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Carvacryl acetate, a derivative of carvacrol, reduces nociceptive and inflammatory response in mice

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ABSTRACT

Aims: The present study aimed to investigate the potential anti-inflammatory and anti-nociceptive effects of carvacryl acetate, a derivative of carvacrol, in mice.
Main methods: The anti-inflammatory activity was evaluated using various phlogistic agents that induce paw edema, peritonitis model, myeloperoxidase (MPO) activity, pro and anti-inflammatory cytokine levels. Evaluation of antinociceptive activity was conducted through acetic acid-induced writhing, hot plate test, formalin test, capsaicin and glutamate tests, as well as evaluation of motor performance on rotarod test.
Key findings: Pretreatment of mice with carvacryl acetate (75 mg/kg) significantly reduced carrageenan-induced paw edema ($P < 0.05$) when compared to vehicle-treated group. Likewise, carvacryl acetate (75 mg/kg) strongly inhibited edema induced by histamine, serotonin, prostaglandin E_2 and compound 48/80. In the peritonitis model, carvacryl acetate significantly decreased total and differential leukocyte counts, and reduced levels of myeloperoxidase and interleukin-1 beta (IL-1β) in the peritoneal exudate. The levels of IL-10, an anti-inflammatory cytokine, were enhanced by carvacryl acetate. Pretreatment with carvacryl acetate also decreased the number of acetic acid-induced writhing, increased the latency time of the animals on the hot plate and decreased paw licking time in the formalin, capsaicin and glutamate tests. The pretreatment with naloxone did not reverse the carvacryl acetate-mediated nociceptive effect.
Significance: In conclusion, the current study demonstrated that carvacryl acetate exhibited anti-inflammatory activity in mice by reducing inflammatory mediators, neutrophil migration and cytokine concentration, and anti-nociceptive activity due to the involvement of capsaicin and glutamate pathways.

Introduction

Inflammation is a dynamic and complex process that arises in response towards cellular injury. It has an important role in tissue repair, yet in some cases it can cause undesirable effects such as tissue damage and loss of function. Inflammation process is characterized by the production of a cascade of mediators that regulate important factors of the inflammatory response, as the increase in vascular permeability and recruitment of leukocytes in the blood (Rodriguez-Vita and Lawrence, 2010). Once released, these inflammatory mediators may activate or sensitize nociceptors adjacent to the injured tissue resulting in pain sensation (Andrade et al., 2012). Currently several analgesics and anti-inflammatory drugs are associated with important side effects, low efficacy and specificity. For this reason, studies are conducted to identify novel therapeutic options to develop and introduce new drugs with greater safety and efficacy.

Medicinal plants are natural products known to be a significant source of new chemical substances with potential therapeutic effects (Calixto, 2005). Among natural products, use of essential oils is a promising option because of proven therapeutic action and for being also commonly added in food to obtain a specific taste (Ipek et al., 2005). In this context, we can include the carvacrol (5-isopropyl-2-methylphenol), a monoterpenic phenol present in essential oils of numerous aromatic plants of the family Laminaceae (Vincenzi et al., 2004). Previous studies have demonstrated antimicrobial (Klein et al., 2013), antioxidant (Yanishlieva et al., 1999; Beena et al., 2013), anti-nociceptive (Melo et al., 2012) and anti-inflammatory (Landa et al., 2009) properties of this compound.

In this study, we decided to investigate a semisynthetic derivative of carvacrol, carvacryl acetate, a compound unexplored as their pharmacological properties. Since carvacrol, as well as other phenols, such as

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thymol, is more toxic than many esters, the synthesis of carvacryl acetate was performed to obtain a derivative of carvacrol with improved pharmacological profile and less toxicity. Thus we suggest that the presence of an ester group, instead of the hydroxyl group of carvacrol, may confer different safety and efficacy features to this compound compared to monortepene carvacryl.

Considering the potential use of natural products in the development of new drugs, the aim of the present study was to investigate the anti-inflammatory and antinociceptive effects of carvacryl acetate, a derivative of carvacrol, in experimental models.

Materials and methods

Drugs and reagents

λ-Carrageenan, indomethacin, serotonin, histamine, compound 48/80, prostaglandin E_2 (PGE_2), acetic acid, formaldehyde and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical (Saint Louis, MO, USA). Heparin and morphine were provided by Merck, Sao Paulo, Brazil. All drugs were dissolved in sterile 0.9% (w/v) NaCl (saline). The carvacryl acetate was dissolved in 2% DMSO. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Compounds

Carvacryl acetate (98% purity; Fig. 1) was obtained by acetylation of carvacrol, using acetic anhydride as acylating agent and pyridine as catalyst. As described below, first in a 50 ml flask, equipped with magnetic stirrer, coupled to a Friedrich condenser and an inert atmosphere was added carvacrol (5 g, 0.033 mol), pyridine (7.5 ml) and acetic anhydride (12.5 ml). Then subjected to magnetic stirring and under constant reflux for 24 h. Continuing the procedure for preparing the reaction mixture was poured into ice water (60 ml) and extracted from reaction product in a separator funnel using chloroform as the solvent (three times 60 ml). The chloroform phases were combined and washed with saturated copper sulphate (three times 60 ml). The chloroform phase was washed with water (three times 60 ml) and dried with anhydrous Na_2SO_4. Subsequently, the solvent was evaporated on a rotary evaporator. The reaction product was subjected to column chromatography using silica gel as stationary phase and a mixture of hexane/ethyl acetate (95:5) as mobile phase. There was obtained 4.779 g (0.025 mol) of carvacryl acetate and 76% yield (Vogel et al., 1996; Moraes et al., 2013). The structural identification of carvacryl acetate was performed by analysis of ^1H and ^{13}C NMR, IR and compared with literature data.

Animals

Male Swiss mice (25–30 g) were housed at a temperature of 25 ± 2 °C under a 12/12-h light/dark cycle with food and water ad libitum. All experiments were performed in accordance with the *Guide for Care and Use of Laboratory Animals* (National Institute of Health, Bethesda, MD, USA) and were approved by the Ethics Committee in Research of the Federal University of Piauí (protocol no. 0066/10).

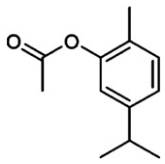

Fig. 1. Chemical structure of carvacryl acetate.

Experimental protocol

Carrageenan-induced paw edema in mice

The animals were randomly divided into six groups ($n = 5$), and edema was induced by injection of 50 μl of a suspension of carrageenan (500 μg/paw) in 0.9% sterile saline into the right hind paw (group I) according Silva et al. (2013). Mice were pretreated intraperitoneally (i.p.) with either 2% DMSO (group II untreated control); indomethacin 10 mg/kg (group III reference control); or carvacryl acetate, 25, 50 or 75 mg/kg (groups IV, V, and VI, respectively). Paw volume was measured immediately before (V_0), and at 1, 2, 3, and 4 h after carrageenan treatment (Vt), using a plethysmometer (Panlab, Barcelona, Spain). The effect of pretreatment was calculated as percent inhibition of edema relative to the paw volume of the DMSO-treated controls by using the following formula (Winter et al., 1962).

$$\%\,\text{inhibition of edema} = \frac{(V_t - V_0)\text{Control} - (V_t - V_0)\text{Treated}}{(V_t - V_0)\text{Control}} \times 100$$

Paw edema induced by different phlogistic agents

To induce edema, the animals were administered 50 μl injections of serotonin (1% w/v), histamine (100 μg/paw), prostaglandin E_2 (3 nmol/paw), or compound 48/80 (12 μg/paw) into the right hind paw (Silva et al., 2013; Chaves et al., 2013; Claudino et al., 2006). The contralateral paw received 50 μl of 2% DMSO and served as an untreated control. In the experiment, the animals were pretreated with carvacryl acetate (75 mg/kg, i.p.) or indomethacin (10 mg/kg, i.p.; reference control) 30 min before these intraplantar injections of phlogistic agents.

Carrageenan-induced peritonitis

For the determination of neutrophil migration into the peritoneal cavity, mice were injected intraperitoneally with 2.0% DMSO, indomethacin 10 mg/kg or carvacryl acetate (75 mg/kg, i.p.). Thirty minutes later, the animals were injected with carrageenan (250 μl; 500 μg/cavity), as adapted from de reports of Chaves et al. (2013). Mice were euthanized 4 h later and the peritoneal cavity was washed with 1.5 ml of heparinized phosphate buffered saline (PBS) to harvest peritoneal cells. The volumes recovered were similar in all experimental groups and were equivalent to ~95% of the injected volume. Total cell counts were performed in a Neubauer chamber, and differential cell counts (100 cells total) were carried out on cytocentrifuge slides stained with hematoxylin and eosin. The results are presented as the number of neutrophils per milliliter of peritoneal exudate. Aliquots of the peritoneal exudates were stored at −70 °C for later analysis of cytokine and myeloperoxidase (MPO) content.

Myeloperoxidase activity assay

MPO assay was based on the method of Bradley et al. (1982) and partly modified. Briefly, 400 μl of the peritoneal exudates was centrifuged at 40,000 ×*g* for 7 min at 4 °C. After, 10 μl of the supernatants were collected and MPO activity was assayed by measuring the change in absorbance at 450 nm using *o*-dianisidin edihydrochloride and 1% hydrogen peroxide. The results were expressed in units/ml. A unit of MPO activity was defined as that converting 1 μmol of hydrogen peroxide to water in 1 min at 22 °C.

Evaluation of TNF-α, IL-1β and IL-10 levels in carrageenan-induced peritonitis

Levels of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-10 (IL-10) were evaluated using sandwich ELISA. Briefly, microliter plates were coated overnight at 4 °C with antibody against mice TNF-α, IL-1β or IL-10 (2 μg/ml, DuoSet ELISA Development kit R&D Systems). Blocking of nonspecific binding sites was accomplished by incubating plates with PBS containing 2% bovine serum albumin (BSA) for 90 min at 37 °C. After blocking the plates,