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Central and peripheral anti-hyperalgesic effects of diosmin in a neuropathic pain model in rats☆

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ABSTRACT

Flavonoids are natural compounds showing anti-hyperalgesic activity in models of pain. Diosmin is a compound poorly studied in the treatment of neuropathic pain. This study evaluates the anti-hyperalgesic actions of diosmin and possible mechanisms of action involved by using a neuropathic pain model in rats. Experimental neuropathic pain was induced by chronic constriction injury (CCI) in male Wistar rats, then aesthesiometric index and plantar tests were assessed to evaluate mechanical and thermal hyperalgesia, respectively, in order to explore the analgesic effects of acute and sub-chronic treatment with diosmin. The $GABA_A$, 5-HT_{1A}, D_2-like and opioid receptors participation, as well as levels of TNF-α, IL-1β and IL-6, were evaluated in the spinal cord and sciatic nerve tissues after acute and subchronic diosmin administration. In addition, the presence of diosmin on cerebral samples was determined by UHPLC–MS analysis. Acute and sub-chronic treatment with diosmin significantly diminished the mechanical and thermal hyperalgesia induced by CCI in rats. This anti-hyperalgesic effects of diosmin were modified in the presence of naloxone (1 mg/kg, i.p.) and haloperidol (0.1 mg/kg, i.p.), but not by $GABA_A$ and 5-HT_{1A} receptor antagonists. The anti-hyperalgesic effects of diosmin were also linked with reduced levels of TNF-α, IL-1β and IL-6. The presence of diosmin in the cerebral samples was confirmed by chromatographic analysis. In conclusion, our results provide evidence that diosmin produces significant anti-hyperalgesic effects acting at central level by an opioid and D_2 dopaminergic receptors participation, and at peripheral level by reducing proinflammatory cytokines.

1. Introduction

Neuropathic pain results of a dysfunction in the nervous system, which is frequently associated with comorbidity since it has been observed in patients with long-standing diabetes, cancer, AIDS, leprosy, and post-surgical events [1]. Conventional therapy for neuropathic pain includes: non-steroidal anti-inflammatory drugs (ketorolac), opioids (morphine), tricyclic antidepressants (amitriptyline, duloxetine), and some anticonvulsants (gabapentin). Although all of these drugs have shown moderate efficacy, they have often been associated with adverse effects and/or withdrawal syndrome [2].

Phytomedicine is considered a useful approach to treat refractory neuropathic pain [3,4]. In regard to this, some natural products that produce antiallodynic and anti-hyperalgesic effects in neuropathic pain, like flavonoids, have been currently reported [5–7]. Diosmin (diosmetin-7-*O*-rutinoside) was firstly isolated in 1925 from *Scrophularia*

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nodosa; and it can be obtained by dehydrogenation of the flavanone glycoside hesperidin [8]. It is abundant in the pericarp of the citrus fruit [9]. This flavonoid is widely recommended and used for the treatment of blood vessel disorders in humans [10]. Its pharmacological properties include antioxidant [11], anxiolytic [12], anti-inflammatory [13], and antidiabetic [14] activities. Its anti-inflammatory properties have been associated to an ability to diminish the over-expression of NF-κB, TNF-α, COX-2, iNOS, and LTB4 [12,15,16]. Its potential for the treatment of neuropathic pain was recently reported by using the chronic constriction injury (CCI) in mice, in the study authors found an involvement of the NO/cGMP/PKG/K_{ATP} channel signaling pathway, glial cells activation and inhibition of spinal cord cytokines like 1L-1β [17].

In the present study, we explored the anti-hyperalgesic actions of diosmin by using the CCI, as neuropathic pain model in rat, and to investigate the possible underlying mechanism of action by screening focused on opioids, $GABA_A$, dopamine D_2, and serotonin 5-HT_{1A} receptors. Additionally, the effects of diosmin on the levels of pro-inflammatory cytokines involved in the development of neuropathic pain were studied.

2. Materials and methods

2.1. Animals

Male Wistar rats (100–120 g) were provided by the animal facilities from the Instituto Nacional de Psiquiatría "Ramón de la Fuente Muñiz" and Centro de Investigación y de Estudios Avanzados (CINVESTAV)-Sede Sur. All the rats were kept in a room under standard conditions of a 12 h light/dark cycle, with food and water available *ad libitum*. All studies followed the Ethical Guidelines for Investigations of Experimental Pain in Animals and were carried out according to a protocol approved by the local Animal Ethics Committee (Project No. NC09-3280.3 and NC12-3280.0), and in compliance with national (NOM-062-ZOO-1999), and international regulations on the care and use of laboratory animals (Publication No. 85-23, revised 1985). The tests were performed during the light phase. The number of experimental animals was kept to a minimum; they were euthanized with a CO_2 overdose at the end of the study.

2.2. Drugs

Diosmin, diosmetin, hesperidin, gabapentin, naloxone, bicuculline, and WAY100635 were purchased from Sigma St. Louis, MO, USA. Also, ketamine (ketaline; Probiomed S.A de C.V, Mexico), xylazine (Procin Equus; PISA agropecuaria S.A de C.V, Mexico), and haloperidol (MP Biomedicals, USA) were used in this study. All drugs were freshly prepared in physiological saline solution (0.9% NaCl, s.s), and administered intraperitoneally (i.p.) in a volume of 0.1 mL/100 g of body weight in rats. Given that diosmin and haloperidol are poorly soluble in water, they were suspended in 0.5% Tween 80 in s.s. to improve its solubility and administration. Doses are referred to the free base. Control animals received vehicle by the same volume and route of administration. Groups consisted of at least six rats for each experimental procedure. Methanol, acetonitrile, phosphoric and acetic acids purchased from J.T. Baker were used for analytical experiments. A 10× phosphate buffered saline (PBS, pH 7.4) was prepared by mixing and dissolving the following substances: Na_2HPO_4 (anhydrous) 10.9 g; NaH_2PO_4 (anhydrous) 3.2 g; NaCl 90 g and distilled water 1000 mL. PBS 10× was stored at room temperature and diluted 1:10 to obtain PBS 1× for the experiments. All these substances were bought from J.T. Baker.

2.3. Chronic constriction injury (CCI) induction

The CCI model was originally described by Bennett and Xie [18]. Animals were anesthetized with a mixture of ketamine (50 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.), and the right thigh was sterilized with iodine solution (Povidone). The right common sciatic nerve was exposed at the level of the mid-thigh and proximal to the sciatic nerve trifurcation. About 7 mm of the nerve was freed from adhering tissue and four ligatures (with black braided silk 3.0) were loosely tied around the sciatic nerve at 1 mm intervals. The incision was sutured and the wound was cleansed with a crystal violet solution. In sham-operated animals, the sciatic nerve was isolated but not ligated. CCI and sham-operated rats were tested simultaneously.

2.4. Experimental design

Surgery was performed on Day 0. A preliminary evaluation was carried out 15 days after surgery to verify the induction of neuropathic nociception by CCI in the sciatic nerve as previously described [18].

In order to understand the minimal and maximal efficacy of diosmin in this model, a therapeutic window was determined by a dose-response exploration of diosmin after acute administration using logarithmic increases among doses; with a 0.25 logarithmic increase between 10 and 1000 mg/kg to complete at least five doses (10, 100, 316.2, 562.3 or 1000 mg/kg, i.p.). To measure the antinociceptive behavior, both the threshold and the latency of paw withdrawal were evaluated. Both parameters were assessed in a temporal course curves from 0 to 120 min at intervals of 30 min after acute administration of each dose (Since treatment for neuropathic pain implicates repetitive administration, acute and subchronic dose of diosmin was explored in this study). To evaluate subchronic treatment, a middle and significant dosage of 316.2 mg/kg, i.p., of diosmin was selected. Effects of diosmin were compared to those produced by vehicle group and the reference drug gabapentin (31.6 mg/kg, i.p.). Under this dosage regimen, treatments were administered daily at midday for 14 days (beginning on the 15th until the 28th day) after evaluation of the behavioral parameter. Nociceptive behavior was assessed on the 15th, 18th, 21st, 24th, and 28th days.

2.4.1. Mechanical hyperalgesia

Mechanical withdrawal thresholds were measured using a Dynamic Plantar Aesthesiometer (Ugo Basile, Italy). The animals were placed in clear acrylic boxes with a metal grid floor, inside a temperature controlled room (at about 25 °C), and they were acclimatized for 30 min before testing. The stimulus was applied with a metal filament (0.5 mm diameter) on the skin of the mid-plantar area of the right hind paw, with an increasing force (1 g/s) of up to 50 g in 50 s, starting below the threshold of detection and increasing continuously up until the rat removed its paw. A response in grams was obtained as the average of three consecutive tests, waiting at least 3 min between measures, modified from [19].

2.4.2. Thermal hyperalgesia

Thermal nociception was evaluated with the Hargreaves assay (Ugo-Basile, Italy) [20]. In this method, a radiant heat source with a locator light is positioned under the plantar surface of a rodent. Rats were acclimated individually in Plexiglas chambers with heated glass floors for 30 min. Latency to withdrawal was measured with three consecutive thermal tests performed at least 3 min apart, one paw at a time. The means of the three tests were estimated. The intensity of the lamp was set at 60 Hz and a cut-off of 30 s was used to avoid tissue damage. The light beam was directed at the plantar surface of the right hind paw until the rat responded or for 30 s, whichever occurred first. Latency to paw withdrawal was recorded with a built-in timer, which displayed reaction time in 0.01 s increments. Data are expressed as withdrawal latency (s) and thermal anti-hyperalgesic effect.

2.4.3. Open-field test

The open-field test modified by Hemsley and Hopwood [21] was used to explore motor alterations. The ambulatory activity of rats was