European Journal of Pharmacology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Mechanisms underlying the antinociceptive effect of mangiferin in the formalin test

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ARTICLE INFO

Article history:
Received 15 April 2013
Received in revised form
15 July 2013
Accepted 3 August 2013

Keywords:
Mangiferin
Antinociception
Opioid receptors
K^+ channels
Formalin test

ABSTRACT

The purpose of this study was to investigate the possible antinociceptive effect of mangiferin, a glucosylxanthone present in *Mangifera indica* L., in inflammatory pain. Furthermore, we sought to investigate the possible mechanisms action that contributes to these effects. The ipsilateral local peripheral (1–30 μg/paw), intrathecal (1–30 μg/rat) and oral (1–30 mg/kg) administration of mangiferin produced a dose-dependent reduction in formalin-induced nociception. The antinociceptive effect of this drug was similar to that induced by diclofenac after oral and local peripheral administration. Furthermore, mangiferin was also able to reduce 0.1% capsaicin- and serotonin-induced nociceptive behavior. The local peripheral antinociceptive effect of mangiferin in the formalin test was blocked by naloxone (50 μg/paw), naltrindole (1 μg/paw), 5-guanidinonaltrindole (5-GNTI, 1 μg/paw), N^G-L-nitro-arginine methyl ester (L-NAME, 100 μg/paw), 1*H*-(1,2,4)-oxadiazolo [4,2-*a*]quinoxalin-1-one (ODQ, 50 μg/paw) and glibenclamide (50 μg/paw), but not by methiothepin (30 μg/paw). These results suggest that the antinociceptive effects induced by mangiferin are mediated by the peripheral opioidergic system involving the activation of δ, κ, and probably μ, receptors, but not serotonergic receptors. Data also suggests that mangiferin activates the NO–cyclic GMP-ATP-sensitive K^+ channels pathway in order to produce its local peripheral antinociceptive effect in the formalin test. Mangiferin may prove to be effective in treating inflammatory pain in humans.

1. Introduction

The standard aqueous stem bark extract of *Mangifera indica* L. (Anacariaceae), also named Vimang®, is composed of a mixture of polyphenols which include phenolic acids (gallic acid, 3,4-dihydroxybenzoic acid and benzoic acid), phenolic esters (methyl gallate, propyl gallate and propyl benzoate), flavan-3-ols (catechin and epicatechin) and the xanthone mangiferin (Fig. 1). This later compound is the predominant component (7.5%) of the extract (Nuñez-Selles et al., 2007). The aqueous extract of *M. indica* L. has shown multiple pharmacological effects such as antioxidant (Sato et al., 1992; Leiro et al., 2003; Pauletti et al., 2003; Pinto et al., 2005), antiviral (Wang et al., 2011), antiallergic (Lee et al., 2009), hepatoprotective (Das et al., 2012), antidiabetic (Ichiki et al., 1998), immunomodulatory (Leiro et al., 2003), anti-inflammatory (Marquez et al., 2012) and antinociceptive (Dar et al., 2005; Garrido-Suarez et al., 2010) effects.

The aqueous extract of *M. indica* L. reduces nociception induced by acetic acid (Ojewole, 2005; Garrido-Suarez et al., 2010) and formalin (Garrido-Suarez et al., 2010) in mice. Also, the extract increases the latency time in the hot-plate test (Ojewole, 2005). The mechanism of action of the extract is still unclear. Previous studies have shown that the extract is capable to reduce tumor necrosis factor alpha (TNF-α) and nitric oxide (NO) production in serum and cell culture (Garrido et al., 2004). Likewise, extract and mangiferin inhibit the release of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) in RAW264.7 and J774 macrophages (Garrido et al., 2006). In vitro treatment with the extract reduces pro-inflammatory cytokines interleukin 1β(IL-1β), TNF-α and colony-stimulating factor (GM-CSF) (Leiro et al., 2003). These data suggest that the extract inhibits the cyclooxygenases and lipoxygenase pathways, and these actions probably account for the

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0014-2999/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ejphar.2013.08.004

antinociceptive effects. Given that mangiferin is the predominant compound of the extract, it could be responsible of the antinociceptive effect induced by the aqueous extract. Therefore, the present study was undertaken to systematically determine the possible antinociceptive activity of mangiferin in the formalin test in rats, as well as the possible mechanisms of action of this compound.

2. Material and methods

2.1. Animals

Experiments were performed on male Wistar rats weighting 200–220 g. Rats were housed in an animal room at 22 ± 2 °C with a 12:12 light-dark cycle and the animals had free access to food and drinking water before experiments. The tests were carried out at 25 ± 1 °C. All experiments followed Guidelines on Ethical Standards for investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the Institutional Animal Care and Use Committee from Universidad Autónoma Metropolitana (UAM) approved the study. Efforts were made to minimize animal suffering and to reduce number of animals, which were used only once. At the end of the experiments, rats were killed in a CO_2 chamber.

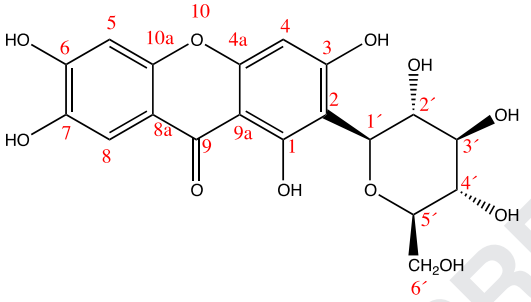

Fig. 1. Structure of mangiferin.

2.2. Drugs

Naltrindole was purchased from Santa Cruz Biotechnology (California, USA). Formaldehyde, serotonin hydrochloride (5-HT) and diclofenac sodium were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methiothepin mesylate (methiothepin), naloxone hydrochloride dihydrate (naloxone), 5-guanidinonaltrindole (5-GNTI), N^G-L-nitro-arginine methyl ester (L-NAME), diazoxide, glibenclamide, 1*H*-[1,2,4]-oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) and capsaicin were a gift of Dr. Vinicio Granados-Soto. Formaldehyde, capsaicin, methiothepin, naloxone, naltrindole, diclofenac, 5-HT, L-NAME, 5-GNTI and glibenclamide were dissolved in saline solution. Diazoxide was dissolved in 100% dimethylsulfoxide (DMSO). Mangiferin was dissolved in 1% DMSO. Capsaicin was dissolved in 10% ethanol, 10% Tween 20, and 80% saline.

Mangiferin was isolated from bark of *M. indica*. The product isolated was compared with commercial sample of mangiferin (Sigma-Aldrich, St. Louis, MO, USA) by thin layer chromatography and identified by comparison with physical and spectroscopic data. Melting point (°C) was determined in a Fisher-Jones apparatus and is uncorrected. Ultraviolet (UV) spectra was measured in a spectrophotometer UV–vis (Perkin Elmer, MA, USA); Infrared (IR) spectra was recorded in a Spectrum RXI in KBr (Perkin Elmer, MA, USA). Optical rotations were measured in a polarimeter model P3002RS (A. KRÜSS Optronic GmbH, Hamburg, Germany) at 589 nm using a 1 dm cell. Specific rotations are given in units of 10–1 deg/cm^2/g (c g/100 cm^3). Mass spectra were obtained by electrospray ionization (ESI) in a GCMate II spectrometer (JEOL Ltd., Tokyo, Japan). 1D and 2D ^{1}H and ^{13}C spectra were obtained at 400 and 100 MHz (Bruker Avance III 400) in pyridine at room temperature with tetramethylsilane (TMS) as the internal reference, chemical shifts (δ) are given in ppm and coupling constants (J) in Hz.

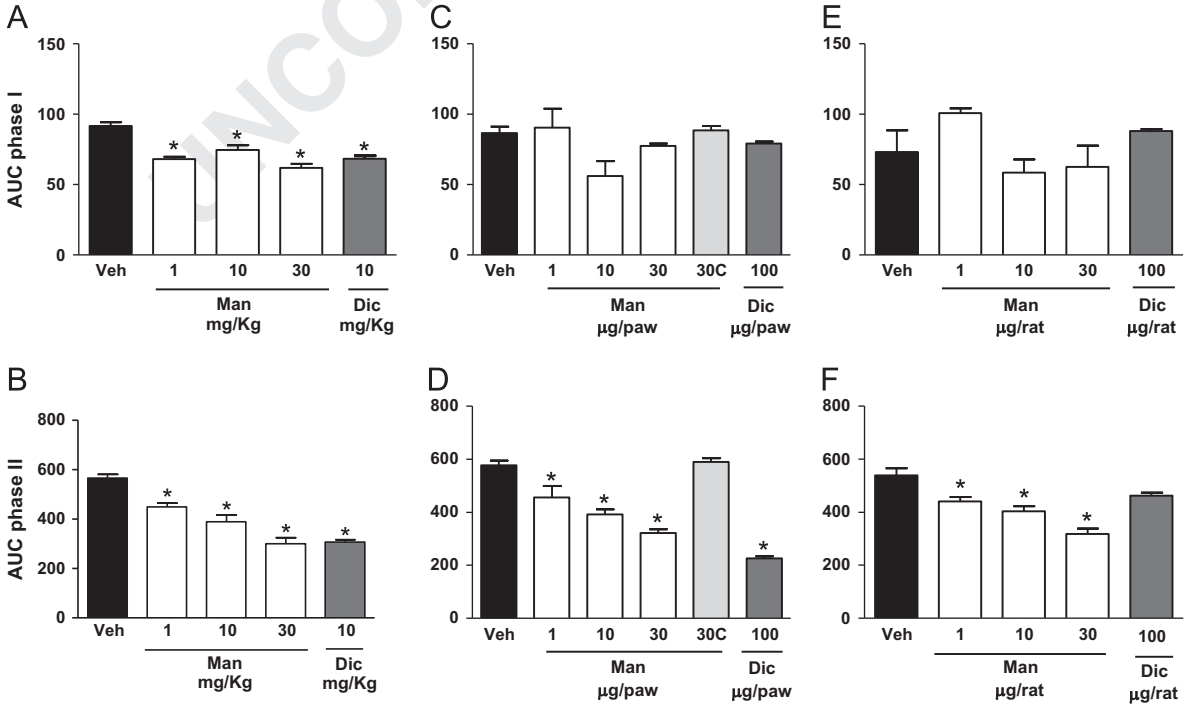

Fig. 2. Effect of systemic ((A) and (B)), local peripheral ((C) and (D)) or spinal ((E) and (F)) pre-treatment (− 10 min) with mangiferin in phase I (upper panels) and phase II (lower panels) of the 1% formalin test in rats. Data are expressed as the mean ± S.E.M. of at least six animals of the area under the number of flinches against time curves (AUC). *$P < 0.05$ vs vehicle group, as determined by one-way analysis of variance followed by the Tukey's test. Abbreviations: Mangiferin: Man; Diclofenac: Dic; Vehicle: Veh.

Please cite this article as: Izquierdo, T., et al., Mechanisms underlying the antinociceptive effect of mangiferin in the formalin test. Eur J Pharmacol (2013), http://dx.doi.org/10.1016/j.ejphar.2013.08.004