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The effect of menthol on acute experimental colitis in rats

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ABSTRACT

Menthol is an aromatic compound with high antiinflammatory activity. The purpose of the current research is to investigate the effectiveness of menthol on acetic acid induced acute colitis in rats. Animals were injected with menthol (20 and 50 and 80 mg/kg, i.p.) 24 h prior to induction of colitis for 3 consecutive days. Menthol at medium and higher doses similar to dexamethasone as a reference drug significantly reduced body weight loss, macroscopic damage score, ulcer area, colon weight, colon length and improved hematocrit in rats with colitis. The histopathological examination also confirmed anti-colitic effects of menthol. Menthol also reduced significantly the colonic levels of tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6) and myeloperoxidase (MPO) activity in inflamed colons. Thus, the findings of the current study provide evidence that menthol may be beneficial in patients suffering from acute ulcerative colitis.

1. Introduction

Ulcerative colitis is a multifactorial intestinal disease with unknown etiology. The quality of life of patients suffering from ulcerative colitis can be impaired by complications including bowel perforation, toxic megacolon, surgical complications and elevated risk of colorectal cancer (Guthrie et al., 2002; Nugent et al., 1991; Zhang et al., 2011). Clinical data suggest that ulcerative colitis may result from excessive immune response to flora that persists in the lumen (Hans et al., 2000) and may lead to the overproduction of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6) and myeloperoxidase (MPO). Some of the colon damage in ulcerative colitis is due to these proinflammatory cytokines (Motaghi et al., 2016).

By now, the unique widely accepted therapy for ulcerative colitis is based on the use of antiinflammatory and immunosuppressive drugs that are sometimes ineffective in some patients or present important adverse effects, therefore, new treatment strategies are required (Hagar et al., 2007).

Menthol is a cyclic terpene alcohol made synthetically or obtained from peppermint, corn mint, or other mint oils used as a flavoring agent in a wide variety of products. Menthol has been used since antiquity for medicinal purposes (MacDougall et al., 2003) and nowadays, is broadly used in liniments, balms, and troches with medical implementation for relief of pain and irritation of airways and skin (Karashima et al., 2007). Although menthol is safe to humans (Gelal et al., 1999) and it is reported to has potent antiinflammatory and antioxidant activities in various in vitro and in vivo models (Li et al., 2009; Wang et al., 2012).

Different rodent models have been established to evaluate efficacy of drugs against ulcerative colitis. Among them, acetic acid-induced ulcerative colitis is a simple and reproducible model, sharing many characteristics with ulcerative colitis in human (MacPherson and Pfeiffer, 1978). Therefore, the aim of the current study was to evaluate the effect of menthol on the acetic acid induced colitis in rats.

2. Materials and methods

2.1. Reagents

Menthol and dexamethasone were gifted from Raha Pharmaceutical Co. (Isfahan, Iran). O-dianisidine dihydrochloride, hexadecyltrimethylammonoium bromide (HTAB) and tween 80 were bought from Sigma Aldrich (St. Louis, USA). Acetic acid was purchased from Merck-Schuchardt (Hohenbrunn, Germany). The ELISA kits for rat TNF-α, IL-1β and IL-6 were all purchased from Boster Co. (Pleasanton, CA, USA).

2.2. Animals

Male Wistar rats weighting 200–240 g were obtained from the animal house of Kurdistan University of Medical Science. The rats were

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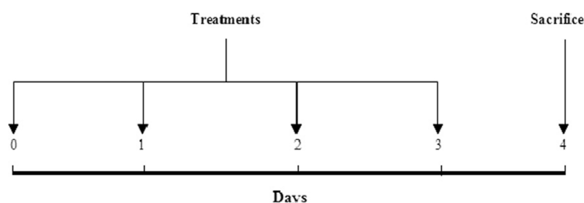

Fig. 1. Schematic of treatment timeline. Colitis was induced by instillation of acetic acid at day 1.

kept in standard laboratory conditions (room temperature of 22–26 °C with a controlled 12/12 h light/dark cycle and free access to animal chow and water). All animal experiments in the present study were performed in compliance with the guidelines of Ethics Committee of Kurdistan University of Medical Science.

2.3. Grouping

The animals were randomized into 6 groups of 6 rats each. (1) Sham group and (2) colitis control group both receiving vehicle (Tween 80 (8%) in normal saline, intraperitoneal) (Rozza et al., 2014). (3), (4) and (5) Menthol treatment groups receiving menthol at 20, 50 and 80 mg/kg, respectively. The doses of menthol were chosen based on a previous report (Rozza et al., 2014). (6) Dexamethasone group was treated with dexamethasone (1 mg/kg, intraperitoneal) as a reference drug. Treatments were given once daily for 3 days starting 24 h before and continued for 2 days after acetic acid instillation. Detailed experimental design is shown in Fig. 1. Ulcerative colitis was induced in all rats except Sham group.

2.4. Induction of acute colitis

The rats were fasted for 24 h with ad libitum access to water. Then, under light ether anesthesia, an 8 cm long tube was introduced into the rectum and 2 ml of acetic acid (3% v/v in normal saline) was slowly infused into the distal colon. The rat was positioned head-down for 30 s to avoid expelling the solution.

2.5. Macroscopic evaluation of colitis

All rats were weighted each day during the experiment.

The rats were euthanized by ether 72 h after acetic acid instillation. Colon length was measured after excision. Eight centimeters long piece of distal colon opened by longitudinal incision, was rinsed with cold normal saline to remove fecal residue, weighted, and scored for inflammation based on the macroscopic features by an independent observer without previous knowledge of the treatments (Minaiyan et al., 2014).

Macroscopic scoring of colon damage was done according to Millar et al. method (Millar et al., 1996) as follow:

0= no macroscopic damage, 1= only erythema in mucosal layer, 2= mild mucosal edema, slight erosion and/or slight bleeding, 3= moderate edema, erosions or bleeding ulcer, and 4= severe erosions, ulceration, edema and/or tissue necrosis.

Furthermore, the colon tissue was placed on a non-absorbent white surface and was photographed with a Canon camera (Powershot G9, 12 megapixel, Japan) to measure percent of ulcer area. Ulcer area was assessed by Fiji-Win 32 software (NIH Image for the Macintosh) (Motaghi et al., 2016).

2.6. Histopathological evaluation

Colon specimens from each animal were fixed and then embedded in paraffin. Five µm thick sections were stained with H & E for mucosal damage assessment. The histological examinations were performed by a pathologist blinded to the treatment groups.

A grading scale was provided for each criteria based on the degree of damage which described previously (Rees, 1998). Total colitis index was the summation of inflammation extent, inflammation severity and crypt damage.

2.7. Biochemical evaluation

2.7.1. MPO activity assay

MPO activity, an indicator of neutrophil infiltration, was assessed in colon according to the method of Bradley et al. with some modification (Bradley et al., 1982). Briefly, colon tissue was weighed and homogenized using polytron homogenizer in 1 ml of ice-cold potassium phosphate buffer (50 mM, pH 6.0) containing HTAB (0.5%) and EDTA (5 mM). Additional buffer solution was added to reach the concentration of 5 ml per 0.1 g of tissue.

The homogenate was subjected to sonication in an ice water bath for 10 s, after which it was freeze-thawed 3 times. Then samples were sonicated again for 10 s, and centrifuged at 10,000*g* for 15 min at 4°C. An aliquot of 100 µL of the supernatant mixture was mixed with 2.9 ml of phosphate buffer (50 mM) containing O-dianisidine dihydrochloride (0.167 mg/ml) and hydrogen peroxide (0.0005%). Then, the changes in absorbance was measured spectrophotometrically at 460 nm.

2.7.2. Assessment of pro-inflammatory cytokines

Briefly, a portion of colon tissue was homogenized in ice-cold potassium phosphate buffer (pH 7.4) by a polytron homogenizer, and the homogenate was saved for the assessment of pro-inflammatory cytokines.

Tissue TNF-α, IL-1β and IL-6 were measured using an ELISA kit according to the manufacturer's instructions (Boster Co., Pleasanton, CA, USA). The cytokine levels were calculated after plotting the standard curves and expressed as ng/g colon tissue. ELISA methods were carried out in triplicate.

2.8. Hematocrit evaluation

The rats were examined 72 h after colitis induction for blood hematocrit by capillary tubes centrifugation (Patel et al., 2014).

2.9. Statistical analysis

Parametric data represented as mean ± S.E.M and analyzed by one-way ANOVA, followed by post hoc Tukey test. Nonparametric data displayed as median (range) and analyzed using Kruskal-Wallis test and post-hoc Mann–Whitney U test. A value of $P < 0.05$ was assigned as statistically significant. Statistical analyses were performed with GraphPad Prism ver. 1.06 (GraphPad software Inc., California, USA).

3. Results

3.1. Effect of menthol on body weight changes of animals

In vehicle-treated rats, acetic acid induced severe colitis that was associated with a significant body weight loss (Fig. 2). Treatment of acetic acid-treated rats with menthol at the doses of 50 and 80 mg/kg significantly (at least $P < 0.05$) prevented the body weight loss.

3.2. Effect of menthol on macroscopic changes of colitis

While colon of Sham group showed normal appearance, intracolonic acetic acid administration induced marked hyperemia, inflammation, and necrosis in the colon in colitis control group (Fig. 3A).

The macroscopic evaluation of colitis indicated that macroscopic damage score (Table 1), ulcer area (Fig. 3B) and colon weight (Fig. 3C) were higher in colitis control group than Sham group ($P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively). Menthol, at medium and high

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