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Anti-hypernociceptive effect of mangiferin in persistent and neuropathic pain models in rats

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ABSTRACT

The present study examines the possible effect of the glucosylxanthone mangiferin (MG) on pain-related behaviors in a tonic acute pain model (formalin test at 5%) and in a chronic constriction injury (CCI) model to clarify the underlying transient and long-term mechanisms. Acute administration of MG (10–100 mg/kg, i.p.) reduced licking/biting exclusivity in the tonic phase of formalin test in a naloxone and yohimbine-sensitive manner. This effect was enhanced by a nonselective nitric oxide synthase (NOS) inhibitor (NG-monomethyl-L-arginine) and by a non-competitive N-methyl-D-aspartate (NMDA) antagonist (ketamine), but it was reversed by the NOS substrate (L-arginine). Pre-treatment with intrathecal yohimbine prevented the anti-hypernociceptive effect of systemic MG. Pre-treatment during 4 days before surgical and 3 days after CCI with MG (50 mg/kg, i.p.) reduced mechanical hypernociception and decreased the signs of Wallerian degeneration (WD) of the sciatic nerve. MG improved the PC-12 cellular viability exposure to glutamate-mediated neuronal death, also involved in neuropathic pain. The findings of this study suggest that MG shows ability to decrease tonic pain in the formalin test. A transient activity of this xanthone on nociceptive pathways mediated by α_2 adrenergic receptors in cooperation with the opioid system could be involved, at least in part, in this effect. Its neuroprotective effect by preventing WD in mononeuropathic rats could be implicated in the mechano-antihypernociceptive long term mechanisms.

1. Introduction

Mangiferin (MG) is a glucosylxanthone broadly distributed in higher plants such as *Mangifera indica* L. (Anacardiaceae) (Núñez-Sellés et al., 2002). This compound is frequently used in traditional medicine and exhibits numerously biological activities (García et al., 2002; Garrido et al., 2004; Pardo-Andreu et al., 2008). Most of these effects are explained, at least in part, by inhibition of NF-κB pathway activation (Leiro et al., 2004a). NF-κB induces the transcription of genes implicated in the expression of some mediators and enzymes involved in inflammation, pain, oxidative stress and synaptic plasticity as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Wei et al., 2007; Lin et al., 2007). In addition, MG shows neuroprotective effects in the glutamate induced neurotoxicity in rat cerebral cortex neurons, preventing neuronal death, oxidative stress and mitochondrial depolarization (Gottlied et al., 2006; Lemus-Molina et al., 2009). This xanthone modulates the activity of the Art and Erk1/2 kinases and neuronal NF-κB nuclear translocation, inhibits calpain activity and maintains the homeostasis of the antioxidant systems after excitotoxic event further other mechanisms (Campos-Esparza et al., 2009). Particularly, MG decreases the glutamate-mediated Ca^{2+} influx through N-methyl-D-aspartate (NMDA) receptor (Gottlied et al., 2006). Also, in view of the relevance of glial activation in neurodegenerative disorders, MG is able to limit microglial activation in terms of attenuation of prostaglandins E_2 (PGE_2) production, reactive oxygen species (ROS) formation and reduction of cyclooxygenase-2 (COX-2) synthesis induced by lipopolysaccharide (Bhatia et al., 2008). These evidences suggest the potentiality of this compound to modulate some of the molecular targets implicated in neuropathic pain mechanisms, especially central sensitization, through of its long term effects mediated mainly by transcriptional changes (Garrido-Suárez et al., 2010). Previously, we advised that MG could be used to treat neuropathic pain supported in these preclinical data and some preliminary clinical reports with *M. indica* L. extract

Abbreviations: 5-HT, serotonin; CCI, chronic constriction injury; COX-2, cyclooxygenase-2; DLPT, dorsolateral pontine tegmentum; DOI, (±)-2.5-dimethoxy-4-iodoamphetamine; GSH, glutathione; HPLC, high performance liquid chromatography; L-NMMA, NG-monomethyl-L-arginine; MAO, mono-amine oxidase; MG, mangiferin; NF-κB, transcription nuclear factor *kappa* B; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; PAG, periaqueductal gray; PGE_2, prostaglandins E_2; pNR1, phosphorylated NMDA receptor subunit 1; ROS, reactive oxygen species; RVM, rostroventromedial medulla; TNFα, necrosis tumoral factor *alpha*; IL-1β, interleukin 1 *beta*; WD, Wallerian degeneration.

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formulations that contain 15–20% of this polyphenol (Garrido-Suárez et al., 2010, 2011). Given that neuroimmune activation, oxidative stress and neuroprotection have been proposed as a new target for its therapeutic intervention (Kim et al., 2004; De Leo et al., 2006; Gao et al., 2007; Bordet and Pruss, 2009). Nevertheless, a transient mechanism of MG on nociceptive pathway could be implicated and the participation of endogenous opioids, K_{ATP} channels and adenosine in acute models of chemical nociception, including formalin test at 1%, is reported (Lopes et al., 2013). On the other hand, a reversible mono-amine oxidase (MAO) inhibitory activity by MG is recognized (Tomić et al., 2005) and its favorable effect on long-term object recognition memory in rats (Pardo-Andreu et al., 2010) could be explained, at least in part, by the modulation of the noradrenergic system also involved in pain transmission. In addition, several lines of evidence have indicated that in the central nervous system, nitric oxide (NO) interacts with noradrenergic mechanisms involved in descending inhibitory pathways, and a reciprocal interplay between NO and α_2-adrenoreceptor in the inhibition of pain in the dorsal horn of the spinal cord has been observed (Xu et al., 1997). Conversely, NO is pivotal in central sensitization and at this time, it recognizes its dual effect on pain transmission and control (Vetter et al., 2001; Cury et al., 2011). L-Arginine–NO–cGMP-sensitive K_{ATP} channel pathway mediates the peripheral and central antinociceptive effect of several compounds such as opioids, non-steroidal anti-inflammatory drugs, α_2-adrenoreceptor agonists as clonidine and plant extracts, in which the most commonly found chemical compounds are natural polyphenols (Cury et al., 2011; Ebrahimi and Schluesener, 2012). Recently, this peripheral mechanism has been reported for MG (Izquierdo et al., 2013).

Q8 The aim of the present study was to evaluate the effect of acute systemic administration of MG in rats on pain-related behaviors in tonic acute pain model (formalin test at 5%), the recommended model when studying antinociceptive activity of NO- and NMDA-related compounds since formalin evoked spinal release of nitrite/nitrate and glutamate in correlation with the biphasic behavior (Okuda et al., 2001), as well as to introduce the study of this xanthone for the first time in chronic constriction injury (CCI) in rats to clarify the underlying transient and long-term mechanism with its subsequent clinical relevance. Additionally, the neuroprotective effect of MG probably implicated in its underlying mechanisms in CCI model was examined in *in vitro* model for studying glutamatergic neurotoxicity.

2. Material and methods

2.1. Drugs

Mangiferin (2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9*H*-xanthen-9-one) was supplied by the Laboratory of Analytical Chemistry, Center of Pharmaceutical Chemistry (Cuba) and had been isolated from the *M. indica* stem bark standardized extract by extraction with methanol yielding a yellow powder with 90% purity as determined by high performance liquid chromatography (HPLC) (Núñez-Sellés et al., 2002). MG was suspended in DMSO (5% in saline solution) for intraperitoneal administration. The solution DMSO + saline has been shown to have no effects on nociception (Colucci et al., 2008). Naloxone hydrochloride, morphine hydrochloride, clonidine hydrochloride, yohimbine hydrochloride, (±)-2.5-dimethoxy-4-iodoamphetamine (DOI), methysergide maleate, NG-monomethyl-L-arginine (L-NMMA), L-arginine and ketamine hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO, USA. Formaldehyde was obtained from Merck (Darmstadt, Germany). All these compounds were dissolved in saline 0.9%.

2.2. Experimental animals

Experimental procedures were carried out in accordance with European regulations on animal protection (Directive 86/609), the Declaration of Helsinki, and/or the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All experimental protocols were approved by the Institutional Animal Care and Ethical Committee from the Center of Drugs Research and Development (CIDEM, La Habana, Cuba). Male Sprague–Dawley (8–10 weeks) rats weighing 200–250 g were obtained from the Center for Experimental Animals Production (CENPALAB, La Habana, Cuba). They were kept in controlled conditions (22 ± 0.5 °C, relative humidity 40–60% a 7 a.m. to 7 p.m. alternate light–dark cycle, food and water *ad libitum*). The experiments took place during the light period and animals belonging to the various treatment groups (n = 6–10 for each group) were tested in randomized order.

2.3. Formalin test. Acute spontaneous nociceptive behavior

The rats were placed individually in an open glass cylindrical chamber (34 × 30 × 28 cm). The animals were habituated to the chamber for 20 min prior to the injection, and returned to the camera following the injection for their observation. Twenty minutes before the formalin injection rats received a single intraperitoneal dose of MG (10, 50, 100 mg/kg, dose volume 1 mL/kg, i.p.) or vehicle (DMSO 5% in saline solution). Formalin (50 μL, s.c.) was inserted into the plantar surface of the right hindpaw of the rat using a microsyringe with a 26-gauge needle (Dubuisson and Dennis, 1977). Licking/biting of the injected paw was recorded using a digital chronometer as the total licking/biting time (s) per 5 min observation periods for 45 min after formalin injection. The response curves for formalin-induced licking/biting behaviors were generated by recording early (0–5 min), latency (5–15 min) and late (15–45 min) phases (Okuda et al., 2001).

2.3.1. Pre-treatment with opioid, alpha 2 noradrenergic and serotonergic receptor antagonists before a single dose of mangiferin in the formalin test

Rats were pre-treated with naloxone (0.1–1 mg/kg, i.p.) a nonselective antagonist of opioid receptors; yohimbine (0.1–1 mg/kg, i.p.) a selective α_2 adrenoreceptor antagonist and methysergide (1–5 mg/kg, i.p.) a nonselective 5-HT receptor antagonist or vehicle. After 15 min, the animals received a dose of MG (100 mg/kg, i.p.) or vehicle, 20 min before formalin injection. Morphine 5 mg/kg, clonidine 0.1 mg/kg and DOI 1 mg/kg were used as positive control, respectively. The agonist and antagonist drugs and doses were selected according to the previous reports (Ahsana et al., 2005; Jürgensen et al., 2005) and on pilot experiments in our laboratory.

2.3.2. Pre-treatments with NOS inhibitor, NOS substrate and NMDA antagonist on tonic phase of formalin test before mangiferin

Rats were pre-treated with NG-monomethyl-L-arginine (L-NMMA) Q10 (3–30 mg/kg or vehicle, i.p.) a nonselective inhibitor of nitric oxide synthase (NOS). After 15 min, the animals received an injection of MG (100 mg/kg or vehicle, i.p.). Following 20 min, formalin s.c. was injected. Other groups of animals were included to perform similar protocol but received pre-treatment with L-arginine (100–300 mg/kg, i.p.) the substrate to NOS, low dose of ketamine (0.3–1 mg/kg, i.p.) a non-competitive NMDA antagonist or their corresponding vehicles.

2.3.3. Pre-treatment with intrathecal yohimbine and L-NMMA before single systemic mangiferin in the formalin test

Additionally, to assess the possible participation of spinal α_2 adrenoreceptor in the systemic action of MG, an intrathecal injection of yohimbine (3–10 μg/20 μL) or vehicle in the same volume (saline) was administered 5 min before MG (100 mg/kg, i.p.). After 20 min intraplantar formalin was administered in right hindpaw. Other groups were performed to verify the involvement of L-arginine–NO pathway in a possible spinal noradrenergic mechanism of systemic MG. The rats received intrathecal injection of L-NMMA (10–20 μg/20 μL) or vehicle (saline) following similar protocol. Intrathecal injections were performed by L6 lumbar puncture under brief anesthesia with ether, whereby

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