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Evaluation of the antinociceptive and anti-inflammatory activities of piperic acid: Involvement of the cholinergic and vanilloid systems

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ABSTRACT

Piperin is the active compound of black pepper (*Piper nigrum*). From the piperine was obtained the molecule of the piperic acid (PAC). The objective of this study was to evaluate the antinociceptive and anti-inflammatory of the compound. The antinociceptive effects of PAC were evaluated by abdominal writhing, formalin, capsaicin and tail-flick tests; while the anti-inflammatory effects were evaluated by paw oedema and air pouch tests, and *in vitro* COX inhibition assay. The possible action mechanism of PAC was evaluated using naloxone, L-NAME, glibenclamide and atropine in tail flick test and by Cholinesterase activity assay and production of TNF-α and IL-1β. PAC significantly reduced the nociceptive effects induced by acetic acid or formalin in mice. PAC also demonstrated an antinociceptive effect in the tail-flick model. The muscarinic receptor antagonist, atropine reduced the antinociceptive effect of PAC in the tail-flick model. PAC was able to inhibit capsaicin-induced nociception, showing involvement of TRPV1. The compound did not alter the motor capacity of the animals, not interfering in the nociceptive response. PAC also showed anti- inflammatory activity by inhibiting the formation of carrageenan-induced paw oedema, leukocyte migration, and cytokine production / release. Atropine reduced the activity of PAC on leukocyte migration, and cytokine production. The compound showed to be able to reduce the cytokine production stimulated by capsaicin. PAC inhibited the COX activity. The results presented suggest that the possible cholinomimetic action and vanilloid agonist of the piperic acid may be responsible by anti-nociceptive and anti- inflammatory effects; these effects are devoid of toxicity.

1. Introduction

The pain is a multifunctional phenomenon involving sensory and emotional aspects. The mediators involved in pain are responsible for the multiplicity of events that occur during the transmission and conduction of pain, in both peripheral and central nervous systems. The transmission of the nociceptive stimulus from the site of tissue injury constitutes an important biological response to protect the organism from further injury. The primary sensory neurons detect and convert mechanical, chemical and thermal stimuli identified by nociceptors in electrical activity and it is led to higher structures (De Prá et al., 2017).

Several studies indicate the inhibition of nociception by the cholinergic system, directly by cholinergic inhibitory descending pathways and by the release of acetylcholine. Modulation of nociception from acetylcholine involves the participation of multiple classes of receptors, including nicotinic and muscarinic receptors (Wess et al., 2003). Other studies show that immune cells express choline acetyltransferase, a direct synthetase for acetylcholine, and other corresponding cholinergic components. Alternatively, the acetylcholine h released from immune cells or cholinergic neurons modulates immune function in an

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autocrine/paracrine manner by acting on its receptors (Rosas-Ballina et al., 2011).

Other systems may have involvement in pain and inflammation, such as the vanilloid system. In this context, TRPV1 (transient receptor potential vanilloid) is a heat activated cation channel that can be stimulated by inflammatory factors and leads to pain. TRPV1 is a non-selective cation channel, but with a certain selectivity by Ca^{+2} (Szallasi et al., 2007). It is expressed in nociceptive fibers, mainly non-myelinated fibers, which increase its importance with regard to pain. Studies evidence the effect of the TRPV1 channel on the activation of macrophages and also show that some TRPV1 antagonists and agonists (receptors desensitization) can suppress the inflammatory response (Ninomiya et al., 2017).

Studies show that species of the Piper genus and piperine, a compound isolated from its extraction, show antinociceptive and anti-inflammatory activities in rodents (Tasleem et al., 2014; Mothana et al., 2016). Piperine is an active alkaloid, an isolated amide of the Piper genus, being the main secondary metabolite of black pepper. The piperamide is the amide that appears in greater concentration in the plant, becoming the main target of biological evaluations (Zarai et al., 2013). Bukhari et al. (2013) demonstrated the antinociceptive and anticonvulsant activities of piperine by the involvement of the opioidergic and gabaergic systems.

A promising compound usually undergoes changes in its chemical structure in order to improve its therapeutic qualities. Piperic acid (PAC) is the acyl part of the piperine molecule, present in different species of the genus Piper, being more abundant in the fruits of *Piper nigrum*. There are reports in the literature on the antidepressant, hepatoprotective, immunomodulatory and antineoplastic activities of piperine. Other studies still demonstrate the ability of piperine to inhibit NO and TNF-α expression (Ferreira et al., 2012). The purpose of this study was to evaluate the antinociceptive and anti-inflammatory profile of Piperic acid, and to investigate its mechanism of action and toxicity.

2. Materials and methods

2.1. Animals

Male *swiss* mice were obtained from the Bioterium of the Department of Physiological Sciences. The experimental protocols for utilization of the animals were approved by ethics committee on the use of animals of the Federal Rural University of Rio de Janeiro, with number 004/2015. The mice were kept in a controlled temperature environment (22 ± 1 °C) and 12 h light-dark cycle. Water and food *ad libitum,* but the food was withdrawn 8 h before oral administration of the drugs in order to avoid interference in the absorption.

2.2. Chemicals

The following substances were used: acetic acid (Vetec, Rio de Janeiro, Brazil); formaldehyde (Merck, Darmstadt, Germany); piperine (purity – ≥ 97%) capsaicin (purity – 63.7%), capsazepine (purity – 98%), dexamethasone (purity - 97%), L-NAME (purity – 98%), acetyl salicylic acid (purity – 99%), λ-carrageenan, and dimethyl sulphoxide (Sigma–Aldrich, St. Louis, MO, USA), and morphine (purity - 97%) (Cristália, São Paulo, Brazil). Piperic acid (Fig. 1) was prepared by hydrolysis of piperine following protocol describing in literature (Ikan, 1991).

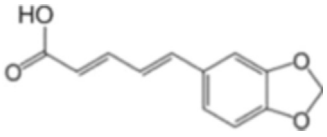

Fig. 1. Structure of piperic acid.

2.3. Treatments

PAC was obtained from the piperine molecule which was purchased from Sigma Chemical. PAC was administered orally at doses of 5, 25 and 50 mmol/kg (Table 1). Morphine was administered orally at the dose of 12.06 μmol/kg in the acetic acid-induced abdominal writhing test, 19.35 μmol/kg in the formalin test and 9.15 μmol/kg in the tail flick and open field tests; while the acetylsalicylic acid was used in the dose of 1.11 mmol/kg in the formalin test. The dose of dexamethasone used in the oedema paw and air pouch tests was 5.73 μmol/kg, subcutaneously. The doses of morphine and acetylsalicylic acid were based on Marinho et al. (2011) and dexamethasone was based on dos Santos et al. (2014). Capsazepine had its dose based (13.3 μmol/kg - i.p.) and was used as described on Lopes et al. (2013) and capsaicin had its dose based (1.6 μg/paw) and was used as described in Sakurada et al. (1998). The control group consisted of animals that received PBS (phosphate buffer saline), while the vehicle group consisted of animals administered with dimethyl sulfoxide solubilized in distilled water (1.5%). The experiment was blinded for all the treatments.

2.4. Acetic acid-induced abdominal writhing test

Model used to screen of the antinociceptive activity (Koster et al., 1959). This model is based on the counts of contractions of the abdominal wall followed by trunk twisting and extension of the hind limbs and contact of the abdomen with the floor of the counting vessel. In this model, intraperitoneal administration of 0.01 ml/g acetic acid (1.2%) was performed 60 min after administration of the substances. The count of the number of writhes was started immediately after injection and remaining for a period of 30 min.

2.5. Formalin test

Model used in the evaluation of inflammatory and non-inflammatory pain (Hunskaar et al., 1986). In this model, 0.02 ml of formalin solution (2.5%) was injected into the right hind paw 60 min after oral administration of the substances. Afterwards, the mice were placed in a container, where the count of the time that animals remained licking the administered paw was made. The time was measured in 2 steps: 1°) neurogenic, performed between 0 and 5 min after formalin injection and 2°) inflammatory, performed between 15 and 30 min after formalin injection.

2.6. Tail flick test

Model used to evaluate antinociceptive activity at the spinal level. The model was performed as described in Moncada et al. (2003). Mice were positioned in the apparatus so that a noxious beam of light was focused approximately 2 cm from the tip of the tail and the latency time of tail removal was recorded. The intensity of the radiant light source was adjusted to latency times between 2 and 5 s; this intensity was not altered and the animals that presented basal values outside these limits were excluded of the experiment. Measures of latency time (LT) were performed with intervals of 20 min between them, totalling 8 measures. The first 2 measurements were taken prior to administration of the substances. The average of these measures is called baseline (BL). The antinociception was quantified by calculating the percentage of increase over the baseline (IBL), obtained by following formula:

$$\text{IBL } \% = 100 - \frac{\text{LTx100}}{\text{BL}}$$

For investigation of the mechanism of action of PAC, antagonists were administered to the mice, intraperitoneally, 15 min prior to oral administration of PAC (50 mmol/kg). Naloxone (non-selective opioid antagonist - evaluation of the involvement of opioid receptors in the antinociceptive activity of PAC) (Stefano et al., 2017), atropine (non-