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Meloxicam-loaded nanocapsules have antinociceptive and antiedematogenic effects in acute models of nociception

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ABSTRACT

Aims: The development of new treatments for inflammation and pain continues to be of high interest, since long-acting effect is critical for patients. The present study investigated whether the polymeric nanocapsules, a drug delivery system, have pharmacological effect on acute nociceptive and inflammatory models in mice.
Main methods: Swiss mice (20–25 g) were previously pre-treated with meloxicam-loaded nanocapsules (M-NC) or free meloxicam (M-F) or suspension without drug (B-NC), at a dose of 5 mg/kg (per oral) at different times (0.5–120 h). Antinociceptive and antiedematogenic effects were evaluated by chemical (acetic acid-induced abdominal writhing, nociception and paw edema induced by formalin and glutamate, croton oil-induced ear edema) and thermal (tail immersion and hot-plate) tests.
Key findings: M-NC reduced the licking time- and paw edema-induced by glutamate and formalin, while M-F did not have an effect. In the acetic acid-induced abdominal writhing and croton oil-induced ear edema, analysis of time-course revealed that M-NC showed a response more prolonged than M-F. In the hot-plate test, a thermal test, the time-course analysis indicated a similar increase in the latency response to thermal stimuli of M-NC and M-F, while in the tail-immersion test M-F had an effect at 0.5 h and M-NC at 24 h.
Significance: Polymeric nanoparticles had antinociceptive, anti-inflammatory and antiedematogenic effects in the formalin and glutamate tests, and prolonged the effect in acetic acid and croton oil tests, but not in thermal tests, supporting the idea that the inflammatory process in tissues facilitates the vectoring of polymeric nanoparticles.

Introduction

Pain is outlined by the International Association for the Study of Pain (IASP) as an unpleasant sensory or emotional experience associated with tissue damage, that can be related or not to inflammation (IASP, 1994). In this context, life quality is negatively affected by pain, which interferes in multiple aspects of health and wellbeing, such as work capacity, relationships and cognitive abilities (Rustoen et al., 2008). Several diseases have pain as major symptom and it begins through noxious thermal, mechanical, or chemical stimulus that excites the peripheral terminals of neurons, called nociceptors (Ferreira et al., 2004). It has been reported that inflammation and pain are related processes that have common effectors and mediators. In inflammation there is the recruitment of leukocytes and plasma proteins to a site of affected tissue as an adaptive response (Medzhitov, 2008). Moreover, classical signs as heat, redness, swelling, pain and consequent function loss of the affected local/organ are present in exacerbated inflammation processes (Lawrence et al., 2002).

Although there is an arsenal of effective analgesics and anti-inflammatory, the clinical use is problematic due to the concern regarding safety, side-effects and bioavailability (Jage, 2005). In this context, new alternatives for the pain pre-treatment have been studied worldwide. Nowadays, nanotechnology is quickly progressing and this area has become a major research field in this century (Arora et al., 2012). During the last decade, nanotechnology has demonstrated priceless advantages in different areas such as pharmacy, medicine, computer science and engineering. In fact, the development of new nanocarrier systems to target the drug delivery is considered the possible future of pharmaceutical therapy (Couvreur and Vauthier, 2006). Over the last few years, biodegradable and biocompatible nanoparticles have been

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reported as the most promising for delivery of pharmacologically active drugs (Adair et al., 2010; Zhang et al., 2011).

Nanocapsules are polymeric nanoparticles that promote drug controlled delivery in specific body sites (Rieux et al., 2006). Several advantages are associated with the incorporation of drugs into polymeric nanocapsules, such as the reduction of side effects (Faraji and Wipf, 2009; Guterres et al., 2001; Schaffazick et al., 2003) and the increase of bioavailability (Couvreur and Vauthier, 2006).

\In this context, many efforts have been made to study the improvement of the pharmacological effects of these systems (Badran et al., 2014; Bender et al., 2012; Cheng et al., 2008). Accordingly, meloxicam-loaded polymeric nanocapsules were effective in protecting learning and memory impairment, neuronal loss and oxidative stress in a mouse model of Alzheimer's disease induced by β-amiloid peptide (Ianiski et al., 2012). Moreover, Khachane et al. (2011) demonstrated that the polymeric nanoparticles containing meloxicam resulted in lesser ulcerogenicity than free suspension.

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that possesses analgesic action and reduces pain and inflammation (Ogino et al., 1997; Pairet et al., 1998). In order to produce its effects, meloxicam inhibits the cyclooxygenase-2 (COX-2) activity (Pairet et al., 1998), blocking the prostaglandin synthesis and arachidonic acid metabolism (Gupta et al., 2002; Lopez-Garcia and Laird, 1998; Vane et al., 1998).

In view of our interest in the pharmacology of nanocarrier systems, the present study aimed to investigate whether polymeric nanocapsules, a drug delivery system, have pharmacological effect on acute nociceptive models in mice, by analysis of time-course of antinociceptive, anti-inflammatory and antiedematogenic responses.

Materials and methods

Materials

Meloxicam and Carbopol® 940 were obtained from Henrifarma (São Paulo, Brazil). Sorbitanmonostearate, polysorbate 80 and poly(ε-caprolactone) M_W = 80,000 were purchased from Sigma-Aldrich (Strasbourg, France). Triglycerides of capric/caprylic acid and triethanolamine were obtained from Via Farma (São Paulo, Brazil), while methylparaben, propylparaben, sorbitol and propylene glycol from Alpha Quimica (Porto Alegre, Brazil).

All drugs used in induction of nociception were dissolved in saline (0.9%). All other chemicals were acquired from standard commercial suppliers.

Nanocapsule preparation

Suspensions of meloxicam-loaded nanocapsules (M-NC) were prepared by the method of interfacial deposition of preformed polymer (adapted from Fessi et al., 1989) at a concentration of 0.3 mg/ml (Ianiski et al., 2012). After preparation of suspensions, polymeric nanoparticles were incorporated into semisolid formulations (Ianiski et al., 2012). The particle diameter was around 283 and 285 nm for M-NC and blank nanocapsules (B-NC), respectively. The zeta potential was approximately −14.53 mV for M-NC and −16.21 mV for B-NC. The content of meloxicam nanocoated was 99.97%.

Animals

Male adult Swiss mice (20–25 g) were obtained from local breeding colony of Federal University of Santa Maria. The mice were maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle (with lights on at 7:00 a.m.). The animals were acclimatized to the behavior room for at least 1 h before test and they were used only once in each test.

The experiment was conducted according to the institutional and international guidelines for the care and use of animals. The Local Committee for Care and Use of Laboratory Animals of the Franciscan University Center (Santa Maria, Brazil) approved the research (code number: 001/2011). The animals were used according to ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). All efforts were taken to minimize the number of animals used and their suffering. The number of animals and intensities of noxius stimuli used were necessarily at the minimum to demonstrate the consistent effects of the drug pre-treatment. At the end of the experimental procedure the mice were killed by cervical dislocation.

The mice were divided into three groups (7–8 animals per group) for each pre-treatment time, as follows: group I received B-NC and served as control (17 ml/kg; oral route), groups II and III received M-NC and free meloxicam (M-F), respectively, at a dose of 5 mg/kg by the oral route (Ianiski et al., 2012). Different groups of animals were used for each nociceptive test. Each time of pre-treatment had a control group that received B-NC. However, all results of group I were condensed (n = 7–8) because they did not have significant difference between animals at different times of pre-treatment.

Acetic acid-induced abdominal writhing

Intraperitoneal (i.p.) injection of acetic acid (1.6%) was used to induce the abdominal writhing (Nogueira et al., 2003). The mice were pre-treated (0.5–72 h) with B-NC, M-NC or M-F before acetic acid injection. After acetic acid injection, the abdominal writhing (full extension of both hind paws) were counted cumulatively over a period of 20 min.

Formalin-induced nociception and paw edema

The formalin test was carried out as described by Hunskaar and Hole (1987). M-NC, M-F or B-NC was administered 0.5–72 h before formalin injection. An intraplantar (i.pl.) injection of formalin (2.5%/paw, 20 μl) (v/v) was administered into the dorsal right hind paw (ipsilateral) of mice. After formalin injection, the time spent licking or biting the injected paw was recorded during the periods of 0–5 min (early phase) and 15–30 min (late phase). The paw edema was measured by comparing the difference between the weight of the formalin-injected paw and the weight of the contralateral paw (non-treated paw). For this purpose, the animals were killed by cervical dislocation 30 min after formalin injection and both paws were cut at the ankle joint and weighed on an analytical balance.

Glutamate-induced nociception and paw edema

The procedure was carried out according to the procedure described previously by Beirith et al.(2002). The mice were pre-treated with M-NC, M-F or B-NC 0.5–72 h before i.pl. injection of glutamate (20 μmol/paw, 20 μl) on the right hind paw. The amount of time spent licking or biting the injected paw was recorded during 15 min following glutamate injection. The difference between the weight of the glutamate-injected paw and the weight of the saline 0.9%-injected paw (contralateral paw) was used as a measure of paw edema. For this purpose, the mice were killed by cervical dislocation 15 min after treatment with glutamate and both paws were cut at the ankle joint and weighed on an analytical balance.

Ear edema induced by croton oil

The antiedematogenic effect of M-NC or M-F was assessed by induction of inflammation by topical application of 2.5% croton oil in acetone (10 μl/ear) in the right ear of each mouse, according to Romay et al. (1998). The mice were pre-treated with M-NC, M-F or B-NC at 0.5–48 h before test. Four hours after phlogistic agent, the animals were killed by cervical dislocation. A segment of 8 mm of each ear was obtained to weighing on an analytical balance. The swelling was determined by comparing the weight difference between the injected ear and untreated ear.