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Hypernociception elicited by tibio-tarsal joint flexion in mice: A novel experimental arthritis model for pharmacological screening

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Abstract

Mice have been used as animal model to study mechanisms underlying inflammatory and immune diseases. The present study describes a model of joint inflammation-induced hypernociception to discriminate pharmacological tests in mice. A polypropylene tip probe with a large area (4.15 mm^2) applied on the plantar surface of the hind paw was used to produce a dorsal flexion of tibio-tarsal joint. Experiments were performed to demonstrate that the probe application did not provoke cutaneous nociception. The decrease in the withdrawal threshold of inflamed joint was used as nociceptive parameter. Administration of zymosan in the tibio-tarsal joint induced a dose and time-dependent hypernociception elicited by articular dorsal flexion movement. Maximal joint hypernociception was detected between 7 and 24 h after zymosan injection, and matched maximal inflammation score as determined by histopathology and neutrophil migration assay. In agreement with the inflammatory hypernociceptive paradigm, flexion-elicited hypernociception induced by zymosan was dose-dependently inhibited by morphine (2–8 mg/kg) and by an effective dose of indomethacin (5 mg/kg). The present study demonstrated that the tibio-tarsal flexion reflex is a behavioral nociceptive model that allows a quantitative evaluation of inflammatory joint hypernociception in mice and its pharmacological modulation.

Keywords: Zymosan; Joint pain; Arthritis; Hypernociception; Hyperalgesia

1. Introduction

A common denominator of inflammatory pain is the sensitization of nociceptors associated with small diameter primary afferent neurons, resulting in a decrease of the nociceptive threshold or shortening of nociceptive behavior end-point (Julius and Basbaum, 2001). This phenomenon may correspond to the clinical state known as hyperalgesia and allodynia, or better defined in animal models as hypernociception (Cunha et al., 2005; Millan, 1999; Parada et al., 2003). Hyperalgesia and stiffness of joints are very frequent symptoms in arthritic patients, which cause limitation of the joint movement. Yet, weight-bearing joint overload aggravates this process (Jacoby et al., 1973). Despite of its clinical relevance, the treatment of the articular hyperalgesia is still limited.

Several experimental models have been used to investigate the pathophysiology of arthritis. Additionally to kaolin, urate crystals, carrageenan or zymosan, the adjuvant and the type II collagen have been used to induce arthritis in rats and mice (Coderre and Wall, 1987; Faires and McCarty, 1962; Helyes et al., 2004; Keystone, 1977; Okuda et al., 1984; Rordorf et al., 1987; Rosenthale et al., 1966; Trentham et al., 1980). However, models of arthritis that allow the study of the joint hyperalgesia

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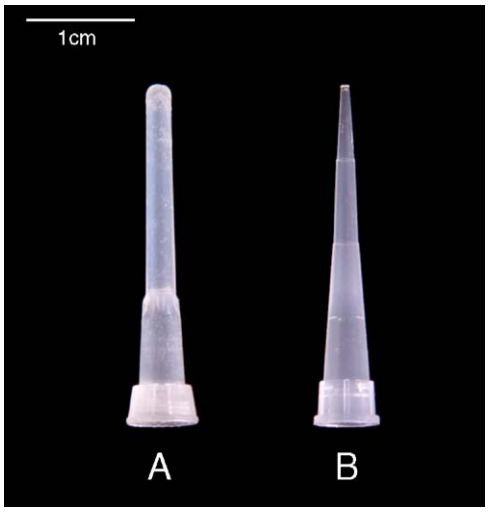

Fig. 1. A polypropylene tip probe with 4.15 mm^2 area size (A) adapted to hand-held force transducer, instead to the standard tip probe (0.5 mm^2, B), was applied on the plantar surface of hind paw to produce tibio-tarsal flexion movement.

are rare and frequently limited. Inflamed joint-induced motor impairment in rats (incapacitation test) is commonly used to evaluate the hypernociception in arthritis. Carrageenan or zymosan-induced articular incapacitation of knee joint of rats is a reproducible test and independent of the observer. It is an appropriate test to study of articular hypernociception associated with deambulation (Rocha et al., 1999; Tonussi and Ferreira, 1992). Moreover, recently, a model to evaluate articular inflammatory hypernociception was developed based on weight-bearing (Pomonis et al., 2005). Although these tests were described for rats, in mice only indirect evaluation of arthritic hypernociception by stimulating the surrounding tissues in the hind paw after adjuvant administration has been used to study joint hypernociception (Chillingworth and Donaldson, 2003). However, this strategy may reflect pain sensitization of cutaneous nociceptors instead of joint nociceptive neurons or even secondary hypernociception.

To our knowledge, there are no models that directly measure joint hypernociception in mice, and it is important to highlight that mice are very important for biological research, in particular those ones involved in immune responses since most bioengineered reagents, such as monoclonal antibodies, are based on mice, and, in addition, a wide range of genetically modified mice are available. Consequently, taking into account this information, the aim of the present study was to develop a novel model to directly evaluate inflammatory joint hypernociception in mice and test its responsiveness by classical anti-hypernociceptive drugs.

2. Materials and methods

2.1. Animals

The experiments were performed on male C57BL/6 mice (20–25 g, University of São Paulo, Ribeirao Preto, SP, Brazil) housed in standard clear plastic cages (five mice per cage) with free access to food and water. All behavioral tests were performed between 9:00 am and 5:00 pm in a temperature-controlled room. Animals' care and handling procedures were in accordance with the International Association for Study of Pain (IASP) guidelines and with the approval of the Ethics Committee of the Faculty of Medicine of Ribeirao Preto (University of São Paulo). All efforts were made to minimize the number of animals used and their suffering.

2.2. Dorsal flexion of the tibio-tarsal joint: assessment by a modified electronic pressure-meter test for mice

Experiments were performed as previously described (Cunha et al., 2004). In a quiet room, mice were placed in acrylic cages (12×10×17 cm high) with a wire grid floor 15–30 min before testing for environmental adaptation. Stimulations were performed only when animals were quiet, without exploratory movements or defecation and not resting on their paws. In these experiments, an electronic pressure-meter was used. It consists of a hand-held force transducer fitted with a polypropylene tip (IITC Inc., Life Science Instruments, Woodland Hills, CA, USA). For this model, a non-standard large tip (4.15 mm^2, Fig. 1A) was adapted to the probe. An increasing perpendicular force was applied to the central area of the plantar surface of the hind paw to induce the dorsal flexion of the tibio-tarsal joint, followed by paw withdrawn. A tilted mirror below the grid provided a clear view of the animal's hind paw. The electronic pressure-meter apparatus automatically recorded the intensity of the force applied when the paw was withdrawn. The test was repeated until 3 subsequently consistent measurements (i.e. the variation among these measurements was less than 1 g). The flexion-elicited withdrawal threshold is expressed in grams (g).

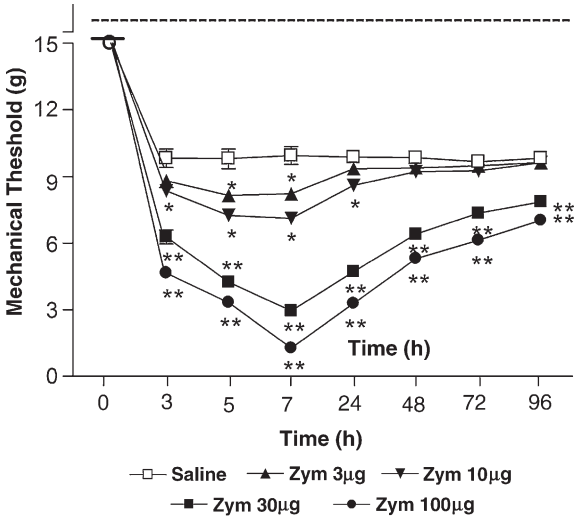

Fig. 2. Dose- and time-dependent flexion-elicited hypernociception of zymosan inflamed tibio-tarsal joints in mice. Zymosan (Zym, 3, 10, 30, 100 μg in 5 μl) or saline (5 μl) was injected into the tibio-tarsal joint, and the flexion elicited hypernociception was evaluated after 3–96 h with electronic pressure-meter test. The results are expressed as the mean±SEM of five animals per group. The symbol * means statistically significant difference when compared with saline treated mice (5 μl) and ** when compared with Zym, 3 and 10 μg, treated mice ($P<0.05$; two-way ANOVA, and one-way ANOVA followed by Bonferroni test).

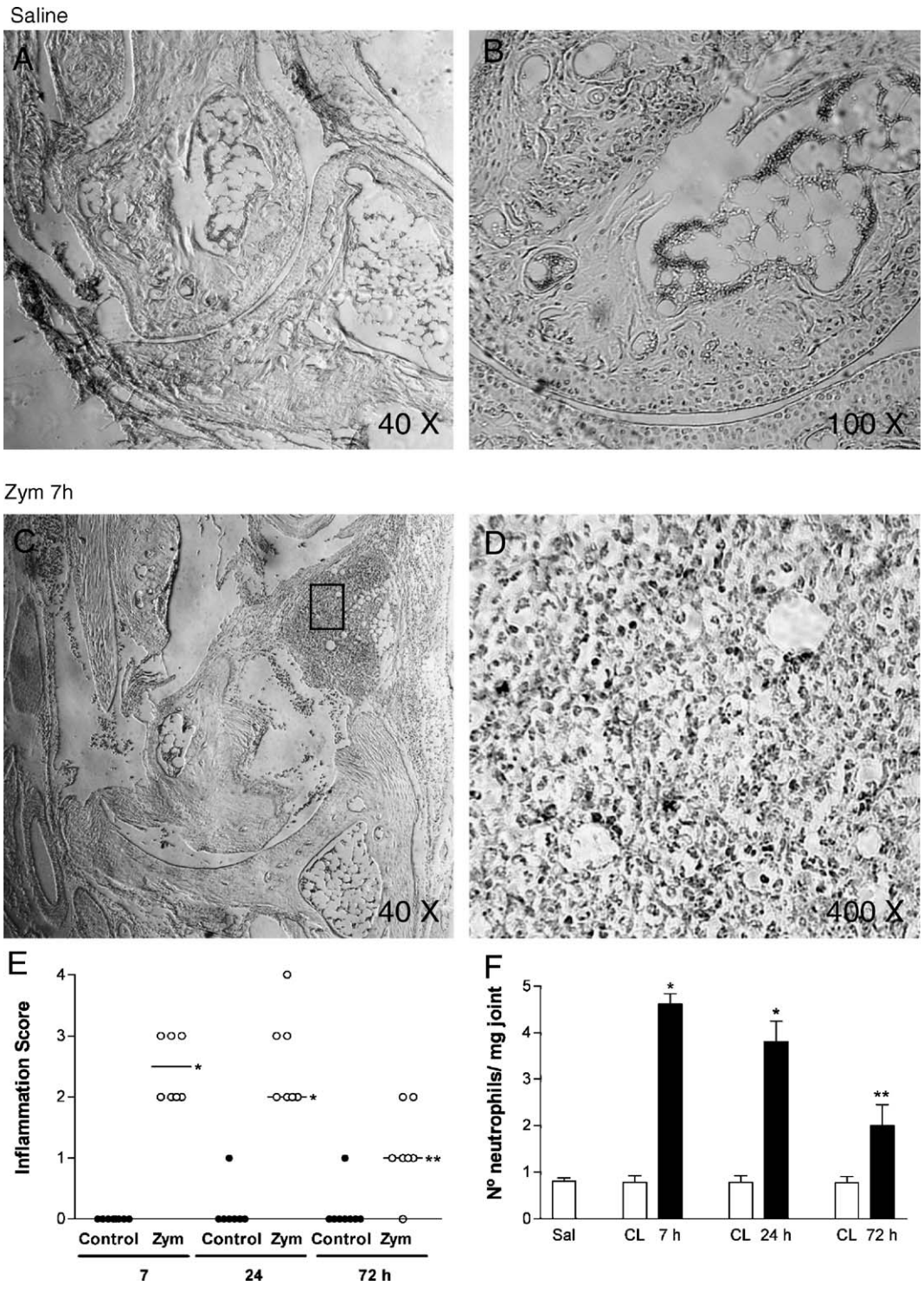

Fig. 3. Histological and MPO evidences of zymosan-induced inflammation of mice tibio-tarsal joint. Zymosan (Zym, 30 μg in 5 μl) or Saline (5 μl) was injected into the tibio-tarsal joint of mice. The articular region was collected after 7, 24 and 72 h for histological analysis as presented by the histological evidence in the 7th h for saline (Panels A and B, 40× and 100×, respectively) and zym (Panels C and D 40× and 400× respectively), the inflammation score (Panel E) or MPO assay as presented by the number of neutrophils/mg of joint (Panel F). The results are expressed as the mean ± SEM of five animals per group. The symbol * means statistically significant difference when compared with saline injection and ** when compared with 7 and 24 h ($P<0.05$ Kruskal–Wallis test and Dunn's post test and ANOVA one-way followed by Bonferroni's test, Panels E and F respectively).

2.3. Induction of tibio-tarsal joint inflammation

Joint inflammation was induced by administration of zymosan (Sigma, St Louis, MO, USA) diluted in 0.9% saline (3, 10, 30 and 100 μg) into the right tibio-tarsal joint region of mice lightly anaesthetized. The volume administered was 5 μl via a 29 G hypodermic needle inserted into the joint. Control animals received a single intra-articular injection of the same volume of sterile saline.

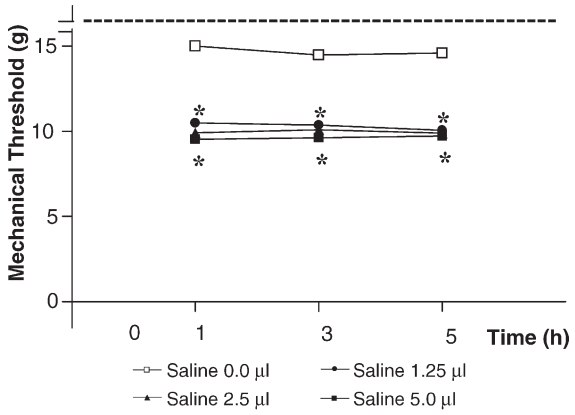

Fig. 4. Volume injection in the tibio-tarsal joint per se elicited a decrease of the flexion movement-induced withdrawal threshold. Mice received a free-volume or saline (1.25–5 µl) injection into the tibio-tarsal joint. The flexion-elicited hypernociception was evaluated after 3–5 h with the electronic pressure-meter test. The results are expressed as the mean±SEM of five animals per group. The symbol * means significant difference when compared with the free-volume group ($P<0.05$ two-way ANOVA, and one-way ANOVA followed by Bonferroni test).

2.4. Histology

The tibio-tarsal bone complex was harvested and fixed in 10% neutral-buffered formalin for 48 h. After decalcification in 10% EDTA pH 7.2 at room temperature for 3 weeks, specimens were embedded by routine histological technique in paraffin and sectioned at 7 µm for hematoxylin and eosin staining. All sectioning were done in a sagittal plane and sections which included entire tibio-tarsal joint were selected for analysis. Samples were harvested at 7, 24 and 72 h after zymosan administration. A minimum of six sections per animal were evaluated by a blinded observer. Histopathological changes in the tibio-tarsal joint were evaluated using the following parameters: cell infiltration in the articular space and synovial tissue, the presence of bone re-absorption, and the synoviocytes proliferation. Each sample was scored for the degree of inflammatory infiltration of the periarticular tissue on following scale: (0) no evidence of inflammation; (1) slight inflammation, characterized by inflammatory cells invading in 1 to 10% of the periarticular tissue; (2) moderate inflammation, characterized by inflammatory cells invading in 10 to 25% of the periarticular tissue; (3) intense inflammation, in 25 to 50% of the periarticular tissue and (4) severe inflammation, inflammatory cells invading more than 50% of the periarticular tissue.

2.5. Myeloperoxidase activity (MPO) assay

The tibio-tarsal joint region was separated from the tibio-tarsal bone complex at 7, 24 and 72 h after zymosan administration. The neutrophil migration to the tibio-tarsal joint region of mice was evaluated by the myeloperoxidase (MPO) kinetic–colorimetric assay as previously described (Graff et al., 1994). Samples were collected in 50 mM K_2HPO_4 buffer (pH 6.0) containing 0.5% hexadecyl trimethylammonium bromide (HTAB) and conserved at −80 °C until. The tissue was homogenized using a Polytron (PT3100) and centrifuged at 16,100 ×g for 4 min. A volume of 10 µl of supernatant were mixed with 200 µl of 50 mM phosphate buffer pH 6.0, containing 0.167 mg/ml *O*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The solution was analysed by spectrophotometry for MPO activity determination at 450 nm (Spectra max), with 3 readings in 1 min. The MPO activity was compared to a standard curve of neutrophils. The results were presented as the MPO activity (number of neutrophils/mg of tissue).

2.6. Drugs

Indomethacin (5 mg/kg) was obtained from Prodome, (São Paulo, Brazil), and made up in 1.2% Tris (Merck, Darmstadt, Germany). Morphine Sulphate (2–8 mg/kg) and 2% Lidocaine Chloride were obtained from Cristalia, (São Paulo, Brazil). Naloxone hydrochloride (1 mg/kg) and zymosan (3, 10, 30 and 100 µg/joint) were obtained from Sigma-Aldrich (St Louis, MO, USA). All compounds, except indomethacin, were dissolved in saline. Indomethacin, morphine and naloxone were administrated intra-peritoneally (i.p.) in a volume of 100 µl. Solution of 2% lidocaine was locally administrated in the subcutaneous plantar tissue in a volume of 25 µl.

2.7. Statistical analysis

Results are presented as means±s.e.m. of measurements performed in 5 mice per group. Two-way analysis of variance (ANOVA) was used to compare the groups and doses at all times (curves) when the hypernociceptive responses were measured at different times after the stimulus injection. The analyzed factors were treatments, time and time versus treatment interaction. When there was a significant time versus

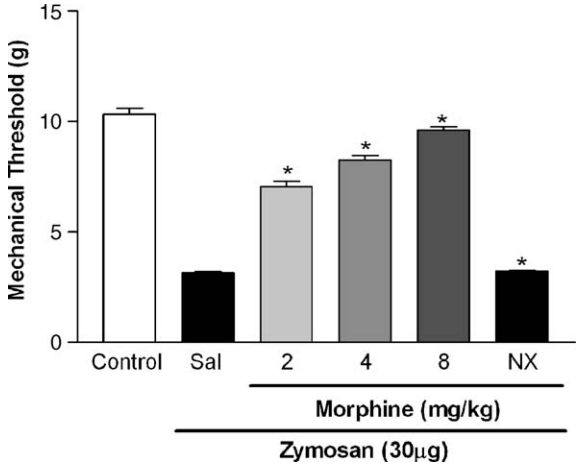

Fig. 5. Morphine inhibits zymosan elicited tibio-tarsal joint hypernociceptive flexion in a naloxone sensitive manner. Zymosan (30 µg in 5 µl) or saline (Control-5 µl) was injected into the tibio-tarsal joint. The animals were post-treated (6 h after stimulus injection) with morphine (2–8 mg/kg) or vehicle (Sal). The effect of naloxone (NX, 1 mg/kg) 30 min before morphine (2 mg/kg) was also demonstrated. The flexion-elicited hypernociception was evaluated after 7 h with the electronic pressure-meter test. The results are expressed as the mean± SEM of five animals per group. The symbol * means significant difference when compared with the free-volume group ($P<0.05$ Two-way ANOVA, and one-way ANOVA followed by Bonferroni test).

treatment interaction, one-way ANOVA followed by Bonferroni's *t* test was performed for each time. On the other hand, when the hypernociceptive responses were measured once after the stimulus injection, the differences between responses were evaluated by one-way ANOVA followed by Bonferroni's *t* test. Differences in the inflammation scores between the groups were expressed as median±s.e.m. and analyzed by the Kruskal–Wallis test and Dunn's post test. Statistical differences were considered to be significant at $P<0.05$.

3. Results

3.1. Kinetics of the hypernociception in the zymosan-induced tibio-tarsal joints inflammation in mice

The administration of 5 μl of zymosan suspension into the tibio-tarsal joint of mice induced a dose- (3, 10, 30 and 100 μg) and time (3, 5, 7, 24, 48, 72 and 96 h) dependent dorsal flexion-elicited hypernociception. The hypernociceptive responses were maximal at 7 h after zymosan administration, coinciding with the maximal neutrophil migration (Figs. 2 and 3, panel F). The sub-maximal dose of zymosan, 30 μg, was used for the next experiments. There was no flexion-elicited hypernociception in the contralateral tibio-tarsal joint (data not shown).

3.2. Tibio-tarsal joint histological analysis and neutrophil migration

As shown in the Fig. 3, zymosan induced significant inflammatory infiltration 7 h after its injection lasting for 24 h (Fig. 3, panels C, D and E), and decreasing after 72 h (Fig. 3, panel E),

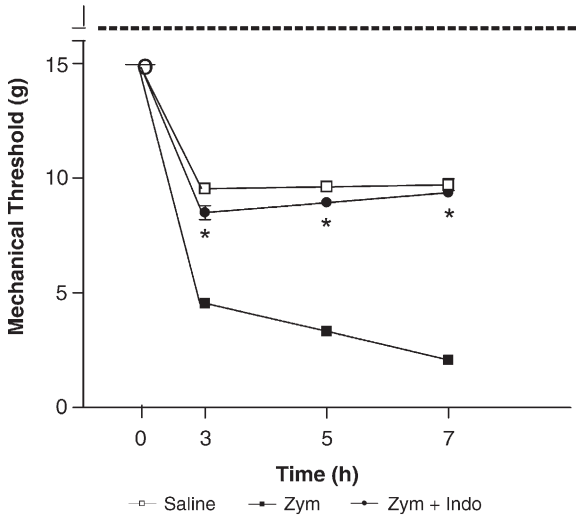

Fig. 6. Zymosan tibio-tarsal joint elicited hypernociceptive-flexion depends on prostanoids. Mice were treated with indomethacin (5 mg/kg/i.p.) or vehicle (200 μl, Tris/HCl buffer) 40 min before the tibio-tarsal joint injection of zymosan (Zym, 30 μg in 5 μl). The flexion-elicited hypernociception was evaluated after 3–7 h with the electronic pressure-meter test. The results are expressed as the mean±SEM of five animals per group. The symbol * means significant difference when compared with vehicle treated mice ($P<0.05$ ANOVA two-way; and ANOVA one-way followed by Bonferroni's *t* test performed for each time).

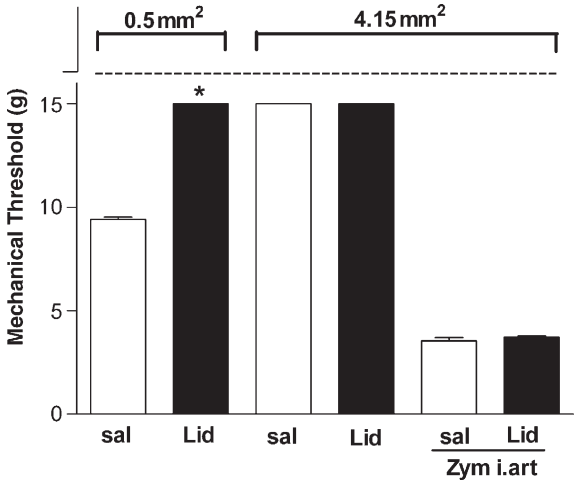

Fig. 7. Effect of i.pl. lidocaine injection on articular hypernociceptive response. Mice received an intraplantar injection of lidocaine (Lid — 2%, 5 μl) or saline (sal — 5 μl) 6.5 h after the i.art. injection of zymosan (Zym, 30 μg in 5 μl). The hypernociception was evaluated 30 min after lidocaine or saline injection with the electronic pressure-meter test using two area size (4.15 mm^2 and 0.5 mm^2) tip probes. The results are expressed as the mean±SEM of five animals per group. The symbol * means significantly difference when compared with the respective saline injected group (Sal). ($P<0.05$, Student *t*-test).

when compared with saline treated group (Fig. 3, panels A and B). It was also observed synovial edema and hyperplasia in the zymosan-treated joint (data not shown). Additionally, the myeloperoxidase activity assay demonstrated a time-dependent increase of neutrophils migration, which was higher between 7 and 24 h post zymosan treatment (Fig. 3, panel F).

3.3. Effect of fluid volume in the hypernociceptive response of the tibio-tarsal joint of mice

This experiment was performed to verify whether the increase of volume in the joint induce hypernociception by itself. As shown in the Fig. 4 saline injection (1.25, 2.5 or 5.0 μl), but not the needle puncture in the tibio-tarsal joint, diminished the mechanical threshold. Intra-articular injection of saline in a volume equal or bigger than 1.25 μl caused similar decreases in the flexion-elicited withdrawal threshold. Pretreatment of the mice with indomethacin (5 mg/kg/i.p./40 min) did not affect the volume elicited flexion hypernociception (data not shown). These results demonstrate that the volume injection in the tibio-tarsal joint decreased the flexion threshold by itself.

3.4. Effect of Morphine in the zymosan-induced tibio-tarsal joint hypernociception in mice

To further support the nociceptive character of the decrease in the flexion-elicited withdrawal response, mice were treated with morphine (2–8 mg/kg; i.p.) 6 h after the injection of zymosan in tibio-tarsal joint (30 μg/joint). The withdrawal response was dose-dependently inhibited by morphine. The treatment with opioid receptor antagonist naloxone (1 mg/kg; s.c.), 30 min before morphine (2 mg/kg) injection, prevented its effect (Fig. 5). The administration of naloxone did not change

the flexion-elicited withdrawal threshold by itself (data not shown).

3.5. Effect of COX inhibition in the zymosan-induced tibio-tarsal joint hypernociception

In order to further characterize the inflammatory component of the joint hypernociception induced by zymosan, a group of mice received the classical cyclooxygenase inhibitor indomethacin (5 mg/kg, i.p; Cunha et al., 2004), 40 min before zymosan injection (30 μg/5 μl/joint). Indomethacin pretreatment significantly reduced the zymosan-induced tibio-tarsal joint hypernociception (Fig. 6). The demonstration that cyclooxygenase products, such as prostaglandins, are, at least in part, involved in the flexion-elicited joint hypernociception induced by zymosan, confirm the ability of this model to detect inflammatory hypernociceptive components of the experimental arthritis.

3.6. Effect of i.pl. lidocaine injection on articular hypernociceptive response

Because the dorsal flexion movement of the tibio-tarsal joint is produced by an increasing perpendicular force applied on the central area of the plantar surface, a set of experiments was performed to verify whether the probe tip applied on the plantar surface of the hind paw stimulates cutaneous nociceptors. To distinguish articular (flexion) from cutaneous nociception (poking), two area size tip probes were adapted on the electronic-pressure meter and applied on the plantar hind paw surface of mice with normal untreated joint or injected with zymosan. As shown in the Fig. 7, the intraplantar injection of lidocaine (2% w/v/25 μl) prevented the withdrawal threshold produced by the standard size tip (0.5 mm^2) and did not alter the flexion movement produced by the large size tip (4.15 mm^2) applied on the plantar surface. The cut off of 15 g was used because above this value the force applied lifts the leg and no response was obtained in all mice tested. These results further support that the standard size tip (0.5 mm^2) application rather than the larger size tip (4.15 mm^2) provokes cutaneous nociception by it self.

To confirm that the nociceptive response induced by intra-articular administration of zymosan is not due to the sensitization of cutaneous nociceptors, mice received an intraplantar injection of either lidocaine (2% w/v/25 μl) or saline. As shown in Fig. 7, the intraplantar injection of lidocaine did not prevent the zymosan inflamed articular hypernociception using the large tip probe. These results reinforce that the large tip probe causes the dorsal flexion movement of the tibio-tarsal joint without the stimulation of cutaneous nociceptors allowing the evaluation of the dorsal flexion-elicited hypernociception during joint inflammation.

4. Discussion

In the present investigation we developed a behavioral model in mice that allows to quantify the inflammatory joint hypernociception and to perform pharmacological screening of analgesic effects of drugs. Although the hyperalgesia is a common clinical symptom associated with arthritis, there are few animal models that allow the evaluation of the hypernociceptive response evoked by joint movement. In this context, an electronic pressure meter device was adapted to detect the hypernociception evoked by dorsal flexion of the inflamed tibio-tarsal joint. The administration of zymosan induced a dose- and time-dependent hypernociception, which was associated with significant inflammatory cells infiltrate and synovial hyperplasia.

Zymosan-induced arthritis (ZIA) in mice was early characterized by polymorphonuclear infiltration and synovial hypertrophy after 72 h, and pannus formation 7 days after its administration (Keystone, 1977). Cartilage destruction associated with synovitis 7 days post zymosan administration was also described in the knee joint of rats (Gegout et al., 1994). Although, those studies evaluated the chronic histological characteristics of arthritis, zymosan has been used to investigate the acute effect of inflammatory mediators in the joint, such as synovial oedema, cell influx, and hypernociception in rats (Rocha et al., 1999).

In the present study, the apparent association between the time-course of the zymosan-induced hypernociception and zymosan-induced neutrophil migration suggests that these both phenomena may be correlated. In fact, the maximal joint hypernociception was detected between 7 and 24 h after the zymosan injection, which corresponds to the maximal neutrophil migration to the inflammatory site. The participation of neutrophils in the pathogenesis of joint tissue lesions has long been recognized (Kitsis and Weissmann, 1991). These cells are predominant in the synovial exudates of a variety of inflammatory arthropathies including gout, Reiter's disease, and rheumatoid arthritis (Harris, 1990). In line with the relevance of neutrophils in the joint inflammation, it was reported these cells participate in the zymosan-induced arthritis in rats through the release of inflammatory mediators such as leukotriene B_4 (da Rocha et al., 2004). Moreover, their contribution for the development of inflammatory hyperalgesia has been suggested by their involvement in C5a- and fMLP-induced hypernociception (Levine et al., 1986).

The articular hypernociception is usually indirectly evaluated by mechanical and thermal tests. For example, the application of a cutaneous nociceptive stimulus on the ipsilateral plantar skin is used to detect primary or even secondary hypernociception (central sensitization) induced by inflammatory agent injected in the joint (Butler et al., 1992; Chillingworth and Donaldson, 2003; Cook and Nickerson, 2005). In the present methodology, the flexion of the tibio-tarsal joint was produced using a non-nociceptive large tip probe applied on the plantar surface of the ipsilateral hind paw. Nevertheless, additional experiments were performed using a standard size tip probe, which typically evokes mechanical nociceptive withdrawal when applied on the plantar skin surface as demonstrated by the subcutaneous administration of 2% lidocaine. In addition, the withdrawal threshold evoked by large tip probe was not affected by the subcutaneous intraplantar treatment with 2% lidocaine after intra articular challenge with zymosan. These findings further support that in the present model only articular nociception is under evaluation. In line with this

statement, the zymosan injected in the tibio-tarsal joint did not induce inflammatory response in the subcutaneous plantar tissue of mice since there was no modification in the MPO activity in the cutaneous tissue (data not shown). It is noteworthy that although it was detected thermal (Hargreaves test) hypernociception after administration of CFA in the tibio-tarsal joint region of mice (Chillingworth and Donaldson, 2003), these response could represent cutaneous sensitization rather than a secondary hypernociception induced by the articular primary hypernociception. This effect could be attributed to a plantar cutaneous inflammation given the higher volume inject of inflammatory stimulus compared with the 5 μl inject in the present study.

Another intriguing issue in the present work is that intra-articular injection of saline (1.25–5 μl) caused a slight reduction of the flexion withdrawal threshold. This response was not prevented by indomethacin, excluding the participation of endogenous prostanoids release in the articular lesion during injection. It is plausible that the increase in joint volume enhances the joint pressure, which may induce nociception triggered by articular movement. In fact, intra-articular volume increases the discharge from the medial articular nerve during movement (Ferrell et al., 1986). Moreover, previous studies demonstrated a relationship between intra-articular pressure and joint position (Guyton, 1966; Nade and Newbold, 1984).

To further confirm that the decrease of withdrawal threshold elicited by dorsal flexion of the tibio-tarsal joint was indicative of a hypernociceptive response, the treatment with morphine was used because it is well described analgesic effect (Maldonado et al., 1994; Nagakura et al., 2003; Verri et al., 2004, 2005). As expected, morphine inhibited in a dose-dependent manner the zymosan elicited hypernociceptive flexion response, which was prevented by naloxone. In fact, intra-articular administration of opioids has demonstrated to be effective in the treatment of joint pain in humans (Stein et al., 1991).

The hypernociception associated with inflammation is, at least in part, a result of the sensitization of the primary nociceptive neurons (nociceptors) caused by the release of inflammatory mediators that ultimately lower the neuronal excitability threshold (Julius and Basbaum, 2001). Among different metabolites of cyclooxygenase, prostaglandin E_2 is considered one of the most important inflammatory mediators associated with the sensitization of nociceptors (Ferreira et al., 1978). In agreement with this statement, indomethacin (non-selective cyclooxygenase inhibitor) reduced the flexion-elicited hypernociception in zymosan-induced joint inflammation. In line with this finding, it has been demonstrated that intra-articular administration of zymosan induces the release of prostanoids in knee joints of mice and rats (Keystone, 1977; Rocha et al., 1999). Actually, zymosan induces the synthesis of prostaglandins by macrophages (Bonney et al., 1978), and the synovial fluids of patients with rheumatoid arthritis contain large amounts of PGE_2 (Trang et al., 1977). This fact may explain the effectiveness of non-steroidal anti-inflammatory drugs in controlling inflammatory articular pain (Higgs et al., 1983).

The present study demonstrates that the tibio-tarsal flexion reflex is a behavioral model that allows quantifying the inflammatory joint hypernociception in mice, as well as its modulation by antinociceptive drugs. Moreover this model permits to study the mechanism underlying the hypernociception induced by joint inflammation in mice.

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