi R IBu – Fisiopatologia

Introduction: Previous studies developed by our group demonstrated a critical role of platelets in hyperalgesia induced by carrageenan or *Bothrops jararaca* venom, since platelet depletion inhibited the hyperalgesic effect of these irritants. Moreover, the injection of platelet releasate (PR) or whole platelets induced hyperalgesia in rats evaluated by the paw pressure test, suggesting that platelets are essential to the genesis of inflammatory pain (Yamashita, *et al*. Journal of Thrombosis and Hemostasis, 9:2057, 2011). In this study, the mechanisms involved in the hyperalgesia induced by PR were evaluated. **Methods:** Male Wistar rats were submitted to the paw pressure test to evaluate pain sensitivity (Ethical Committee for the Use of Animals of Institute Butantan, protocol number 848/11) after intraplantar injection of PR (100 µL), with the following counts: $0.2x10^9$, $2x10^9$ and $200x10^9$ platelets/L. Hyperalgesia was assessed after 1, 2 and 4 hours. The platelet count of $200x10^9$/L PR was used to evaluate the time-response curve and edema activity. The mediation of hyperalgesic response induced by the PR was measured by treating animals with anti-rat platelets, fucoidin (selectin inhibitor), and methysergide (antagonist of serotonin receptor). **Results:** PR did not induce a dose-response, since the hyperalgesia observed was similar at all concentrations tested. Hyperalgesia was no longer observed 6h after treatment with PR ($200x10^9$ platelets/L). PR induced a slight edema, and a statistically significant difference was observed compared to the control only at 30 min after inoculation (11%). Treatment of animals with anti-rat platelet, induced depletion of circulating platelets (92%) and also completely reversed hyperalgesia entailed by PR at all times evaluated. Pretreatment with fucoidin inhibited hyperalgesia induced by PR. Furthermore, pretreatment with methysergide failed to interfere in the hyperalgesic effect of PR. **Discussion:** The results demonstrate that PR did not induce a concentration-dependent hyperalgesic effect, at least under the concentrations employed. Hyperalgesia caused by PR is not long-lasting since this effect was only observed up to 4 hours after treatment. Our results also show that edema is not directly associated with hyperalgesia. Our results suggest that besides the hyperalgesic components present in PR, circulating platelets have a key role in the development of hyperalgesia associated with PR. Neutrophils are also involved in the hyperalgesic response induced by PR. Moreover, results from methysergide pretreatment indicate that 5-HT receptors are not directly associated with the hyperalgesic effect, suggesting that serotonin is not responsible for mediating hyperalgesia induced by platelets releasate. **Financial support**: PIBIC/CNPq and FAPESP (Proc: 2012/24621-1)

05.017 Superoxide anion induces mechanical hyperalgesia via spinal activation of MAP kinases and PI3K. Carvalho TT[1], Ribeiro FAP[1], Campos CC[1], Casagrande R[2], Verri Junior WA[1] [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas

Introduction: Mitogen-activated protein kinases (MAPKs) transduce signals from the cell membrane to the nucleus thus controlling cellular proliferation, differentiation and survival. MAPKs are grouped into subfamilies including ERK, JNK and p38. The PI_3K also regulates key cellular functions and activates MAPKs. The spinal inhibition of ERK1/2, JNK, p38 and PI_3K inhibits peripheral hyperalgesia induced by carrageenan and cytokines, formalin-induced overt-pain and nerve lesion-induced neuropathic pain in mice. Thus, inhibition of spinal MAPKs and PI_3K reduces inflammatory and neuropathic pain. However, it is not known whether peripheral superoxide anion induces mechanical hyperalgesia via spinal activation of MAP kinases and PI_3K. The aim of this study was to evaluate if the mechanical hyperalgesia induced by peripheral injection of KO_2 (superoxide anion donor) depends on spinal activation of ERK 1/2, JNK, p38 and PI_3K in mice. **Methods:** Swiss mice (n = 5 per group) were used in this study with the approval of the Ethics Committee for Animal Use from UEL, process nº 71.2012.68. KO_2 (30 µg/25µL) or Vehicle (saline) was injected intraplantar and mechanical hyperalgesia was evaluated through an electronic version of the von Frey filaments test. The groups that received stimulus by KO_2 were pretreated (30 minutes before) intrathecally with PD98059 (MEK 1/2 inhibitor - 1, 3 or 10 µg), SP600125 (JNK inhibitor - 1, 3 and 10 µg), SB202190 (p38 inhibitor - 1, 3 or 10 µg), Wortmannin (PI3K inhibitor - 0.3, 1 or 3 µg) or vehicle (2% DMSO in saline, 5 µL) and the measurements were made at 30 min, 1, 3, 5 and 7 hours after stimulation. **Results:** KO_2 induced significant mechanical hyperalgesia in mice that was reduced by treatment with PD98059 up to 67.9%, SP600125 up to 71.2%, SB202190 up to 71.0% and Wortmannin up to 91.0%. **Discussion:** These results demonstrate that these pathways are activated by superoxide anion, leading to inflammatory pain. Therefore, the results indicate that inhibiting spinal MAPKs and PI3K might be an efficient therapeutic approach to reduce hyperalgesia induced by peripheral production of reactive oxygen species such as superoxide anion. **Financial support:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), MCTI, SETI/Fundação Araucária and Paraná State Government, Brazil.

05.018 Antinociceptive and anti-inflammatory activity of *Condalia buxifolia* in mice. Simões RR[1], Junqueira SC[2], Maldaner G[3], Morel AF[3], Zanchet EM[1], Santos ARS[2] [1]UFSM – Fisiologia e Farmacologia, [2]UFSC – Ciências Fisiológicas, [3]UFSM – Química

Introduction: *Condalia buxifolia* belongs to the family Rhamnaceae, comprising 18 species distributed throughout the American continent. It is popularly known as "coronilha-folha-de-buxo or espinilho". It is used in folk medicine as antipyretic, anti-inflammatory and against dysentery (Bastos, Pesquisa Botânica, v. 40, p.69, 1989). However, to date no pharmacological study has been performed concerning the anti-inflammatory action for this species; hence, this study evaluates the possible antinociceptive and anti-inflammatory effect of the methanolic extract of *C. buxifolia* (MECb) and investigates some of the mechanisms underlying this effect. **Methods:** Male Swiss mice were used (26-30g). Dry aerial parts of *C. buxifolia* were extracted with MeOH in a Soxhlet apparatus for 12 h. After the plant material was filtered and the residue rejected, being the solvent evaporated under reduced pressure to obtain the methanolic extract of *C. buxifolia* (MECb). The antinociceptive and anti-inflammatory effects of orally administered of MECb were evaluated in mice subjected to the formalin (2.5%), capsaiscin (1,6 µg/paw, a TRPV1 agonist), cinnamaldehyde (10nmol /paw, a TRPA1 agonist), acetic acid (0.6%) and acidified saline (5.0pH/paw, an ASIC agonist) models. We also evaluated the effect of MECb in temperature and oedema of the injected paw with formalin and the locomotor activity the animals were performed in the open field test. In addition, the time-course of antinociceptive effect of MECb (100 mg/kg, p.o.) was analyzed in the formalin test. Animals were habituated to the laboratory conditions for at least one hour before testing, and treated orally with the MECb (10-300 mg/kg) or vehicle (5% ethanol and saline, 10 ml/kg). The statistical significance of differences between groups was detected by One-way Anova followed by post hoc test Kruskal-Wallis, and all protocols used were approved by CEUA-UFSM (protocol number 046/2012). **Results:** The oral administration of MECb (30-300 mg/kg) significantly inhibited the acetic acid (35 ± 3%) and late (inflammatory, 53 ± 2% inhibition at dose of 100 mg/kg) phase of formalin-induced licking, without affecting responses of the first (neurogenic) phase. However, MECb did not alter the increased temperature and paw edema induced by formalin. Moreover, MECb (100 mg/kg, p.o.) significantly reduced the time of licking/biting caused by capsaicin (55 ± 2%) or acidified saline (52 ± 3%), but did not altered the response caused by cinnamaldehyde. In the open field model, MECb did not show change in the locomotor activity. **Discussion:** These data show for the first time that MECb has significant antinociceptive and anti-inflammatory actions in mice, which appear to be mediated by an inhibition of the activation of TRPV1 and ASICs. Such findings are of interest because they support, at least, the use of *Condalia buxifolia* in popular medicine. Funding: PRONEX / FAPERGS and CAPES.

05.019 Vitexin inhibits inflammatory pain in mice by targeting TRPV1, oxidative stress, and cytokines. Hohmann MSN[1], Borghi SM[1], Carvalho TT[1], Staurengo-Ferrari L[1], Pinge-Filho P[1], Casagrande R[2], Verri Junior WA[1] [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas

Introduction: The flavonoid vitexin is a flavone C-glycoside (apigenin-8-*C*-β-d-glucopyranoside) present in several medicinal and other plants. Plant extracts containing vitexin are reported to possess antinociceptive, anti-inflammatory, and antioxidant activities. However, the only evidence of its antinociceptive activity was demonstrated in the acetic acid-induced writhing model. Therefore, the analgesic effects and mechanisms of vitexin were evaluated. **Methods:** Male Swiss mice (25-30 g) were treated intraperitonealy (ip, 1, 3 and 10 mg/kg) with vitexin or vehicle (saline) 30 min before or 24 h after inflammatory stimulus. The writhing response was evaluated for 20 min after ip injection of acetic acid (0.8%) or phenyl-*p*-benzoquinone (1890 µg/kg). The paw flinching and licking nociceptive responses were quantified for 30 min after formalin 1.5% (25 µL/paw) or CFA (10 µL/paw) injection, and for 5 min after capsaicin injection (1.6 ng/paw). Mechanical and thermal hyperalgesia were evaluated 1-5 h after carrageenan (300 mg/paw), capsaicin (1.6 µg/paw) or 1-8 days after CFA (10 µL/paw) stimulus. Antioxidant capacity, measured by reduced glutathione (GSH) levels, Ferric reducing antioxidant power (FRAP) and 2,2´-azinobis (3-ethylbenzothiazoline 6-sulfonate radical cation, $ABTS^{\cdot+}$) scavenging ability, and cytokine levels (TNFα, IL-1β, IL-6, IL-33 and IL-10) were determined 3 h after carrageenan (300 mg/paw) injection. License number of The Research and Ethics Committee of Universidade Estadual de Londrina: 32813.2012.03. **Results:** Vitexin dose-dependently inhibited acetic acid-induced writhing (up to 86.1%). Furthermore, it also inhibited pain-like behavior induced by PBQ (up to 67.7%), CFA (up to 72.7%), capsaicin (up to 72.8%), and both phases of the formalin test (up to 50.0 and 72.0% in the first and second phase, respectively), in addition to carrageenan-, capsaicin- and chronic CFA-induced mechanical (up to 83.9, 52,8 and 37.4%, respectively) and thermal (up to 100.0, 67.4 and 60.8%, respectively) hyperalgesia. Vitexin prevented the reduction of GSH levels (50.2%), FRAP (58.0%), and $ABTS^{\cdot+}$ scavenging ability (85.1%), inhibited the production of TNF-α (48.3%), IL-1β (88.0%), IL-6 (39.8%), and IL-33 (40.6%), and up-regulated the levels of IL-10 (107.0%). **Discussion:** Vitexin presents analgesic effect in a variety of inflammatory pain. Its antinociceptive action seems to be dependent on: targeting TRPV1 channel activity as observed by inhibition of capsaicin (a TRPV1 agonist), inhibition of antioxidant depletion, and modulation of cytokine production with concomitant inhibition of pro-hyperalgesic (TNF-α, IL-1β, IL-6, and IL-33) and stimulation of anti-hyperalgesic (IL-10) cytokine production. **Financial Support:** CAPES, CNPq, MCTI, SETI / Fundação Araucária, and Governo do Estado do Paraná (Brazil).

05.020 Anti-hyperalgesic effect of World Health Organization (WHO) analgesics ladder in a model of paclitaxel-induced pain syndrome. Pinheiro KV[1], Rigo FK[2], Oliveira SM[3], Ferreira J[3] [1]UFSM – Farmacologia, [2]Santa Casa BH, [3]UFSM – Bioquímica Toxicológica

Introduction: Paclitaxel has been widely used against a variety of solid tumors. However its use is limited by side effects, specially a pain syndrome characterized by acute arthralgia/myalgia and chronic neuropathy. Unfortunately, there are no effective therapies to reduce paclitaxel-induced pain syndrome. Thus, we assessed the efficacy of analgesics used in the WHO ladder, which are extensively used to treat cancer-related pain, in an experimental model of paclitaxel-induced pain syndrome. **Methods:** Male adult Wistar rats (N=6-8, 180-250g) were used in this study (Ethics Committee: 23081.005024/2010-88). Hyperalgesia was measured with von Frey filaments and used as a parameter of nociception. Paclitaxel-induced pain syndrome was induced by four injections of paclitaxel (1 mg/kg, i.p.) on alternate days (days 1, 3, 5, and 7). The acute and chronic phases were assessed 24 h and 15 days after the first injection, respectively. Rats were treated orally with vehicle, acetaminophen (step 1 of the ladder), codeine alone or plus acetaminophen (step 2) and morphine (step 3) after acute or chronic phases assessment. **Results and discussion:** Paclitaxel caused hyperalgesia 24 h after administration. Acetaminophen alone reverted paclitaxel-induced acute hyperalgesia from 60 to 240 min after administration, with values of effective dose 50 (ED_{50}) and maximum inhibition (I_{max}) of 7(5-10) mg/kg and 91 ± 7%, respectively. Codeine alone also reduced the acute hyperalgesia induced by paclitaxel from 30 up to 120 min after treatment, with an ED_{50}=0.7(0.4-1.2) mg/kg and I_{max}=100%. Codeine plus acetaminophen reverted acute hyperalgesia without changes in drugs potency (ED_{50}=0,72(0,5-1,0)+7,2(5-10,2) mg/kg) and efficacy (I_{max}=100%), but producing a longer lasting anti-hyperalgesia (up to 360 min). Morphine presented anti-hyperalgesic effect in paclitaxel-induced acute hyperalgesia only 30 min after its treatment, with an ED_{50}=1.4(0.4-5.3) mg/kg and Imax=86 ± 3%. The repeated treatment with paclitaxel also led to a marked hyperalgesia 15 days after the first injection. Acetaminophen alone reverted chronic paclitaxel-induced hyperalgesia with duration (up to 240 min), potency (ED_{50}=10(3-30) mg/kg) and efficacy (I_{max}=82 ± 4%) similar to that presented in acute phase. Codeine alone also inhibited chronic hyperalgesia with a duration similar to the acute phase, but with a lower potency (ED_{50}=6(3-13) mg/kg) and efficacy (I_{max}=75 ± 6%). The combination of codeine plus acetaminophen inhibited chronic hyperalgesia, with increased efficacy (I_{max}=100%) and potency of codeine (ED_{50}=0,46(0,3-0,6)+ 4,6 (3,6-6,5) mg/kg). Finally, morphine (3 mg/kg) reversed chronic hyperalgesia just 60 min after administration, without significant changes in its potency (ED_{50}=2(1-5) mg/kg) and efficacy (I_{max}=72 ± 17%). Together, analgesics of WHO ladder are capable of reverting both acute and chronic phases of paclitaxel-induced hyperalgesia in rats, with the step 2 analgesics combination presenting more potent, efficacious and long-lasting effect. Thus, WHO analgesics ladder could also be useful to treat paclitaxel-induced pain syndrome. **Financial Support**: CNPq, CAPES.

05.021 Differential nociceptive response induced by TRPM8 agonist in streptozotocin-diabetic rats. Jesus CHA, Scarante FF, Schreiber AK, Cunha JM UFPR – Farmacologia

Introduction: Neuropathic pain is a major complication of diabetes, characterized by spontaneous pain, hyperalgesia and/or allodynia, induced by stimuli of different natures, including mechanical and thermal (cold and heat). Additionally, it is reported that allodynia to cold is a common complaint of diabetics and that the abnormalities in response to thermal stimuli of cold is the most sensitive test for early detection of diabetic polyneuropathy. Despite of the importance of this clinical sign, little has been explored about the mechanisms involved in the establishment of cold allodynia in diabetes as well as the involvement of TRPM8 receptor, which is the goal of this work. **Methods:** Male *Wistar* rats (180-220 g, n = 8-10) were used in all protocols, which were previously approved by the UFPR's Committee on the Ethical Use of Animals (authorization #649). Animals were treated intraperitoneally with citrate buffer (10 mM, pH 4.5, NGL, normoglycemic control group) or streptozotocin (STZ, 50mg/kg, diluted in citrate buffer, DBT-diabetic group). For assessment of cold allodynia, both NGL and DBT animals were instilled with 100 µL of acetone (TRPM8 receptor agonist) in the center of one of their hind paw prior to administration of STZ (Baseline 0, B0) and weekly until week 4 after induction of diabetes (Basal 4, B4). The nociceptive behavior of shaking paw (flinches) was recorded for 2 minutes after instillation. To evaluate the involvement of TRPM8 receptor, NGL and DBT animals received intraplantar injection (i.pl) of menthol, TRPM8 agonist (0.1, 0.5 or 1%, 50 µL/paw) or vehicle (corn oil) at 4 weeks after STZ. The direct nociceptive response (flinches) was evaluated for 20 minutes starting from menthol injection. **Results:** The number of flinches induced by acetone instillation was significantly higher in DBT animals only in week 4 after induction of diabetes (37%). After menthol i.pl. injection, DBT animals exhibited no difference (dose of 0.1%) or significant decreased of number of flinches (when considered menthol at doses of 0.5 and 1%) when compared to NGL animals (mean±SEM; DBT vehicle: 7.6 ± 1.5; DBT menthol 0.1%: 23.9 ± 1.9; DBT menthol 0.5%: 16.9 ± 1.8; DBT menthol 1%: 28.2 ± 3.9; NGL vehicle: 7.7 ± 1.6; NGL menthol 0.1%: 24 ± 1.5; NGL menthol 0.5%: 45.4 ± 4.3; NGL menthol 1%: 45.7 ± 6.3). **Conclusion:** Taken together, our data, for the first time in the literature, show that DBT animals are hyporesponsive to menthol, a TRPM8 agonist. Interesting, parallel in our group, demonstrated that menthol induced exacerbated responses in a different animal model of neuropathic pain (chronic constriction injury of sciatic nerve), emphasizing that the pathophysiologic mechanisms of neuropathic pain can change depending on the etiology. Finally, further studies still need to be conducted (using a specific TRPM8 antagonist), to confirm the role of this receptor on the development of cold allodynia associated with diabetes.

05.022 Effects of warm water immersion therapy on persistent inflammatory pain model: **Analyze of action mechanism**. Britto RN[1], Stramosk J[1,2], Cesar-Martins T[2], Batisti AP[2,3], Santos ARS[4], Piovezan AP[2], Martins DF[1,2] [1]Unisul – Fisioterapia, [2]Unisul – Neurociência Experimental, [3]Unisul – Naturologia Aplicada, [4]UFSC – Neurobiologia da Dor e Inflamação

Aim: Immersion therapy is commonly used in physical therapy with many known benefits; balneotherapy sessions appear to reduce pain and increase mobility dynamics in women with fibromyalgia. In addition, the combination of exercise therapy with the warm water reduces pain in this population with greater benefits than exercise by itself and with longer effects on pain management. The aim of the present study was to investigate the effects of only warm water immersion therapy (WWIT), upon persistent inflammatory pain. **Methods:** The inflammatory pain was induced by an intraplantar (i.pl.) injection of 20 mL of complete Freund's adjuvant (CFA) in adult Swiss mice (25–35 g). Twenty-four hours after CFA i.pl. injection, mouse pain threshold was evaluated through tactile allodynia, using Von Frey Hair (VFH) filaments. Further analyses performed in CFA injected mice were paw oedema measurement. The mice were subjected to WWIT for different times. Withdrawal frequency to mechanical stimuli was assessed 24 hours after CFA injection and 30 minutes after WWIT (daily), or control treatments. The opioidergic and adenosinergic system was assessed by systemic peripheral i.pl. administration of naloxone (5mg/paw, a non selective opioid receptor antagonist) or caffeine (150 nmol/paw, a non selective adenosine receptor antagonist). Behavioral tests were analyzed using one or two-way analysis of variance following the Student Newman-Keuls and Bonferroni test, respectively. **Results** were presented as the mean ± S.E.M. for each group. $P<.05$ was considered significant. **Results:** The treatment with WWIT did not modify paw oedema, but significantly ($P< 0.05$) decreased the paw withdrawal response in persistent inflammatory pain and this effect was reversed by pretreatment of the animals with naloxone ($P< 0.05$) or caffeine ($P< 0.05$) given by intraplantar. **Conclusions:** Our results indicate that the warm water immersion therapy reduces persistent inflammatory pain by activation of the neurologic pathways such as opioid and adenosinergic system. A better understanding of the peripheral mechanisms of warm water immersion could stimulate therapists to integrate WWIT with strategies also known to influence endogenous pain control, such as exercise or acupuncture to potentiate endogenous analgesia. **Sources of research support:** UNISUL.

05.023 Anti-nociceptive effects of essential oil *Piper rivinoides* Kunth. Costa NF[1], Nascimento DD[1], Siqueira AM[1], Calheiros AS[1], Souza SP[2], Valverde SS[2], Frutuoso VS[1], Castro-Faria-Neto HC[1] [1]Fiocruz – Imunofarmacologia, [2]Fiocruz – Farmanguinhos

Introduction: The family Piperaceae has 10-12 genus and about 1,200 species, which 700 correspond to the *piper* genus. These species are used for medicinal purposes and the most relevant biological activities described are antitumor, analgesic, anxiety and insomnia. No phytochemical study was found for the specie *Piper rivinoides*. The aim of this study was to investigate the antinociceptive effect of the essential oil of *Piper rivinoides* Kunth (OEPR). **Methods**: Analysis and identification of volatile components was peformed using two gas chromatographs: GC-2010 (GC1 e GC2) and one quadrupolo MS-QP2010 (MDGC). Acetic acid writhing test: mice received i. p. injection of 0.8% acetic acid, writhing numbers was counted for 10 minutes. Capsaicin-induced nociceptive: mice was stimulated with capsaicin in the right hind paw (1,6 µg/paw). The time licking spent was counted for 5 minutes. Rota Rod Model: Mice were selected 24 h before the experiment, choosing only those ones who remained for 60s twice on the equipment. Animals were treated (o. p). with OEPR (0.1, 1, 10, 100 mg / kg) or vehicle one hour before each experiment. Morphine (10 mg / kg, i.p.), diclofenac (50 mg / kg, o.p.) and phenobarbital (50 mg / kg, o.p.) were used as standard drugs. Macrophages Culture, MTT: Peritoneal macrophages were cultured with OEPR (1, 10, 50, and 100 µg/mL), 20h after, 10 µL of MTT (5mg/mL) was added and the toxicity was verified doses in plate reader (570nm). All experiments had been performed in accordance to the Fiocruz Council Animal Care (CEUA), number 033/09. **Results** and discussion: 93.23% of the essential oil were analyzed, in which 19 compounds were found, e.g. α-pinene (32.93%), β-pinene (20.74%), caryophyllene (7.6%), germacrene B (6.72%). The OEPR effect was dose dependent, which 100 mg / kg (21.7 ± 10.0, n> 12) reduced the number of writhes in relation to control group (50.0 ± 13.3 , n> 12), ED50 = 43.72 mg / kg. The same pattern was observed in the model of capsaicin, in which the animals treated with OEPR at a dose of 100 mg / kg (19.17 ± 36.5, n> 10) showed the inhibition of paw licking time in relation control (79.78 +24.4) and ED50 = 43.11 mg / kg, suggesting a possible inhibition on neurogenic pain. In the rota rod model, the highest dose (100mg/kg) did not present significant effect, 120s ± 0, n>10 compared to the saline group (114.65 ± 15.2, n> 10), suggesting that OEPR do not have sedative effect. In the macrophages culture, OEPR showed no cytotoxic effect even on the highest dose (100µg/mL), (0.17 $\pm$ 0.05, n = 3) compared to the control group (0.17 $\pm$ 0.03, n = 3). Financial support: CNPq-FIOCRUZ.

05.024 Quercetin attenuates tactile allodynia in rats with sciatic constriction injury. Lopes EM[1], Piauilino CA[1], Brandão DCBS[1], Gomes BS[1], Brito SMRC[1] [1]UFPI – Biochemistry and Pharmacology

Introduction: Peripheral neuropathic pain, resulting from nerve injury due to trauma or disease, is recognized as one of the most difficult types of pain to treat. Several medications are used to relieve nerve pain, but they don't work for everyone and most have side effects that limit their usefulness. Therefore, alternative therapies are still needed. Studies indicate that quercetin may have therapeutic value in the treatment of neuropathy (Kandhare, Biomed & Aging Pathol. (2), 173, 2012; Narenjkar, 2(3): 51, Neuroscience). The present study was designed to evaluate the effect of quercetin in rats with sciatic constriction injury. **Methods:** Male Wistar rats (190-260 g) were used submitted to an unilateral sciatic nerve constriction injury. The biceps *femoris* and the *gluteus superficialis* (right side) were separated and the sciatic nerve exposed, isolated, and compressed (Mosconi, Pain 64: 37,1996). Animals were treated with quercetin (5, 10 or 15 mg/kg, i.p.) or vehicle (2% dimethylsulfoxide) daily for 7 days. The mechanical nociceptive threshold (MNT) was determined by means of von Frey filaments before and in the 4th and 8th days after nerve constriction. In sham-operated animals, the right sciatic nerve was exposed but not compressed. All experimental protocols were approved by Ethics Committee of Animal Experimentation, CEEA/PI n° 008/2012). Statistical analyzes were performed using ANOVA (one way) followed by Tukey test, $p<0.05$. **Results and Discussion:** Data were expressed as mean ± S.E.M., $^{a}p<0,05$ *vs* Control, $^{b}p<0,05$ *vs* Vehicle , $^{c}p<0,05$ *vs* Sham. MNT values (g): Control (10.3 ± 1.1 ; 13.1 ± 1.0 and 11.6 ± 0.9, n=10) Sham-group (11.9 ± 1.1; 7,0 ± 0.8 and 10.0 ± 0.9, n=10), Vehicle (8,6 ± 1,0; 2.8 ± 0.8 and 2.5 ± 0.5), Quercetin 5 mg/kg (11.2 ± 1.1 ; 8.5 ± 0.8 and 9.5 ± 0.6, n= 10), Quercetin 10 mg/kg (9.8 ± 0.9 ; 10.2 ± 0.8 and 10.4 ± 0.8, n=10) and Quercetin 15 mg/kg (11.2 ± 1.5 ; 11.2 ± 1.5; 10.6 ± 1.1, n= 5). In sham-operated animals there was no difference in nociceptive behaviors when compared with control. The quercetin groups showed decreased MNT at the 4th and 8th day after nerve injury when compared with vehicle. The results suggest that administration of quercetin (5, 10 or 15 mg/kg) significantly attenuated sciatic nerve constriction-induced tactile allodynia, assessed by paw withdrawal threshold. In conclusion, the results of the present study suggest antinociceptives effects of quercetin in this model of neuropathic pain in rat. **Financial support:** UFPI / CAPES

05.025 Role of TRPV1 on the development of acute gout attacks. Hoffmeister C[1], Silva MA[2], Rossato MF[2], Trevisan G[2], Oliveira SM[2], Guerra GP[3], Silva CR[2], Ferreira J[4] [1]UFSM – Farmacologia, [2]UFSM – Química, [3]UTFPR – Alimentos, [4]UFSC – Farmacologia

Introduction: The gout is one of the inflammatory painful conditions that most affect the world population. Despite the large number of clinically available drugs, many patients are refractory to standard treatments or suffer from the side effects of them. Thus, we intend to investigate whether nociception and inflammation observed in a reliable model with the clinic (monosodium urate (MSU) crystals administration in the tibio-tarsal joint of rodents) are mediated by the TRPV1 receptor. **Methods:** Adult male Wistar rats were used in this study (250-300 g) (Ethics Committee process number 108/2011). Initially we characterize the nociceptive and inflammatory responses triggered by MSU intra-articular (i.a.) injection. For this, the animals were submitted to i.a. injection of 50 µL of MSU suspension (1.25 mg/site) or vehicle (PBS). The nociceptive effects were evaluated by analyzing mechanical allodynia (using von Frey hair filaments), heat hyperalgesia (using Hargreaves, 1988), and spontaneous nociceptive score (0 to 4 grade scale) from 1 up to 48 hours after MSU injection. Joint inflammation was evaluated by measuring the edema formation joint (digital calliper) from 1 up to 72 hours and also plasma extravasation (protein content), leukocyte infiltration (Neubauer chamber), myeloperoxidase (MPO) activity and also IL-1β production (enzyme immunoassay kit) in the synovial lavage was measured 1 up to 24hs after MSU injection. Subsequently, experiments were conducted to evaluate the participation of TRPV1 in MSU i.a. responses using a selective antagonist of this receptor (SB366791, co-injected with MSU (1.25 mg/site) or vehicle), systemic defunctionalization of TRPV1-positive sensory fibers (Resiniferatoxin, subcutaneous injection), and verification of immunoreactivity of this receptor (analyzed by western blotting). **Results:** We observed that MSU elicited ongoing pain-like behaviour, hyperalgesia and allodynia, as well as plasma extravasation, leukocyte infiltration, MPO activity and IL-1β production in the joint lavage fluid , with maximal effect around 4h after MSU injection. All these responses were inhibited by the co-administration of SB366791 (10 nmol/site) in 82 ± 9, 70 ± 16, 100, 74 ± 8, 98 ± 3, 49 ± 7 and 78 ± 24%, respectively. Also, 4h after MSU injection there was an increase in the immunoreactivity of the TRPV1 receptor (60 ± 4%). Furthermore, the defunctionalization of TRPV1-positive sensory fibers significantly reduced MSU-induced ongoing pain-like behaviour (68 ± 15%), hyperalgesia (65 ± 16%), allodynia (97 ± 19%) and edema (60 ± 7%).

Discussion: We demonstrate that TRPV1 acts on sensory neurons and plays a relevant role in the nociception and inflammation induced by intra-articular MSU, indicating it as a potential target to treat acute gout attacks. **Research support:** CNPq, CAPES.

05.026 Bioassay-guided fractionation of hexanic extract from *Pterodon polygalaeflorus* by its antinociceptive activity. Pinto FA, Vigliano MV, Velozo L, Sabino KCC, Coelho MGP
UERJ – Bioquímica

The secondary metabolites produced by plants had a fundamental key to the development of modern synthetic organic chemistry. Historically, the development occurred in parallel with the study of plants, mainly from the nineteenth century (Montanari CA, Química Nova 24: 105, 2001). Seeds of the genus *Pterodon*are commercially available in the medicinal flora market, where are being widely used for its pharmacological properties. Our laboratory showed low acute toxicity for the ethanolic extract from *P. pubescens* and also that these extract and its fractions exhibit anti-edematogenic and antinociceptive activities (Coelho PL, J. Ethnopharmacol. 98: 109, 2005). The aim of this study was to analyze the fractionation of the *Pterodon polygalaeflorus* hexane extract (EHxPpg) and evaluate its antinociceptive potential. Fractions and subfractions were evaluated in the acetic acid-induced writhing test. Male SW mice were pre-treated by oral route with fractions/subfractions or vehicle and 1 h later, the acetic acid 0.6% was injected intraperitoneally, and the constrictions were counted after 5 min during 10 min. Subfractions were also assayed in the capsaicin test. After orally pre-treatment with test substance male SW mice received an injection of 2.5 capsaicin in PBS pH 7.4 (50 µL, 1.6 µg paw) in the right hind paw and shortly after, the time (s) of licks and bites were counted over a period of 0-5 min. All animal experiments were approved by the ethics committee of IBRAG-UERJ by protocol 052009 and 072013. The fractionation of EHxPpg on silica gel 60 column produced four fractions. Fr2Ppg exhibited a high antinociceptive activity at doses 0.1 and 1 mg/kg with 92.7% and 55.8% inhibition, respectively, in the writhing test. So, this fraction was selected to carry out bioactivity guided fractionation in order to identify and isolate the compounds. The fractionation of Fr2Ppg on a silica gel 60 column generated five subfractions (SF2.1 a SF2.5). The highest activity on the writhing test was exhibited by SF2.5 at doses of 0.01, 0.1 and 1 mg/kg, with inhibitions of 74.2%, 54.4% and 48.3% respectively. SF2.5 was further fractionated on silica gel 60 column producing five subfractions (SF2.5.1 a 2.5.5). SF2.5.4 presented the highest activity on the writhing test reducing 69.6% (0.01 mg/kg), 47.3% (0.1 mg/kg) and 38.9% (1 mg/kg) of contortions while SF2.5.5 inhibited 30.7% (0.1 mg/kg) and 56.0% (1 mg/kg) of writhes. SF2.5.4 presented a high antinociceptive effect at the doses 0.01 mg/kg (72.4%), 0.1 mg/kg (78.7 %) e 1 mg/kg (71.6%) while SF2.5.5 shows a significant antinociceptive effect of lower intensity at doses 0.01 mg/ kg (30.2%) and 1 mg/kg (38.6%) using the capsaicin model. These subfractions (SF2.5.4 and SF2.5.5) showed higher activity in the acetic acid-induced writhing test and capsaicin test suggesting antinociceptive properties, and are being analyzed by chromatographic techniques aimed the isolation / identification of its active principles to evaluate its mechanisms of action. The monitoring of the chemistry of the plant is necessary to ensure the pharmacological and quality to the development of a new herbal. Support: CNPq, FAPERJ, CAPES and UERJ.

05.027 Activation of P2X3 and P2X2/3 receptors in gastrocnemius muscle of rats induces pro-inflammatory cytokines release and neutrophil migration. Schiavuzzo JG[1], Melo B[1], Santos DFS[1], Teixeira JM[2], Parada CA[2], Fusaro MCGO[1] [1]FCA-UNICAMP, [2]IB-UNICAMP

Introduction: We have recently demonstrated that activation of P2X3 receptors in gastrocnemius muscle of rats by α,β-meATP induces mechanical hyperalgesia by an indirect sensitization of the primary afferent nociceptor mediated by bradykinin, sympathetic amines and prostaglandins. The involvement of P2X3 receptors was confirmed by the intramuscular administration of the selective P2X3 and P2X2/3 receptors, A317491, which blocked the mechanical hyperalgesia induced by α,β-meATP. The aim of this study was to verify whether activation of P2X3 receptors in the gastrocnemius muscle of rats induces mechanical hyperalgesia also mediated by bradykinin B2 receptor and neutrophil migration. We also evaluated whether α,β-meATP was able to induce release of pro-inflammatory cytokines and neutrophil migration in gastrocnemius muscle of rats. **Methods**: Male Wistar rats (2 months old, 200-250g) were used in this study and all experimental procedures were approved by the Ethics Committee in Animal Research at the UNICAMP (license number 2518-1). The mechanical hyperalgesia was quantified 2 hours post intramuscular administration of a,b-meATP by the pressure analgesimeter Randal Sellito. To investigate the role of bradykinin B2 receptor and neutrophil migration in the mechanical hyperalgesia induced by a,b-meATP, Bradyzide, the selective B2 receptor antagonist, or the non-specific selectin inhibitor Fucoidan were used. The local concentrations of TNF-α, IL-1β, IL-6 and CINC-1 were measured by Enzyme-linked Immunosorbent Assay (ELISA) and neutrophil migration was quantified by the method of the myeloperoxidase enzyme (MPO). The muscle tissues were removed 2 hours post administration of a,b-meATP.

Results: Administration of the Bradyzide (1.5µg, n=6) in the ipsilateral but not in the contralateral gastrocnemius muscle prevented ($p<0.05$, One Way ANOVA, test Tukey) the mechanical hyperalgesia induced by a,b-meATP (1000µg/muscle). Pre-treatment with Fucoidan (25mg/kg, i.v., 20 min) also prevented ($p<0.05$, One Way ANOVA, test Tukey) the mechanical hyperalgesia induced by a,b-meATP (1000µg/muscle). The administration of α,β-meATP (1000mg/muscle) significantly increased local concentration of TNF-α (Mean ± SEM: 384.73 ± 90.47pg/mL, n=6), IL-1β (586.02 ± 29.28pg/mL, n=6), IL-6 (8745.94 ± 432.80pg/mL, n=6) and CINC-1 (972.35 ± 85.86 pg/mL, n=6) when compared with 0.9% NaCl administration ($p<0.05$, Tukey test). The administration of α,β-meATP (1000mg/muscle) significantly increased (2355730,91 ± 347209,49 neutrophils/muscle, n=6) the MPO activity when compared with 0.9% NaCl administration ($p<0.05$, Tukey test). The pre-treatment with fucoidan (25 mg/kg, i.v., 20 min) significantly reduced ($p<0.05$, Tukey test) this MPO activity. **Discussion:** The results suggest that activation of P2X3 receptors in gastrocnemius muscle of rats by α,β-meATP induces mechanical hyperalgesia also mediated by bradykinin B2 receptor and neutrophils. Also, this study demonstrated that activation of P2X3 receptors induces release of important pro-inflammatory cytokines and neutrophil migration. This study points out P2X3 receptor as important target to control inflammatory muscle pain. **Financial Support:** FAPESP (2011/13884-9).

05.028 NLCR4/ASC/caspase-1 inflammasome assembling participates in the genesis of inflammatory pain. Lopes AHP, Talbot J, Silva RL, França RFO, Zamboni DS, Ferreira SH, Cunha FQ, Cunha TM FMRP-USP

Introduction: The inflammatory hyperalgesia is a complex process that depends on the sensitization of nociceptive fibers mediated by the release of mediators such as interleukin-1β. We have previously demonstrated that the release of IL-1β during carrageenan-induced peripheral inflammation (standard inflammatory pain model) depends on the activation of caspase-1. There are also evidence that caspase-1 activation is mediated by the assembling of a molecular protein platform known as inflammasomes. Thus, the aim of this study was to evaluate the participation of inflamassome activation, specifically the role of NLRC4, NLRP3, Caspase-1 and ASC adaptor molecule in the genesis of inflammatory hyperalgesia. **Methods**: Male C57BL/6 (20-30g, N=5) wild type (WT) and deficient mice in NLRC4-/-, NLRP3-/-, Casp1-/- and adaptor molecule ASC-/- were used in this study. All animal experiments were approved by the local ethical committee for animal use (CETEA-FMRP 149/2011). Peripheral inflammation was induced by carrageenan (100 µg/paw) or its vehicle (0.9% NaCl) injected in the plantar surface of the mice hindpaw. Mechanical inflammatory hyperalgesia was evaluated using an electronic version of the von Frey test. The production of cytokines, IL-1β, TNF-α, CXCL1/KC and neutrophil migration were evaluated by ELISA and myeloperoxidase activity, respectively. The interleukin (IL)-1β and Caspase-1 protein expression were evaluated by western blotting. The volume of the mice hindpaw (oedema mm^3/paw) was measured with a plesthismometer (Ugo Basil, Italy). *In vitro* studies were performed in cultivated macrophages, extracted from the peritoneum of naive animals, incubated with carrageenan (300ug/ml). **Results**: In the present study we observed that mechanical hyperalgesia and oedema induced by intraplantar injection of carrageenan was reduced in IPAF-/- and ASC-/- mice, but not in NLRP3-/- mice compared to WT animals. The mechanical hyperalgesia induced by paw injection of IL-1β and PGE2 in ASC-/- and NLRC4-/- mice showed no significant differences compared to WT mice. Interestingly, ASC -/-, NLRC4-/- and NLRP3-/- mice did not show impaired neutrophil recruitment, suggesting that the reduction in hyperalgesia were not dependent on reduction of neutrophil migration. Activation of caspase-1 *in vivo*, as evidenced by the expression of the mature form of caspase-1 (~ 20kDa), was also reduced in ASC-/ - and NLRC4-/- mice, compared to WT. *In vitro*, the production of IL-1β by carrageenan-stimulated macrophage was reduced in cells from caspase-1-/-, ASC-/- and NLRC4-/- mice compared to WT mice. **Discussion**: The present data demonstrates that ASC and NLRC4 molecules are crucial to caspase-1 inflammasome activation in carrageenan-induced peripheral inflammation and mediates the production of pro-nociceptive mediator IL-1β. In conclusion, it is likely that inhibition of NLRC4 inflammasome assembling might be a target to control inflammatory pain. **Financial support**: CAPES, FAPESP.

05.029 Antinociceptive activity of aggregatin D isolated from *Sinningia aggregata* in a model of mechanical hyperalgesia in mice. Souza GV[1], Bastos-Pereira AL[1], Ribas JLC[1], Stefanello ME[2], Zampronio AR[1] [1]UFPR – Farmacologia, [2]UFPR – Química

Aim: Aggregatin D (AgD) is a naphthoquinone isolated from *S. aggregata*. Previous studies showed that oral administration of AgD reduced the second phase of formalin and local injection of this compound reduced the mechanical hyperalgesia induced by carrageenan (Cg). However, its mechanism of action is unknown. Cg injection in the mice's hindpaw induces the release of both tumor necrosis factor-α (TNF-α) and keratinocyte-derived chemokine (KC) (Cunha et al., Proc. Natl. Acad. Sci, 102:1755, 2005). TNF-α promotes the release of Interleukin-1β (IL-1β), that promotes the release of prostaglandins (PGs) which are responsible for the induction of hyperalgesia. Conversely, KC promotes mechanical hyperalgesia through the release of IL-1β, but also directly through the release of sympathetic amines. Bradykinin (BK) can also mediate Cg-induced hyperalgesia by releasing PGs and sympathetic amines but not cytokines (Cunha et al., Eur. J. Pharmacol., 573:221, 2007). This study evaluated the effect of AgD on the mechanical hyperalgesia induced by some of these mediators in mice and on the release of NO by peritoneal macrophages which may provide evidences about its mechanism of action. **Methods:** Male Swiss mice (20-30 g) were used. Experimental protocol was approved by the Ethics Committee for Animal Use of the institution (# 628). Mechanical threshold was assessed using von Frey monofilaments and the Up-and-Down paradigm as previously described (Mori et al., Phytomedicine, 18:143, 2011). Basal mechanical threshold was assessed and then animals were treated with AgD (0.07, 0.7, 7 ng/paw) and after 30 min they received an intraplantar injection of Cg (300 µg). AgD 7 ng/paw was selected for subsequent experiments and mechanical hyperalgesia was induced by TNF-α (1 pg/paw), IL-1β (0.5 pg/paw), PGE_2 (100 pg/paw). In all experiments, dipyrone (Dip, 320 µg/paw) was used as positive control. We also evaluated the effect of AgD on NO production by mice peritoneal macrophages stimulated for 4 h with LPS (100 and 10 ng/mL) in the presence of AgD at 4 to 4000 ng/mL. **Results**: Pretreatment of the animals with AgD in the paw reversed dose-dependently the mechanical hyperalgesia induced by Cg (inhibition of 23, 40 , And 66 %). AgD 7 ng/paw significantly reduced the mechanical hyperalgesia induced by BK (90%), TNF-α (56%), IL-1β (59%) and PGE_2 (66%). Dip had similar effects (25%,64%, 50% and 78%, respectively). AgD alone reduced basal NO release by macrophages in all concentrations tested (around 30 %) without affecting viability. AgD (4000 ng/ml) reduced NO production in cells stimulated with the lower dose of LPS (10 ng/ml, 45%). **Conclusions:** AgD showed an important antinociceptive activity, and its effect is partly related to the inhibition of the hyperalgesic effects of BK, TNF-α and IL-1β. It is unlikely that AgD act as a cyclooxigenase inhibitor, since it also reduced the mechanical hyperalgesia induced by PGE_2. Inhibition of NO production by AgD in non-stimulated cells suggests an anti-oxidative effect. Changes in LPS-induced NO production only occurred at higher concentrations which may suggest that NO is not involved in the mechanism of action of AgD. However, further studies are necessary to clarify this point. **Financial support**: CNPq, CAPES and Araucária Foundation.

05.030 Effects of the pro-resolving lipid mediator lipoid A4 on neuropathic pain following spinal cord hemi section in rats. Martini AC, Forner S, Rae GA UFSC – Farmacologia
Introduction The majority of traumatic spinal cord injury (SCI) patients develop chronic central pain syndromes which impact on their daily routines and quality of life and are refractory to treatment by neurosurgical, pharmacological and behavioral therapeutic strategies. Immune and inflammatory responses to SCI have been associated to onset of neuropathic pain via activation of a broad spectrum of factors and signaling pathways. Lipoxin A4 (LXA4), an eicosanoid endowed with anti-inflammatory and pro-resolution properties, exerts neuroprotective and antihyperalgesic effects. The present study aims to assess the potential therapeutic effect of LXA4 on relief of SCI-induced pain. **Methods** Spinal cord left side hemisection was carried out at T10 level with a 15 scalpel blade in anesthetized adult male Wistar rats. At 4 and 24 h after SCI, rats received two intrathecal injections of LXA4 (150 or 300 pmol) or vehicle. Mechanical sensitivity of hind paws and recovery from motor impairment were evaluated weekly for up to 4 weeks after SCI, using 4.56 and 5.18 von Frey hairs and the Basso, Beattie and Bresnahan locomotor scale, respectively. All procedures were approved by UFSC's Ethics Committee (#PP00686). **Results** Sham-operated control rats showed normal mechanical responsiveness and motor activity following surgery. SCI increased the frequency of responses to mechanical stimulation with 4.56 von Frey monofilament only of the contralateral hind paw on days 7, 14, 21 and 28. The mechanical responsiveness to 5.18 Von Frey hair was increased in the ipsilateral hind paw on days 7, 14, 21 and 28. SCI rats presented BBB scores of 2 to 3 on day 2 after surgery, which gradually improved over the following 3 weeks to reach a final score of 16. Both doses of LXA4 significantly attenuated the mechanical sensitivity changes induced by SCI. LXA4 also significantly enhanced recovery of locomotor performance on days 7 and 14. **Discussion** These results demonstrate the strong protective effect of intrathecal LXA4 on the development of nociception induced by SCI, indicating that it might provide effective pain relief in subjects afflicted by neuropathies following spinal cord injury. **Financial Support:** CNPq, CAPES, FAPESC, PRONEX.

05.031 New method for evaluation of articular disability in experimental arthritis: **investigation the role of glial cells**. Quadros AU, Fonseca MD, Pinto LG, Ferreira SH, Cunha TM FMRP-USP – Farmacologia

Introduction: The evaluation of articular nociception and disability is a challenge. The methods available so far have been limited and subject to analyzer influence. Searching for help in this problem, the purpose of this work was standardize the use of dynamic weight bearing (DWB), as a new device, for assessment of articular nociception and disability in experimental models of arthritis. In this system, the animal can walk freely and without analyst interference by 5 minutes in a platform with sensors, which capture the difference between the weight distributions of each animal limb. In rheumatoid arthritis (RA) the pain is severe in 60% of patients and cause incapacitation in 70% of them (CORBACHO & DAPUETO, 2010). Is a multifactorial pain and needs more researches that provide new treatments possibilities. One of them may is going to the control of glial cells activity. The involvement of glial cells in the chronic pain and disability in experimental models of arthritis has been reported, but there isn't description of this contribution in function of the time. Objectives: Standardize DWB for evaluate articular nociception and disability in animal models of arthritis and investigate the role of glial cells in this process on function of the time. **Methods and Results**: Animal care and handling procedures were in accordance with the guidelines of the IASP and with the approval of the Animal Ethics Committee of the University of Sao Paulo with number 115/2011. In AIA model DWB showed reduction until 43% ± 2.5% between 7 and 24 hours after the intrarticular challenge with mBSA. The disability proved was predominantly inflammatory, once four different anti-inflammatory drugs (indomethacin, dexamethasone, etoricoxib and infliximab) totally recovered the articular function. After that, there is a relation between pain and disability, since the treatment with morphine also restored the articular function. Employing pharmacological (minocycline and fluorocitrate) and molecular techniques, as real time PCR and Western Blotting (expression of GFAP and Iba1) was showed that there is a previous activation of satellite cells followed by astrocytes and microglia. The intrathecal treatment with IL-1Ra and infliximab, showed that this cells act by IL-1β and TNFα release in spinal cord and dorsal root ganglia, long term sensitizing the neurons. **Conclusion**: The DWB is an effective and predictable method to study pain and disability in AIA model. Glial cells participate as both induction and maintenance of pain and disability in AIA model. **Financial support**: CNPq and FAEPA

05.032 Evaluation of the analgesic Effect of systemic and topical citral. Antunes AMP, Rocha NP IBB-Unesp – Pharmacology

Introduction: Citral is a major component of lemongrass oil that is widely used as a food flavoring, as a perfume and for its analgesic and anti-inflammatory purposes. Several reports had shown that citral is an activator of all vanilloid receptors (TRP channels) found in sensory neurons and produces subsequent long-lasting inhibition of TRPV1–3 and TRPM8, while transiently blocking TRPV4 and TRPA1(PLoS ONE, 3:1,2008). To assess its potential usefulness in pain treatment, we examined citral's systemic and topical actions on mechanical inflammatory and neuropathic hyperalgesia (Randal-Sellito) and neuropathic cold allodynia in rats. Systemic citral was also assayed on thermal nociception mice (hot plate and tail-flick). **Methods:** All experimental protocols were approved by Ethics Committee on Animal Experimentation (CEEA/IBB, UNESP nº034/04). Male Wistar rats (180-250 g) were oral or topically (paw) treated with citral (400 mg/kg, or 10 (w/v), respectively) 1h before 1% carrageenan (50 ml/paw) or 15 and -22 days after sciatic nerve constriction injury (SNCI). Carrageenan - induced mechanical hyperalgesia (3h) and neuropathic mechanical hyperalgesia (1, 3 and 24h) were determined using a Randall-Selitto anesthesiometer. Acetone test was used to assay cold allodynia in SNCI rats (3 and 24h). Hot plate and tail-flick latencies (55°C) were observed (0, 1, 2 and 3h) in mice treated with oral citral (400 mg/kg). The results were expressed as mean±SEM and considered significant when $p<0.05$ (n=5-6 animals). **Results:** There were no significant differences between control or citral treated rats (oral or topical) in mechanical hyperalgesias induced by intraplantar injection of carrageenan or SNCI. Citral - treated mice have significantly enhanced latency only 3h after treatment (Control: 4.0 ± 1,0 sec ; Citral: 8,00 ± 2.2 sec) in tail-flick test. However, topical pretreated rats with citral significantly have reduced responses to cold 15 and 22 days after SNCI. The inhibition percentage of time of paw shaking was: 57% (1h), 70% (3h) and 0% (24h) after 10% citral paw application. **Discussion:** Citral showed weak central antinociception or mechanical antihyperalgesic action, but it was very strong against cold allodynia seen in neuropathic injury. Because of the already known citral's broad spectrum actions on TRP and the prolonged sensory inhibition, this substance may be useful as a new analgesic agent for treatment of cold allodynia, itch or other types of acute and chronic pain involving superficial sensory nerves. **Financial Support**: Capes

05.033 Aldehyde dehydrogenase 2 activation reduces neuropathic pain in rats. Neto BS[1], Ferreira JC[2], Mochly-Rosen D[3], Cury Y[1], Zambelli VO[1] [1]IBu – Dor e Sinalização, [2]ICB-USP, [3]Stanford University – Chemistry and Systems Biology

Introduction: Neuropathic pain control remains a challenge and an unmet clinical need. Aldehyde-dehydrogenase 2 (ALDH2) is a mitochondrial enzyme responsible for the metabolism of reactive aldehydes. Aldehydes accumulation has been recently related to increased pain. Recent data from our group has been shown that activation of ALDH2, using a small molecule called Alda1, displays a potent antinoceptive effect in a model of carrageenan-induced hyperalgesia (intraplantar, i.pl) in rats. ALDH2 activation induces analgesia by reducing aldehydic load. However, the role of ALDH2 in neuropathic pain control is still unknown. Therefore, we propose to investigate the involvement of ALDH2 in neuropathic pain, using Alda-1, an ALDH2 pharmacological agonist which selectively enhances the activity of ALDH2. **Methods**: The experiments were conducted in C57/BL mice following the protocols approved by the Butantan Institute Ethical Committee (976/12). Neuropathic pain was induced by sciatic nerve chronic constriction injury (CCI). The nociceptive threshold was determined, before and 14 days after surgery, using the electronic von Frey method. Fourteen days after surgery, a dose response curve for Alda-1 was performed (5, 10 and 20 mg/kg, s.c. route) and the pain threshold evaluated. **Results:** CCI decreased the pain threshold when compared to values obtained before surgery (67%). Alda1 (5 mg/kg) decreased CCI-induced nociception at 1 and 2 hours after its administration (33 and 94%, respectively, compared to baseline). Alda1 (10 and 20 mg/kg) also increased nociceptive threshold (88 and 91% at 1h, respectively, compared to baseline and 84 and 95% at 2 h, compared to baseline, respectively). No differences in pain threshold were detected 3 h after Alda1 injection. Alda1 vehicle treatment did not modify pain threshold. **Discussion:** The results indicate that activation of ALDH2 by Alda1 reduces CCI-induced nociception. This effect was not dose-dependent and lasted 2 hours. Our data propose a novel mitochondrial target for neuropathic pain control. Therefore, Alda-1 may be a novel therapeutic drug class to reduce neuropathic pain. **Support:** FAPESP (2011/08873-8, 2012/05035-4)

05.034 FAR infrared emitted by bioceramics reduces hypernociception of inflammatory origin in mice. Emer AA[1], Lenfers B[2], Cidral-Filho F[1], Martins DF[1] [1]Unisul – Neurociência Experimental, [2]UFSC – Neurociências.

Aim: Bioceramics are minerals that have photo thermal properties: when heated emit and/or reflect far infrared thermal radiation. This type of low level light radiation promotes molecular vibration leading to increased cellular metabolism and cell membrane permeability, triggering biochemical changes that stimulate the exchange of metabolites as well as ATP synthesis, up-regulation of chemical mediators which play a role in edema formation, pH regulation, free radicals metabolism and microcirculation (HONDA, Int J of Biomet, v. 2, p. 92, 1988.); resulting in physiological effects essential to the healing process, i.e., pain relief, acceleration of inflammatory processes, re-absorption of edema and nerve or lymphatic vessel regeneration (GRECO, Bio Biop Res Commun. v. 163, p. 1428, 1989.; VACCA Bio Biop Res Commun, v. 195, p. 704, 1993; VACCA Bio Biop Res Commun, v. 203, p. 991, 1994.; BAXTER, London: 1995; SKINNER, Aust Dent J, v. 41, p. 188, 1996). The aim of this study was to evaluate the effect of far infrared radiation emitted / reflected by bioceramics in a BioPower ® Pad - a health pad made of 80% BioCorn PVC and 20% bioceramics - on pain of inflammatory origin as well as on paw temperature increase and edema formation in an experimental model of inflammation in mice. **Methods**: Experiments were conducted using adult male Swiss mice weighing 25-35 g, housed at 22°C under a 12-h light/12-h dark cycle (lights on at 06:00), with access to food and water ad libitum. The experiments were performed after approval of the protocol by the Ethics Committee of the Universidade do Sul de Santa Catarina (UNISUL). The animals (n = 8) underwent intraplantar injection (right hind paw) of a solution containing 20 µl of Freud's complete adjuvant (CFA, 70%). For treatment a Biopower® Pad was placed inside the animals box. After 24 h of exposure to the product, mechanical nociceptive threshold was assessed as response frequency to 10 presentations of a 0.4g von frey filament applied to the animals right hind paw. The evaluations were performed daily for 10 days - after each evaluation, the animals were put back in their boxes and re-exposed to the Pad until the subsequent evaluation (24 hours). In addition, the volume (edema formation) and the temperature of the right hind paws were evaluated on experimental days 1, 3 and 10 with a Pleithsmometer and a digital thermometer respectively. Control animals were placed on a Sham Pad - consisting of 100% BioCorn PVC (without bioceramics) and underwent the same experimental protocol. **Results**: The results show that the i.pl. injection of CFA induced mechanical hypernociception ($P < 0.001$) which was significantly reduced by acute exposure to the BioPower® pad containing bioceramics. The analgesia lasted for up to 2 hours with peak effect 30 min after treatment ($P < 0.001$ - maximum inhibition of 53 ± 11%). Chronic treatment with the Biopower® Pad reduced mechanical hypernociception on all evaluation days. In addition, the treatment significantly decreased paw temperature on days 1 and 3 day, 8 ± 1% ($P < 0.001$) and 5 ± 1% ($P < 0.05$) respectively, when compared with the control group. Conclusion: Far infrared radiation emitted / reflected by bioceramics in the BioPower ® Pad reduced mechanical hypernociception of inflammatory origin as well as the increase of paw temperature induced by intraplantar injection of CFA in mice. Sources of research support: UNISUL.

05.035 Effect of topical beta-myrcene in chronic neuropathic pain and acute inflammatory hyperalgesia. Dias MC, Rocha NP IBB-Unesp-Botucatu – Farmacologia

Introduction: The monoterpene □-myrcene is an important analgesic component of the essential oil of many plants, such as *Cymbopogon citratus.* Previous studies have shown that systemic□-myrcene induced antihyperalgesic effect possibly mediated by activation of the arginine /NO/cGMP pathway (Eur J Pharmacol 217;225-7, 1992). We have previously demonstrated that oral □-myrcene treatment inhibit neuropathic mechanical hyperalgesia induced by chronic constriction injury (CCI) of sciatic nerve in rats. The aim of this study was to evaluate the analgesic effect of topically applied □eta-myrcene in rats with mechanical acute inflammatory hyperalgesia or mechanical and cold neuropathic allodynias induced by CCI. **Methods:** Neuropathic pain was induced in male Wistar rats (200 – 250 g), anesthetized with the association of ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) which were submitted to a chronic constriction injury (CCI). The von Frey filaments and the acetone test were used to assay mechanical and cold allodynias 15days after CCI at 0, 3, 24 and 48 hours after □eta-myrcene treatment (v.o: 400 mg/kg or topical (paw): 10% (w/v) in mineral oil. Withdrawal of the hind limb was considered a positive response either to mechanical allodynia (in grams) and cold allodynia (time of paw shaking in seconds). Mechanical hyperalgesia was determined using a Randall-Selitto anesthesiometer 3 and 4 h after intraplantar injection of 50 µl solution of 1% carrageenan 30 min after topical treatment with 10% or 20% □-myrcene. Values were expressed as mean ± S.E.M of 5 measurements. This study was approved by Ethics Committee on Animal Experimentation (CEEA/IBB,UNESP nº 030/04) .**Results:** Topical treatment of CCI animals with 10% □□□□-myrcene significantly enhanced mechanical threshold (Control – 0h : 13,3 ± 4 g; Treated rats -3h: 18,4 ± 2 g; 24h: 19,1 ± 3 g,;48h: 16,6 ± 5 g, $p<0,05$) and reduced cold allodynia (Control – 0h : 0,96 ± 0,2 sec ; Treated rats -3h: 0,39 ± 0,05 sec; 24h: 0, 40 ± 0,04 sec; 48h: 0,46 ± 0,05 sec, $p<0,05$) .Per oral or topical pretreatment of animals with □□□□-myrcene did not reduce mechanical allodynia in CCI-rats and hyperalgesia induced by intraplantar injection of carrageenan, respectively. Oral □□□□-myrcene significantly reduced cold allodynia induced by CCI. The inhibition percentage was:3h: 80%; 24h: 32% and 48h: 47%. **Discussion:** Topical □eta-myrcene treatment showed an excellent analgesic action against mechanical and cold allodynias induced by CCI. These effects were long-lasting until 48h after a single dose.In contrast, oral □eta-myrcene only showed a weak mechanical antiallodynic action in CCI- induced neuropathic pain, but it was also very strong against cold allodynia. Acute inflammatory hyperalgesia was not reduced by topical □-myrcene. Others studies are needed to elucidate the mechanisms involved in antiallodynic actions seen in neuropathic pain, mainly the arginine /NO/cGMP pathway or vanilloid receptors. **Financial support:** Capes

05.036 Analgesic and/or anti-inflammatory effects of two new compounds derived from pyrazole. Oliveira LP[1], Florentino IF[1], Sousa LV[1], Silva DPB[1], Menegatti R[2], Costa EA[1] [1]UFG – Fisiologia e Farmacologia, [2]UFG – Farmácia

Introduction: Inflammation is a series of responses of vascularized tissues of the body to injury. Most of anti-inflammatory drugs on market are inhibitors of prostaglandins (PGs) synthesis. These mediators are synthesized through the actions of phospholipase $A_2$and cyclooxygenases (COXs) on the arachidonic acid released from cellular membrane phospholipids. Due to their ability to inhibit cyclooxygenase activity and the arachidonic acid cascade, pyrazole-derived compounds, including dipyrone, have anti-inflammatory, antipyretic and analgesic properties, being perhaps most important, inhibition of prostaglandin biosynthesis in the cyclooxygenase step. The aim of this study is to analyze the analgesic and/or anti-inflammatory effects of two new compounds derived from pyrazole. **Material and Methods:** This study was performed using LQFM020 and LQFM039, molecules synthesized in 'Laboratório de Química Farmacêutica e Medicinal/FF/UFG' (LQFM). Experiments were performed using male *Swiss* albino mice (25–30 g). The antinociceptive and anti-inflammatory activities of LQFM020 and LQFM039 were evaluated by the methods of: acetic acid-induced writhing, formalin-induced pain; and the "in vitro" cyclooxygenase inhibition assay were also used. The experimental protocols were approved by the Ethic Commission of UFG (number: 017/13). **Results and Discussion:** In the acetic acid-induced writhing test, the LQFM020 (9, 17.5 and 35 mg/kg, p.o.) and LQFM039 (17.5, 35 and 70 mg/kg, p.o.) and indometacin reduced the number of writhes by 40, 45, 60, 35, 40, 53 and 43 %, respectively, when compared with the control value of 88.9 ± 2.23. LQFM020 (17.5 mg/kg, p.o.) and morphine reduced the licking time in the neurogenic phase (0–5 min) by 35 and 92 %, respectively, in relation to the control value of 78 ± 5.58 s. In the inflammatory phase (15–30 min), treatment with LQFM020 (17.5 mg/kg), indomethacin and morphine reduced the licking time from control value of 150 ± 15.38 s by 35, 42 and 98 %, respectively. LQFM039 (35 mg/kg, p.o.) and morphine reduced the licking time in the neurogenic phase (0–5 min) by 34 and 90 %, respectively, in relation to the control value of 72 ± 2.17 s. In the inflammatory phase (15–30 min), treatment with LQFM039 (35 mg/kg), indomethacin and morphine reduced the licking time from control value of 128 ± 5.98 s by 42, 32 and 96 %, respectively. LQFM020 and LQFM039 has IC50 estimate above the concentration of 2.78 mM; thus ruling out the inhibition of COXs as a possible mechanism of action. In conclusion, LQFM020 and LQFM039 have significant antinociceptive and inflammatory activities; pharmacological studies are continuing in order to characterize the mechanisms of action responsible for these action. **Financial Support**: CAPES, CNPq, FAPEG and FUNAPE/UFG.

05.037 Preliminary evaluation of the antinociceptive activity from bark of fruit of *Platonia insignis* Mart. (Clusiaceae). Souza RDS[1], Freitas FFBP[1], Nunes MGL[1], Santos BIS[1], Sousa SEL[1], Reis Filho AC[1], Costa ICG[2], Chaves MH[2], Almeida FRC[1] [1]UFPI – Plantas Medicinais, [2]UFPI – Química

Introduction: *Platonia insignis* Mart. is a member of the Clusiaceae family commonly known as bacuri, is a native species of the Brazilian Amazon and its fruits are appreciated like juice, cream and ice cream among others. It has been used in folk medicine to treat skin diseases in humans and animals, and the seed decoction to treat diarrheal and inflammatory diseases. The aim of the present study was to evaluate the antinociceptive effect of the ethanolic extract from bark of fruit from this species (EEPI) and its partition fraction (ethyl acetate - FAcOEt) in models of acute nociception as well as some of the possible mechanisms involved. **Methods:** Male and female Swiss mice (20-30 g, n= 6-10) were used in the evaluation of acute toxicity test. The animals showed no acute toxicity after the treatment with EEPI (up to 2 g/kg, po), so it was not possible to calculate the LD_{50}. Male Swiss mice were treated with EEPI (100-400 mg/kg, po) 60 min before the intraplantar injection of formalin (2 %) or capsaicin (2 µg). Control animals received vehicle (C- 2 % Tween 80 in saline) or morphine (5 mg/kg sc) and the nociception was evaluated by quantifying paw licking time after formalin (5 min (first phase) and 15–30 min (second phase)) or capsaicin (5 min). In glutamate test mice were treated with EEPI (100, 200 and 400 mg/kg, po), FAcOEt (50, 100 and 200 mg/kg, po) 60 min before injection of glutamate (10 µmol), vehicle or MK 801 (0.03 mg/kg ip) and the nociception was evaluated by 15 min. To study some mechanisms of action in the glutamate test, the animals were pretreated (20 min before FAcOEt) with naloxone (opioid antagonist, 2 mg/kg ip) and glibenclamide (ATP- sensitive K+ channels inhibitor, 3 mg/kg ip). The locomotor activity was evaluated in the open field task. The protocols were approved by the Animal Ethics Committee/UFPI (n°. 008/2012) and were carried out in accordance with the current ethical guidelines for investigation of experimental pain in conscious animals. **Results:** EEPI (200 and 400 mg/kg) significantly reduced the formalin response in second phase of the test when compared with vehicle. A significant reduction in time length spent on licking the paw was observed with EEPI (200 mg/kg) in the capsaicin test. In glutamate test, EEPI (400 mg/kg) and FAcOEt (100 mg/kg) showed a significant antinociceptive effect ($p<0.05$). The FAcOEt antinociceptive effect was reversed by naloxone and glibenclamide. In the open field test, EEPI (200 and 400 mg/kg) did not change the frequency of crossings. **Discussion:** These results provided for the first time, evidence that oral administration of the EEPI exerted pronounced antinociception when assessed in formalin (second phase), glutamate or capsaicin induced nociception models in mice and the FAcOEt also exerted antinociceptive effect in glutamate test. The antinociceptive action of FAcOEt possibly involves the participation of opioid system and potassium channels. It is unlikely that the observed effects are due to central depressant activity since it was unable to modify the motor behavior in open field test. **Financial Support:** UFPI, CAPES/Brazil.

05.038 Anti-inflammatory and antinociceptive activities of LQFM046 molecule. Silva DPB[1], Florentino IF[1], Galdino PM[1], Oliveira LP[1], Menegatti R[2], Costa EA[1] [1]UFG – Fisiologia e Farmacologia, [2]UFG – Farmácia

Introduction: Nonsteroidal anti-inflammatory drugs (NSAIDs) have been commonly used due to its anti-inflammatory, antipyretic and analgesic effects, but these drugs have serious side effects such as gastrointestinal ulcers and bleeding. In an attempt to improve the effectiveness and reduce the side effects of anti-inflammatory drugs, the Laboratory of Pharmaceutical Medicinal Chemistry designed and synthesized the molecule LQFM 046, which is a molecular hybridization of clopirac and acetaminophen (paracetamol), both NSAIDs. This strategy aims to maintain the therapeutic activities of starter in the new compound. The objective of this study was to evaluate the anti-inflammatory and antinociceptive activities of LQFM 046, seeking to understand the mechanisms involved. **Methods:** The animals used were adult male *Swiss* mice weighing 35 - 40g (n= 9). The antinociceptive and anti-inflammatory effects of LQFM 046 were evaluated by the methods of acetic acid-induced writhing and formalin-induced pain, in animals with or without naloxone pretreatment. "*In vitro*" assay of COX-1 enzyme to verify concentration versus effect of LQFM046. All experimental protocols were approved by the Research Ethics Committee of the UFG (Protocol Nº. 182/10). **Results and Discussion:** LQFM046 (300 mg/kg, p.o.) reduced the number of acetic acid-induced writhes from control value of 92.3 ± 6.1 by 62.2 ± 7.04. LQFM046 (150 and 300 mg/kg, p.o.) reduced the licking time in the neurogenic phase (0–5 min) by 33 and 44 %, respectively, in relation to the control value of 63.3 ± 3.2 s. In the inflammatory phase (15–30 min), the treatment with LQFM046 (75, 150 and 300 mg/kg), reduced the licking time from control value of 211.7 ± 21.5 s by 47, 64 and 66%, respectively. The pretreatment with naloxone (3 mg/kg p.o.) did not reduce the antinociceptive activity of LQFM046 (150 mg/kg p.o.) in the first or second test of formalin-induced pain. In the "in vitro" evaluation the LQFM 046 was able to reduce the COX-1 activity in a concentration-dependent manner (IC_{50} = 20 μM). The reduction in writhes suggests that LQFM046 has an analgesic effect; this effect was confirmed in the formalin test. However, the analgesic effect in the first and second phase was not blocked by an opioid antagonist, suggesting that LQFM046 effect is independent of opioid receptors activation. The inhibition of COX-1 may explain, in part, the analgesic effects of LQFM046. In conclusion, LQFM046 has antinociceptive and anti-inflammatory effects that may be due to COX-1 inhibition. **Financial Support**: CAPES, CNPq, FAPEG and FUNAPE/UFG

05.039 Immune cell infiltration and production of inflammatory mediators in dorsal root ganglion, but not in spinal cord, are related to murine herpetic hyperalgesia. Silva JR, Talbot J, Lopes AHP, Cunha TM, Cunha FQ FMRP-USP – Farmacologia

Introduction: Herpes Zoster (HZ) is a disease caused by the reactivation of latent herpesvirus Varicella Zoster (VZV) in the sensory ganglion, characterized by dermal rash and severe pain. VZV infects only humans, and there are no animal models available to study the disease. However, when mice are inoculated with herpes simplex virus type-1 (HSV-1) on the skin of the hind paw, they develop HZ-like skin lesions and show pain-related responses to noxious mechanical stimulation and innocuous tactile stimulus. For this reason, this model has been used to study the pathophysiology of herpes zoster. So far, there are no data available about the immune response in dorsal root ganglion (DRG) of mice infected with HSV-1 in this model, neither the relation between inflammatory response and hyperalgesia development. Thus, the aim of this study was to evaluate immune cells and inflammatory mediators present in DRGs and its relationship with herpetic hyperalgesia. **Methods:** Briefly, mice were depilated with a chemical depilatory and three days later 2 x 10^5 plaque forming unities (PFU) of HSV-1 were inoculated in the skin of the right hind paw after scarification. Mice were observed daily and behavioral tests were performed from 0-21 day post inoculation. The DRGs L1-L6 were collected at 7, 15 and 21 days post infection (dpi) to evaluation of cellular infiltration (flow citometry), western blot analysis (GFAP and COX-2 expression) and PCR (TNF-a and COX-2 mRNA expression).Viral load was measured by quantitative Real-Time PCR. In some groups mice were treated with anti-TNF-a (1ug/i.t/day). All experiments were approved by the Animal Ethics Committee from FMRP/USP (nº 105/2010). **Results and Discussion:** Mice developed hyperalgesia from 3 to 21 dpi in the ipsilateral (ips) paws, but not in the contralateral (cl) paws. At 12 dpi all mice recovered from zoosteriform skin lesions. However, approximately 50% of mice showed persistent hyperalgesia behavior without zoosteriform skin lesions until 45dpi. A higher viral load was detected in DRGs L4, L5 and L6 of infected mice at 7 dpi, when compared to control or naive mice. We also observed an intense activation of satellite glial cells in ips DRGs (GFAP expression). Moreover, we observed, by flow cytometry, an intense inflammatory infiltrate composed by neutrophils and macrophages in ips DRGs but not in cl DRGs or spinal cord at 7dpi. Lymphocytes (CD4+ and CD8+) infiltration was detected at 15 and 21dpi in ips DRGs but not at the spinal cord. On infected mice, a higher mRNA expression of COX-2 and TNF-a was detected in ips DRGs. Moreover, blockage of TNF-a reduced the development of herpetic hyperalgesia. **Conclusions:** Our results show the presence of an intense inflammatory infiltrate in DRGs of infected mice, and the early expression of inflammatory mediators in this local that contribute for the induction of herpetic hiperalgesia. **Financial support:** FAPESP (2010/12309-8), FAEPA, TIMER.

05.040 The role of NOD1 and NOD2 during peripheral neuropathy, glial activation and release of pronociceptive cytokines. Ferreira DW[1], Santa-Cecília FV[1], Cunha FQ[1], Ferreira SH[1], Zamboni DS[2], Cunha TM[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Biologia Celular e Molecular e Bioagentes Patogênicos

Introduction: Among pattern recognition receptors (PRRs), the Toll-like receptors (TLRs) and NOD-like receptors (NLRs) are the most important in recognizing the pathogen-associated molecular patterns (PAMPs). Upon recognition of PAMPs, NLRs, such as NOD1 and NOD2, recruit directly RIPK2 (receptor-interacting serine/threonine-protein kinase 2), an adaptor protein important in NLRs-mediated NFκB activation. A previous work has indicated that TLRs play a crucial role in the activation of spinal cord glial cells in the induction and maintenance of chronic inflammatory and neuropathic pain. Furthermore, in models of inflammation/infection of the central nervous system, NLRs and TLRs cooperate in the activation of glial cells. Thus, it is possible that both receptors may be important for microglia activation in chronic pain models. In the present study, we aimed to evaluate the role of receptors NOD1 and NOD2 in the genesis of neuropathic chronic pain, focusing on their signaling pathways (RIPK2) and release of pronociceptive cytokines, such as IL-1β, IL-6 and TNF-α. **Methods:** The experiments were carried out on male C57BL/6 (WT) mice or male NOD1, NOD2, RIPK2 and DKO deficient mice. All animal care and experimental procedures were conducted according to the guidelines of the Ethics Committee (106/2011) of the School of Medicine of Ribeirão Preto (University of São Paulo, São Paulo, Brazil) and according to the IASP guidelines on the use of laboratory animals. Mice were anesthetized with isoflurane and induced to the Spared Nerve Injury neuropathy model (SNI). Spinal activation of NOD2 was performed by intrathecal administration of MDP (5 µl volume). The mechanical threshold (in grams) was determined by application of von Frey filaments to the hindpaws. The levels of gene expression were determined by RT-PCR and the levels of protein expression were determined by ELISA and Western blot in spinal cord samples (segments L4, L5 and L6). **Results:** The results demonstrate that NOD1, NOD2, RIPK2 deficient mice, as well as NOD1/2 double deficient mice (DKO) showed a significant reduction of mechanical hypersensitivity (from 5th day to 21th day) after peripheral neuropathy (SNI). At 7th day after peripheral neuropathy, the analysis of the activation profile of the spinal cord glial cells showed increased expression of Iba-1 and GFAP in WT mice. Additionally, it was observed a reduction transcriptional levels of Iba-1 and GFAP in NOD1, NOD2, RIPK2 and DKO mice, and a reduced production of IL-1β and TNF-α. Nevertheless, the levels of IL-1β, IL-6 and TNF-α mRNA are increased in the spinal cord after acute administration of MDP (NOD2 ligand). Moreover, daily administration of MDP (for 5 days) increased levels of IL-1β, TNF-α and Iba-1 mRNA. **Discussion:** The results suggest that NOD1 and NOD2, via RIPK2, contribute to neuropathic pain, possibly by mediating the release of pronociceptive cytokines and increased glial activation. These mechanisms represent a novel approach for elucidating the pathophysiology of chronic pain, and a target for the development of drugs for the treatment of neuropathic pain. **Financial Support:** CNPq, FAPESP.

05.041 LASSBio-1247 is an effective prototype drug candidate to treat rheumatoid arthritis pain. Santos EAP[1], de Sá Alves FR[1], Lima CKF[1], Fraga CAM[2], Barreiro EJ[1], Miranda ALP[1] [1]UFRJ – Fármacos, [2]ICB-UFRJ

Introduction: Rheumatoid arthritis (RA) is characterized by persistent synovitis with cartilage and bone damage at multiple joints. Most of these changes are mediated by cytokines produced by leukocytes, as TNF-α, IL-1β and IL-6. Besides cell chemotaxis, TNF-α plays crucial role in pathogenesis and associated pain of RA (FELDMAN, Nat Rev Imm,2,364,2002). Thus, the reduction of TNF-α is an important strategy to treat RA. Previous study showed that LASSBio-1247 decreases TNF-α*in vitro*, has a relevant anti-hypernociceptive effect and reduces MPO level in an inflammatory carrageenan model (SANTOS, Dissertação-Ms,84,2009). The present study aimed to deepen the anti-hypernociceptive and anti-inflammatory properties of LASSBio-1247 and to evaluate in RA model. **Methods:** To access cell viability (MTT test) and TNF-α production, macrophages were stimulated by LPS and incubated with LASSBio-1247 at different concentrations to determine the IC_{50}. Cell migration was accessed by neutrophil chemotaxis induced by fMLP in a Boyden chamber (FIERRO, J Immun,170,2688,2003). The anti-hypernociceptive effect was evaluated on thermal (hot-plate) LPS- and capsaicin-induced hypernociception (VAJJA, Int Imm,4,901,2004; MIZUSHIMA, Pain,113,51,2005) and on mechanical hypernociception in the mBSA-induced delayed-type hypersensibility RA model. Total leucocytes count of the articular washes was determined by optical microscopy (CUNHA, Eur J Pain, 12,1059,2008). The stomachs were removed in order to evaluate the gastric damage in treated animals. Animal protocols were approved by UFRJ ethical animal care committee (FARMACIA02/CEUA/UFRJ). **Results** are expressed in mean ± standard error and were statistically analyzed using ANOVA-oneway (n=6-8 animals/group;*$p<0.05$). **Results and Discussion:** LASSBio-1247 showed a IC_{50}=12,9μM on the TNF-α production and the inhibition is not associated with cell death, since cell viability was reduced only by 30% at 100μM, a concentration 10 times higher than its IC_{50}. LASSBio-1247 inhibited 55%* the LPS-induced thermal hypernociception that is mediated by TNF-α, IL-1β and IL-6 (VAJJA, Int Imm,4,901,2004). LASSBio-1247 at 100μmol/kg orally twice/day (5 days) didn't induce stomach damage in the hypernociceptive test induced by mBSA. Moreover, the compound reversed hypersensitivity at several points of the acute and chronic phases*, reduced the total number of leukocytes at the joint* and also the neutrophil chemotaxis *in vitro* by 50%*. LASSBio-1247 inhibited by 70%* the hypernociception induced by capsaicin, a response mediated by the p38MAPK and TRPV1 activation (MIZUSHIMA, Pain,113,51,2005). In order to evaluate TRPV1 antagonism, calcium-imaging assay showed LASSBio-1247 at 10μM couldn't reduce Ca^{+2} influx in HEK293 cells expressing hTRPV1 after capsaicin stimulation. Preliminary results indicate LASSBio-1247 can reduce TNF-α level at plasma and synovial fluid from mBSA treated animals. So, the anti-hypernociceptive and anti-inflammatory effects of LASSBio-1247 could be in part due to the decrease of TNF-α, leading to a decrease of cell migration. Furthermore, it may be acting on the p38MAPK signaling pathway, whose effect will be investigated. These results point out LASSBio-1247 as a promising and interesting prototype of a drug candidate to treat RA. **Financial support**: CAPES INCT-INOFAR FAPERJ

05.042 The medial plantar nerve ligation (MPNL) as a new model of neuropathic pain. Sant'Anna MBM, Souza GR, Bozzo TA, Ferreira SH, Cunha FQ, Cunha TM FMRP-USP – Farmacologia.

Introduction: Neuropathic pain is a condition of maladaptation caused by structural and functional changes of central and peripheral sensory pathways that produce changes in processing nociceptive information. **Objectives:** In an attempt to better understand these events, our laboratory has developed a model of neuropathic pain consisting of a minimally invasive surgery. The animals quickly recover from surgery (one day) allowing us to study not only the events that occur when the neuropathy is already installed but also in the early days after nerve injury. **Materials and methods:** Under anesthesia (2% isoflurane) a small incision of approximately 0.5 cm was performed in the right ankle to expose the medial plantar nerve, which is then tied up with a catgut suture. The nociceptive evaluation was performed using the von Frey filaments during twenty-five days after surgery. Motor coordination was evaluated by rota-rod and foot print recording and analysis. **Results:** Firstly, it was observed that MPNL produced a reductionin the mechanical nociceptive threshold of mice paw. Interestingly, animals subjected to MPNL present a fast recovery and they did not demonstrated any motor impairment as opposed to other well recognized models of neuropathic pain. In addition, the pharmacological treatments with indomethacin, morphine, and gabapentin at 3 days after surgery reduced mechanical hypersensitivity. However, only gabapentin treatment was able to reduced mechanical hypersensitivity at 14 days after surgery. Moreover, it was observed that MPNL caused an up-regulation of GFAP and ATF-3 expression in the dorsal root ganglion. **Conclusion:** These results indicate that MPNL is a reasonable new model to study chronic pain in mice, presenting similar pharmacological and phenotypic characteristics to other existing neuropathic pain models.