Life Sciences 139 (2015) 83–90

Contents lists available at ScienceDirect

Life Sciences

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

Anti-inflammatory and antinociceptive effects of racemic goniothalamin, a styryl lactone

Débora Barbosa Vendramini-Costa [a,b,*], Humberto Moreira Spindola [b], Glaucia Coelho de Mello [c], Edson Antunes [c], Ronaldo Aloise Pilli [a], João Ernesto de Carvalho [b,d]

[a] University of Campinas, Department of Organic Chemistry, Institute of Chemistry, Rua Josué de Castro s/n, Cidade Universitária Zeferino Vaz, CP 6154, Campinas, São Paulo 13081-970, Brazil
[b] University of Campinas, Chemical, Biological and Agricultural Pluridisciplinary Research Center, CPQBA, Rua Alexandre Cazelatto, 999, Vila Betel, Paulínia, São Paulo 13148-218, Brazil
[c] University of Campinas, Cidade Universitária Zeferino Vaz, Department of Pharmacology, Faculty of Medical Sciences, Campinas, São Paulo 13081-970, Brazil
[d] University of Campinas, Cidade Universitária Zeferino Vaz, Faculty of Pharmaceutical Sciences, Campinas, São Paulo 13081-970, Brazil

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 18 July 2015
Accepted 11 August 2015
Available online 19 August 2015

Keywords:
Goniothalamin
Styryl lactones
Inflammation
Antinociception
Mice
Paw edema

ABSTRACT

Aims: The present study aimed to further investigate the anti-inflammatory activity of goniothalamin (GTN), a styryl lactone, as well as its antinociceptive effects.
Main methods: The anti-inflammatory activity was evaluated in models of paw edema induced by different mediators in mice and carrageenan-induced peritonitis. Evaluation of the antinociceptive effect was performed through acetic acid-induced writhing test and formalin test. Activity of GTN on gene expression levels of interleukin-1beta (IL-1β), induced nitric oxidase synthase (iNOS) and cyclooxygenase-2 (COX-2) were evaluated in vitro in lipopolysaccharide (LPS)-stimulated macrophage (RAW 264.7), as well as gene expression and protein levels of tumor necrosis factor-alpha (TNF-α).
Key findings: Pretreatment with GTN (300 mg/kg) significantly reduced paw edema induced by compound 48/80, prostaglandin E_2, phospholipase A_2 and bradykinin. GTN (10, 30 and 100 mg/kg) inhibited leukocyte migration in the peritonitis model and gene expression levels of IL-1β, iNOS and TNF-α, as well as TNF-α protein levels, in LPS-stimulated macrophages, without affecting COX-2 gene expression levels. GTN inhibited nociception induced by acetic acid in the writhing model and in the formalin test, when both neurogenic and inflammatory phases were inhibited.
Significance: For the first time the acute anti-inflammatory profile of GTN is characterized and its antinociceptive activity reported. The current study shows that GTN inhibits both vascular and cellular phases of inflammation, with bradykinin and PLA_2 induced inflammation being the most affected by GTN. Its anti-inflammatory effects also involved the in vitro inhibition of gene expression of alarm cytokines and mediators as IL-1β, iNOS and TNF-α.

1. Introduction

Inflammation is a component of the body's response to internal and external environmental stimuli. When tissue homeostasis is perturbed, macrophages and mast cells release mediators such as cytokines, chemokines, reactive oxygen species (ROS), which induce leukocytes mobilization and infiltration at the site of injury, thus stabilizing the inflammatory process associated with generation of pain [13,32]. If the inflammatory response is maintained for a short period, it has therapeutic consequences. However, if it becomes chronic, it can cause diseases, such as diabetes, rheumatoid arthritis, Alzheimer's, cardiovascular and lung diseases, autoimmune disorders and cancer [1,20]. Although several anti-inflammatory drugs are available, many of them can cause side effects after long-term use. Thus, studies focusing on the discovery of new anti-inflammatory drugs are desirable and nature has been an important source for new compounds.

The styryl lactones comprise a class of structurally simple compounds with a broad spectrum of biological activities [14]. Among them, goniothalamin (GTN, Fig. 1), a secondary metabolite originally isolated from several species of *Goniothalamus* (*Annonaceae*), exhibited antiproliferative and cytotoxic activity against a variety of tumor cell lines in its (*R*), (*S*) and racemic forms [2,8,12,14,28,29]. Several studies were conducted with GTN analogs, reporting its antiproliferative potential and in all of them the importance of the α,β-unsaturated δ-lactone moiety was highlighted, which acts as a Michael acceptor for cysteine residues or other nucleophiles in biological systems [3,4,6].

While several in vitro studies have been described for GTN, there is little information concerning its in vivo pharmacological activity. Recently, our group reported the gastroprotective effects of racemic GTN on chemically-induced gastric ulcers in rats, suggesting that GTN may act as a mild irritant, inducing the production of sulfhydryl compounds and prostaglandins, in a process known as adaptive

* Corresponding author at: Fox Chase Cancer Center, Cancer Prevention and Control (P2149), 333 Cottman Avenue, Philadelphia, PA, 19111, United States.
E-mail address: vendramini.debora@gmail.com (D.B. Vendramini-Costa).

http://dx.doi.org/10.1016/j.lfs.2015.08.010
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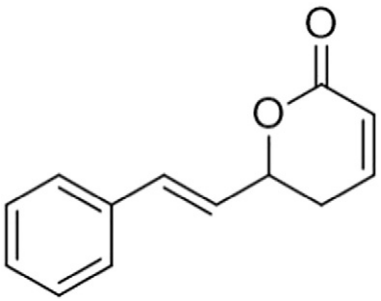

Fig. 1. Chemical structure of racemic goniothalamin.

cytoprotection [34]. Our group also reported the in vivo antiproliferative activity of racemic GTN on Ehrlich solid and ascitic tumor models in mice, without signs of toxicity at the effective doses [3,33].

Previously we have shown the anti-inflammatory activity of racemic GTN in the carrageenan-induced paw edema model in mice [33]. In this study, treatment with 300 mg/kg of GTN inhibited all phases of inflammation (up to 72 h after inflammatory stimulus), whereas doses of 30 and 100 mg/kg were effective only after 24 and 48 h from the inflammatory stimulus respectively. Although we have evaluated the anti-inflammatory profile of GTN on carrageenan-induced paw edema, we do not know which mediators are involved in this activity.

Bearing in mind the promising antiproliferative and anti-inflammatory activities of GTN and the lack of information concerning its pharmacological activity, the objective of this study was to investigate the effect of racemic GTN in models of inflammation induced by different mediators. As inflammation and nociception are closely related, the antinociceptive activity of GTN was also evaluated.

2. Material and methods

2.1. Synthesis of racemic goniothalamin

The racemic form of GTN was synthesized according to previously described methodology from our group, except for the utilization of allylmagnesium bromide in substitution to the enantioselective allylation step [12,14]. Racemic GTN was obtained in 65% overall yield and >95% purity, confirmed by ^{1}H- and ^{13}C-analyses and melting point comparison (m.p. = 82–84 °C; lit. 81–82 °C) [12,34].

2.2. Animals

Experiments were conducted with Balb/C and Swiss mice (20–30 g, 60 days) from the Multidisciplinary Center for Biological Investigation on Laboratory Animals Sciences (CEMIB–UNICAMP). Animals were maintained at the Animal Facilities of Pharmacology and Toxicology Division, CPQBA, University of Campinas (Campinas, Brazil), in a room with controlled temperature 25 ± 2 °C for 12 h light/dark cycle, with free access to food and water.

Research and animal euthanasia protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Institute of Biology/UNICAMP — Ethical Committee for Animal Research (2126-1, 3431-1, 3436-1). Euthanasia was performed by overdose of anesthesia (Ketamine/Xylazine) followed by cervical dislocation.

2.3. Drugs

Goniothalamin was emulsifield with 1% Tween 80 (Sigma™) and dissolved in phosphate–saline buffer (PBS). Vehicle was 1% Tween 80 in PBS, 10 mL/kg, for all experiments. Cyproheptadine (Periactin™), Piroxicam (Indocid™) and dexamethasone (DEX-Sigma™) were dissolved in PBS + 1% Tween 80 and used as positive controls. Acetic acid, formaldehyde, phospholipase A_2 from *Naja naja* venom (PLA_2), compound 48/80 (C48/80), prostaglandin E_2 (PGE_2), bradykinin (BK) and λ-carrageenan from Sigma™ were dissolved in PBS. Lipopolysaccharide (LPS) from Sigma™ was dissolved in DMSO (Sigma™).

2.4. Acute toxicity

Balb/C and Swiss mice (n = 6) were treated intraperitoneally (i.p.) with GTN (50, 100, 300, 600 and 1000 mg/kg), observed during 4 h and then daily for 15 days. General toxicity signals like body weight loss, locomotion on an open field apparatus, agitation, lethargy, respiration, salivation, tearing eyes, cyanosis and mortality were evaluated [18].

2.5. Paw edema induced by phospholipase A_2 (PLA_2), compound 48/80, bradykinin and PGE_2

Balb/C mice were divided into three groups (n = 8) and treated i.p. with vehicle, positive control or GTN (300 mg/kg). Thirty minutes later, edema was induced by intraplantar injection (right hind paw) of the inflammatory compounds and the volume of the paws was measured before and 15, 30, 60, 90 and 120 min after inflammation induction. Inflammatory inducers and positive controls, when used: PLA_2 (5 μg/paw, positive control dexamethasone — 1 mg/kg, i.p.) [15], compound 48/80 (10 μg/paw, positive control ciproheptadin — 4 mg/kg, i.p.) [9], bradykinin (50 μg/paw) [9], PGE_2 (1 μg/paw, positive control piroxicam — 20 mg/kg, i.p.) [10].

The volume of the edema in milliliters was registered using a plethysmometer (Ugo Basile, Italy), where the right hind paw was submerged until the tibio-tarsal joint in the measuring chamber of the device. The volume of fluid displaced was recorded and considered the volume of the paw. The results were expressed as the difference between the volume of the paw at the referred time intervals and the basal volume. The percentage of inhibition of edema was calculated as follows: $[(A - B) / A] \times 100$, where A = edema volume of negative control group and B = edema volume of experimental group.

2.6. Carrageenan-induced leukocyte migration in the mouse peritoneal cavity (peritonitis)

Balb/C mice (n = 8) were treated by subcutaneous route with vehicle, GTN (10, 30 and 100 mg/kg) or dexamethasone (1 mg/kg). Peritonitis was induced 45 min later, by i.p. injection of carrageenan solution (500 μg/250 μL) or 250 μL of PBS (negative control) [16]. Four hours later, mice were euthanized, and the peritoneal cavity was washed with 5 mL of PBS containing heparin 5 IU/mL. The peritoneal fluid was recovered for the analysis of total leukocyte numbers in a cell counter.

2.7. Writhing test

Groups of Swiss mice (n = 6) were treated i.p with vehicle and 10, 30, 100 and 300 mg/kg of GTN. Writhings were induced by an i.p. injection of 0.8% acetic acid solution (10 mL/kg), 30 min after treatment. Then, the numbers of writhings (abdominal constrictions) were cumulatively counted over 15 min, for nociception evaluation [30]. Data represent the mean of writhings observed per dose administrated.

2.8. Formalin test

The formalin test was carried out as described by [36] with few changes in the protocol [30]. Groups of Swiss mice (n = 6) were treated i.p. with vehicle and GTN (50, 100 and 150 mg/kg). After 30 min, animals were injected with 20 μL of a formalin solution (formaldehyde 1.2%, in PBS) into the plantar surface of the right hind paw. The total time spent by animal licking or biting the injected paw, an index of nociception, was recorded for the following 40 min.