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Antinociceptive and anti-arthritic effects of krameсyne

Angel Josabad Alonso-Castro, Miguel Angel Zavala-Sánchez, Julia Pérez-Ramos, Ernesto Sánchez-Mendoza, Salud Pérez-Gutiérrez *

Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana — Xochimilco, Mexico

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ABSTRACT

Aims: The aim of this study was to evaluate the antinociceptive (acute assays) and anti-inflammatory (chronic assays) effects of krameсyne (KACY), a peroxide isolated from *Krameria cytisoides*.
Main methods: The antinociceptive activity of KACY was evaluated using the hot plate, acetic acid and formalin tests. The effects of KACY on heat-induced hemolysis in rat erythrocytes were also evaluated. The *in vivo* anti-inflammatory assays were performed using the chronic TPA (12-O-tetradecanoylphorbol 13-acetate) method to induce ear edema and carrageenan-kaolin induced arthritis (CKIA). In the CKIA model, the hot plate test was performed, serum samples were obtained for the quantitation of pro-inflammatory (IL-1β, IL-6, IL-12 and TNF-α) and anti-inflammatory (IL-4 and IL-10) cytokines.
Key findings: KACY possess antinociceptive effects with comparable activity to naproxen (NPX). KACY inhibited hemolysis (EC_{50} = 180 μg/mL), in comparison to the untreated group and with a higher potency than NPX (EC_{50} = 263 μg/mL). KACY at 50 mg/kg decreased inflammation by 38% (chronic TPA-induced edema model) and by 26% (CKIA model), in comparison with the vehicle group and with similar activity to the positive controls 8 mg/kg indomethacin (IND) and 1 mg/kg methotrexate (MTX), respectively. In the CKIA model, KACY increased the release of anti-inflammatory (IL-4 and IL-10) cytokines but reduced the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-12 and TNF-α). KACY at 50 and 100 mg/kg showed antinociceptive effects by 27% and 23%, respectively, in mice with mono-arthritis.
Significance: KACY might be a good alternative for the treatment of rheumatoid arthritis (RA) due its antinociceptive and anti-inflammatory activities.

Chemical compounds studied in this article

Indomethacin (PubChem CID 3715)
Methotrexate (PubChem CID 126941)
12-O-tetradecanoylphorbol 13-acetate (PubChem CID 27924)
Carrageenan (PubChem CID 6850766)
Kaolin (PubChem CID 16703273)
Naproxen sodium (PubChem CID 23681059)

Introduction

RA is a chronic and systemic autoimmune inflammatory disease linked to synovial hyperplasia that causes articular destruction and functional disability, affecting approximately 1 to 2% of the population [24]. The drugs commonly used for the treatment of RA are steroids and non-steroidal anti-inflammatory drugs (NSAIDs), as well as disease-modifying anti-rheumatic drugs (DMARDs) [2]. However, all these drugs have severe adverse effects. Steroids cause adrenal suppression, whereas NSAIDs induce gastric ulceration and kidney damage. DMARDs, such as MTX, cause myelosuppression, gastric ulcerations, and damage to the liver and kidneys [2]. Because of the side effects produced by anti-arthritic drugs, many patients discontinue their treatment [15,32]. Therefore, it is highly necessary to find new anti-inflammatory agents with antinociceptive effects and with similar or higher activity than currently used drugs but with fewer toxic effects.

Plants have traditionally been an important source of anti-inflammatory drugs [29]. For instance, salicylic acid was obtained from the tree *Salix alba* [27,33]. Recently, KACY (Fig. 1) was isolated from leaves of *Krameria cytisoides* Cav (Krameriaceae) [21]. In acute assays, KACY showed a lethal dose 50 (LD_{50}) higher than 5000 mg/kg p.o. and when administered at 2 mg/ear inhibited acute TPA-induced ear edema in mice (6 h), with similar effects to those found in IND at 2 mg/ear [21]. KACY at 50 mg/kg p.o. decreased carrageenan-induced inflammation in paw edema in mice in an acute test (6 h) with similar effects to those found in IND at 8 mg/kg p.o. [21]. Furthermore, KACY inhibited the transcriptional production of iNOS, COX-2, NO, TNF-α and IL-6 in LPS-stimulated macrophages [25]. Our previous studies were carried out in assays of short duration (hours). In addition, the detailed mechanisms by which KACY exerts its *in vivo* anti-inflammatory

* Corresponding author at: Calzada del Hueso 1100, Col. Villa Quietud, Coyoacan 04960, Mexico. Tel.: +52 54837263.
E-mail address: msperez@correo.xoc.uam.mx (S. Pérez-Gutiérrez).

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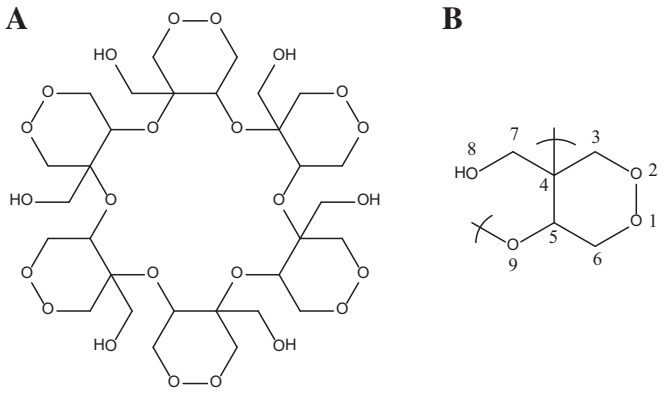

Fig. 1. Chemical structure of KACY a) cyclic polymer and b) monomer.

effects remain unclear. This study shows for the first time, that KACY exerts anti-arthritic and antinociceptive effects. Furthermore, our research provides information of the mechanism of anti-arthritic effect of KACY. We also showed the antinociceptive effects of KACY in mice with CKIA.

Materials and methods

Reagents

Ketamine and xylazine were obtained from Pisa Agropecuaria (Atitalaquia, Hidalgo, Mexico), aluminum silicate (kaolin), TPA and IND were purchased from Sigma (St. Louis, MO, USA). NPX was obtained from Tripharma (Distrito Federal, Mexico). MTX was obtained from Teva Pharmaceutical Mexico (Huixquilucan, State of Mexico, Mexico). ELISA kits for the quantitation of IL-4 and IL-12 (eBioscience, Vienna, Austria), IL-1β, IL-6, IL-10 and TNF-α (Peprotech Inc., Rocky Hill, NJ, USA) were acquired.

Plant material

K. cytisoides was collected in june 2009, in Las Comadres, which belongs to the municipality of Guadalcazar, San Luis Potosi State, Mexico. The identification of the plant was confirmed by José García Pérez (Instituto de Zonas Desérticas, Universidad Autónoma de San Luis Potosí, México). A voucher specimen (SPLM44560) was deposited in the Isidro Palacios Herbarium of the Universidad Autónoma de San Luis Potosi (SLPM).

Isolation of KACY

The isolation of KACY was carried out as described previously [21]. Dried leaves of *K. cytisoides* were reduced to powder. A portion (200 g) was defatted with hexane (2 L) under reflux for 4 h and then extracted with MeOH (2 L) under reflux for 4 h. The methanol extract was concentrated to half the original volume under reduced pressure, and a dark brown solid was obtained with 3% yield (m.p. 172 °C, dec.). The compound purity was determined by thin-layer chromatography.

Animals

CD-1 male mice weighing 25 to 30 g or Wistar male rats weighing 170 to 220 g, from the Universidad Autónoma Metropolitana — Xochimilco animal facility, were housed in isolated cages at 24 °C under a light–dark cycle of 12:12. The animals were supplied with food (Purina, Cuautitlan Izcalli, State of Mexico, Mexico) and water *ad libitum*. The experiments were carried out according to Official Mexican Norm NOM 062-ZOO-1999 (Technical specifications for the production, care and use of laboratory animals). The research also followed the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals [37]. All animal procedures were approved by the Research Bioethics Committee of Universidad Autónoma Metropolitana — Xochimilco.

Antinociceptive activity

Hot plate

The hot plate test was conducted on a thermostatically controlled heated metal plate (Analgesiometer, Ugo basile, Italy) at a temperature of 55 ± 1 °C according to the method of Turner [31]. The time (in seconds) that elapsed between placing the rat on the hot plate and the manifestation of signs of acute discomfort, such as licking of the hind paw or jumping in an attempt to escape from the heat, was taken as the reaction time or latency. Rats exhibiting latency time between 3 and 8 s were chosen. The latency time was determined at 60, 120 and 240 min after the oral administration of the following test samples (n = 8 each group): vehicle (saline solution), 100 mg/kg NPX or 10, 50 and 100 mg/kg KACY. A cut off time of 10 s was allowed to avoid thermal injury to the paws. The percentage antinociceptive response was calculated according to the following formula:

$$\%\ \text{Antinociceptive response} = \frac{\text{Reaction time} - \text{basal latency}}{\text{Cut off latency} - \text{basal latency}} \times 100.$$

Formalin

The formalin test was carried out as described by Hunskaar and Hole [7]. One hour prior to formalin injection, mice (n = 8 per group) orally received a) 100 mg/kg of NPX, b) KACY administered at doses of 10, 50 and 100 mg/kg and c) saline solution (vehicle group). Mice were injected with 30 μL of 1% formalin (in 0.9% saline) into the subplantar space of the right hind paw and individually placed into glass cylinders. The duration of paw licking was recorded at 0–15 min (first phase) and 15–45 min (second phase) after formalin injection.

Acetic acid-induced constrictions

The acetic acid method was carried out as described by Koster et al. [12]. One hour prior to the acetic acid injection, mice (n = 8 per group) orally received 100 mg/kg of NPX, KACY at doses of 10, 50 and 100 mg/kg and saline solution (vehicle group). Each group was administered with 10 mL/kg body weight (i.p.) of an aqueous solution of acetic acid (1.0%). The mice were individually placed into glass cylinders and observed for the number of abdominal constrictions, counted over a period of 0–30 min.

Anti-inflammatory activity

Membrane stabilizing effect in erythrocytes

Wistar rats were anesthetized, and their blood was collected by cardiac puncture under aseptic conditions. The experiment was carried out using heat-induced hemolysis of rat erythrocytes, as described by Pérez et al. [20]. Briefly, the vials containing 20 μL of fresh rat blood in 1 mL of PBS were treated in quintuplicate with NPX (50 to 300 μg/mL), as a positive control, or with KACY at concentrations ranging from 50 to 300 μg/mL. Vials were incubated for 15 min at 37 °C, followed by 54 °C for 15 min. Thereafter, vials were centrifuged, and the absorbance of the supernatant was measured at 540 nm spectrophotometrically. The percentage of hemolysis inhibition, compared to the control was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{Abs control} - \text{Abs treatment}}{\text{Abs control}} \times 100.$$

The effective concentration 50 (EC_{50}) of KACY and NPX for the plasma membrane stabilization effect was calculated using the plot of concentration of the KACY or NPX vs percentage inhibition of hemolysis.