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Protective effect of carvacrol on acetic acid-induced colitis

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ABSTRACT

The pharmacological therapy for inflammatory bowel diseases continues to be problematic, and requires new alternative options. In this study, we tested the hypothesis that carvacrol (CAR), a phenolic monoterpene with anti-inflammatory and antioxidant activities, can treat experimental colitis in mice. C57BL/6 mice (n = 8/group) were subjected to intrarectal administration of acetic acid (5%) to induce colitis. Mice were pretreated with CAR (25, 50 or 100 mg/kg, p.o.) every 12 h for three days prior to the induction. Abdominal hyperalgesia, macroscopic and microscopic colon damage, myeloperoxidase (MPO) activity, tumor necrosis factor (TNF)-α and interleukin (IL)-1β levels, oxidative stress markers, and antioxidant enzyme activities were evaluated. Pretreatment with all doses of CAR significantly decreased abdominal hyperalgesia and colon MPO activity and TNF-α and IL-1β levels. A reduction in macroscopic and microscopic damage ($p < 0.05$) was observed at doses of 50 and 100 mg/kg CAR. Pretreatment with CAR significantly reduced lipid peroxidation (for all doses) and increased sulfhydryl groups (at 100 mg/kg). This effect was accompanied by a significant increase in catalase, superoxide dismutase, and glutathione peroxidase activities. These findings indicate that CAR protected mice from acetic acid-induced colitis by reducing inflammatory, nociceptive, and oxidative damages.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that leads to disruption in homeostasis in the digestive tract and uncontrolled intestinal inflammation [1,2]. Unlike Crohn's disease, UC is restricted to the colon and rectum [3]. The main symptoms of UC include diarrhea, abdominal cramps, and recurrent blood in the stools caused by mucosal ulcers [4]. Although the etiology of UC is still not completely established, it is known that multiple factors are associated with its pathogenesis, such as genetic susceptibility, environmental and microbial factors [5], and an imbalance between the immune system and the intestinal microbes [6,7]. It was showed that the cytokine tumor necrosis factor (TNF)-α is a key mediator in the onset of UC and the presence of the 308 G/A allele confers a 3.62-fold risk for the development of UC when compared to the risk in the general population [8].

Epidemiologic data have shown increasing global incidence and prevalence of UC in recent decades. In a military population-based study in the USA, Porter et al. [9] found a prevalence of 21.9/100 000 cases of UC per year, which in turn appeared to have a positive correlation with the elevated number of life stressors. Recently, Santos et al. [10] showed that up to 20% of patients with UC in Brazil needed to undergo colectomy due to complications caused by the disease.

The current pharmacological therapy for patients with UC includes non-selective anti-inflammatory drugs and corticosteroids\immunosuppressants [11], as well as anti-TNF-α agents [12]. These drugs are used to maintain long-term remission, reduction of abnormal colonic inflammation, and control of clinical symptoms, such as diarrhea, rectal bleeding, and abdominal pain. However, the continuous use of these medications can cause serious side effects to patients (e.g., gastric ulcer formation, hyperglycemia, glaucoma, muscle weakness [13], and increased rates of infections, malignancies and other adverse events [14]). Thus, a great effort has been made to develop new drugs to treat UC.

Reports from the literature indicate that the use of medicinal plants is an alternative approach for the treatment of experimental UC [15]. Medicinal plants are sources of diverse pharmacologically active compounds, such as monoterpenes, which can be obtained from a number of aromatic plants, including those from the genera Lippia and Origanum [16]. A previous study showed that the essential oil of *Origanum*

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onites L. reduced colonic damage in colitis induced by 2,4,6-trinitrobenzenesulfonic acid, and carvacrol was identified as its major constituent [17].

Carvacrol (5-isopropyl-2-methylphenol) is a phenol monoterpene that possesses a variety of pharmacological properties, including antioxidant, antibacterial, hepatoprotective, anti-inflammatory, antinociceptive, and gastroprotective activities, among others [18–21]. However, its effect on colitis is not entirely clear. In this study, we evaluated the effects of carvacrol against acid acetic-induced colitis in mice.

2. Material and methods

2.1. Reagents

5-Aminosalicylic acid, 2,4-dinitrochlorobenzene, adrenaline, aprotinin A, Bradford reagent, benzomethonium chloride, bovine serum albumin, catalase, cremophor, carvacrol, dinitricbenzoic acid, glutathione reductase, glutathione, hexadecyltrimethylammonium bromide (HTAB), *o*-dianisidine hydrochloride, ethylenediaminetetraacetic acid (EDTA), tert-butyl hydroperoxide, horseradish peroxidase, nicotinamide adenine dinucleotide phosphate reductase (NADPH), and phenylmethylsulfonyl fluoride propionyl chloride were purchased from Sigma (St. Louis, MO, USA). Enzyme-linked immunosorbent assay (ELISA) kits for quantitative analysis of mouse TNF-α and IL-1β were obtained from R & D Systems (Minneapolis, MN, USA). Isoflurane was obtained from Cristália (Itapira, SP, Brazil). Acetic acid and sulfuric acid were obtained from Êxodo Científica (Hortolândia, SP, Brazil). All other reagents were obtained from Merck (São Paulo, SP, Brazil).

2.2. Animals

Male C57BL/6 mice (25–30 g) were obtained from the Animal Center of Federal University of Sergipe. They were randomly housed in appropriate cages under a temperature of 22 ± 2 °C and a 12-h light/dark cycle (lights on from 6:00 a.m. to 6:00 p.m.), with free access to food (Purina, Brazil) and tap water. The animals were randomly distributed among the experimental groups. All experimental and euthanasia procedures were performed according to the National Laboratory Health and Use Guide (NIH) and International Council for Laboratory Animal Science (ICLAS) standards, and were approved by the Research Ethics Committee of the Federal University of Sergipe, approval no. 15/2014. The visual observers who carried out the nociceptive test and macroscopic and microscopic analysis were blinded from identification of the experimental groups.

2.3. Experimental design and colitis induction

Animals were divided into six groups (n = 8). Mice in group I (Vehicle + Saline) were pretreated with cremophor (0.05% prepared in 0.9% saline, as the vehicle for CAR) every 12 h, per os (p.o.), dosed at 0.1 mL/10 g of body weight for three days. In group II (Vehicle + Colitis), mice were pretreated with cremophor similarly. Groups III, IV, and V (CAR + Colitis) were subjected to pretreatment with CAR at 25, 50 and 100 mg/kg [26,27], respectively (every 12 h, p.o., 0.1 mL/10 g, for three days), and group VI (5-ASA + Colitis) received pretreatment with 5-ASA (100 mg/kg, every 24 h, for three days) [22].

On the third day, one hour after the last pretreatment, animals from groups II to VI were subjected to colitis induction. Mice were subjected to isoflurane (1–2%) anesthesia, and colon lavage was performed with 2 mL of 0.9% saline solution, with the aid of a flexible catheter (3.5 F), which was inserted approximately 3 cm deep into the proximal portion of the anus. Next, 150 μL of a 5% acetic acid solution (v/v) prepared in saline was intrarectally injected into the lumen of the colon [23]. After administration of acetic acid, mice were maintained in a supine position for 30 s to prevent leakage of the solution [24]. Animals from group I were subjected to the same procedure using an equal volume of saline instead of acetic acid solution. Twenty-four hours later, the animals were killed by excess of inhaled isoflurane (5%), and colon tissue was collected for analysis.

2.4. Evaluation of colon macroscopic damage

Thereafter, colons were immediately removed and washed in 10 mmol/L phosphate-buffered saline (PBS) solution (pH 7.4). The severity of the colonic lesions was evaluated using a macroscopic scale with the following scores: 0 (no macroscopic changes), 1 (only mucosal erythema), 2 (mild mucosal edema, slight hemorrhage or minor erosion), 3 (mild edema, mild ulcers, bleeding or erosions), and 4 (severe ulceration, edema, and tissue necrosis), according to previous studies [23,25].

2.5. Evaluation of inflammatory biomarkers

Myeloperoxidase (MPO) activity was measured using the technique described by Bradley et al. [26] with minor modifications. Colon samples (~100 mg) were collected, weighed, cut into small pieces, and homogenized in potassium phosphate buffer (50 mmol/L, pH 6.0, containing 0.5% HTAB). Next, 1-mL aliquots of the homogenate were centrifuged (10 min, 18,000*g*, 4 °C) to obtain the supernatant. In a 96-well plate, 20 μL of the supernatant was pipetted and mixed with 200 μL of a solution containing *o*-dianisidine dihydrochloride (0.167 mg/mL, prepared in 50 mmol/L of potassium phosphate buffer containing 0.005% of H_2O_2). The results are expressed as units of MPO per mg of tissue (UMPO/mg tissue).

The levels of TNF-α and IL-1β were quantified in colon tissues through enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's instructions. For this, the colons were removed and homogenized in PBS (NaCl 137 mmol/L; KCl 2.7 mmol/L; Na_2HPO_4 8.1 mmol/L; KH_2PO_4 1.5 mmol/L, pH 7.2) containing Tween 20 (0.05%), phenylmethylsulfonyl fluoride (PMSF; 0.1 mmol/L), benzomethonium chloride (0.1 mmol/L), EDTA (10 mmol/L), and aprotinin A (2 ng/mL). The amount of protein in each sample was measured via the Bradford method, and the results are expressed in pg of cytokine/mg of protein.

2.6. Histological analysis

For the histological evaluation, the colons were removed and fixed with 10% buffered formalin. These samples were then embedded in paraffin, following routine histological processing, cut into 5-μm-thick sections, and stained with hematoxylin and eosin. Architectural loss, cellular infiltration into the mucosa, muscle submucosa thickening, abscess and hemorrhage formation in the crypts, and depletion of goblet cells were assessed by a qualitative score as follows: 0 (no detectable changes), 1 (mild injury), 2 (moderate injury), and 3 (severe injury) [27]. Qualitative analyses were performed on 400× magnified images.

2.7. Measurement of abdominal hyperalgesia

The magnitude of mechanical abdominal hyperalgesia was evaluated using the electronic von Frey test [28]. Animals were acclimated in individual cages for 30 min prior to the test after which mechanical stimuli were applied to the abdomen of each animal. The first measurement was performed prior to the colitis induction with acetic acid (basal measurement) and the second measurement was performed 23 h after the induction.

For each measurement, the electronic von Frey tip was applied as a stimulus of increasing pressure in the lower abdominal region until the mice presented a withdrawal response. The force required to cause this response was considered the threshold value. This procedure was