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Original article

The effect of theophylline on acetic acid induced ulcerative colitis in rats

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ABSTRACT

Background: Ulcerative colitis is a relapsing inflammatory disorder of the colon. There is a need to explore the new treatments for this disorder. Theophylline, a competitive inhibitor of phosphodiesterase, is shown to have anti-inflammatory properties. However, the effect of theophylline on ulcerative colitis has not yet been investigated. The present study evaluated the effect of theophylline on acetic acid induced ulcerative colitis in rats.
Materials and methods: Colitis was induced by instillation of 2 ml of acetic acid solution (3%). Colon samples were evaluated grossly and microscopically and assayed for myeloperoxidase (MPO) activity and proinflammatory cytokine concentrations.
Results: Treatment with theophylline at the doses of 20 and 50 mg/kg attenuated acetic acid induced ulcerative colitis as shown by improvement in body weight loss, macroscopic score, ulcer area, hematocrit and histopathological score. Theophylline treatment also reduced MPO activity and tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1 β) and interleukin 6 (IL-6) concentrations in inflamed colon.
Conclusion: Theophylline has a protective effect in acetic acid-induced ulcerative colitis which might be due to its anti-inflammatory activities. Therefore, theophylline has the potential to be used for successful treatment of ulcerative colitis.

1. Introduction

Ulcerative colitis is an inflammatory disease affecting the mucosal layer of the distal colon and rectum [1]. Proinflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin 1β (IL-1 β) and interleukin-6 (IL-6) are involved in the immunopathogenesis of ulcerative colitis [2,3]. Thus, the therapies which reduce the proinflammatory cytokines could be beneficial for the treatment of ulcerative colitis. Although 5-aminosalicylic acid, glucocorticoids and immunosuppressive drugs are effective to some extent, some patients are still refractory or experience serious side effects, so new treatments are needed [4].

Various works have shown that phosphodiesterase (PDE) inhibitors have cell protecting properties [5] and are effective in improvement of different inflammatory conditions such as arthritis [6] and autoimmune encephalitis [7]. Inhibition of PDE elevates the level of intracellular cyclic adenosine monophosphate (cAMP) in white blood cells which then reduce proinflammatory cytokines [6,8,9] and inhibits reactive oxygen species [10] and nitric oxide generation [6].

Theophylline as a non-specific PDE inhibitor is widely used worldwide as a bronchodilator in patients with asthma and chronic obstructive pulmonary disease (COPD) for over 60 years [11]. Several studies have shown that the positive effects of theophylline in patient with asthma are not entirely explainable by bronchodilatory effects [12]. Theophylline inhibited lung carcinogenesis in animal model [13]. Moreover, theophylline showed anti-inflammatory activity both *in vitro* [6,8,9] and *in vivo*. *In vivo* studies showed that theophylline attenuates the response to allergen [14] and reduces bronchial mucosal eosinophils in patients with mild asthma [15]. It has been also shown that theophylline may exert various effects on tissues other than lung such as cartilage [6], hearth [16], kidney [17] and esophagus [18]. However, the effect of theophylline on inflamed colon was not yet evaluated. Therefore,

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current study investigated the effect of theophylline on acetic acid induced ulcerative colitis, which is a chemically induced model of colitis that shows various pathophysiological similarity to the ulcerative colitis in human [19].

2. Materials and methods

2.1. Reagents

Dexamethasone and theophylline were gifted from Raha Pharmaceutical Co. (Isfahan, Iran). Hexadecyltrimethylammonium bromide (HTAB) and O-dianisidine dihydrochloride were procured from Sigma–Aldrich (St. Louis, USA). Acetic acid was bought from Merck-Schuchardt (Hohenbrunn, Germany). Enzyme-linked immunosorbent assay (ELISA) kits for rat TNF-α, IL-1β and IL-6 were all purchased from Boster Co. (Pleasanton, CA, USA).

2.2. Animals and grouping

Adult male Wistar rats (210–240 g) were obtained from the animal house of Kurdistan University of Medical Sciences. The animals were housed three per cage in a regulated environment (23–25 °C and 12/12 h light/dark cycle) with free access to standard chow and water. After one week of adaptation, the rats were treated intraperitoneally once daily with 10, 20 or 50 mg/kg of theophylline (treatment), or dexamethasone as a reference drug 24 h before acetic acid instillation and for the subsequent 3 days. Sham and colitis control animals received vehicle (normal saline, 1 ml/kg, i.p.). Each group composed of 6 rats. The doses of theophylline were chosen based on our pilot study and a previous report [6].

2.3. Colitis induction

All rats were fasted for 24 h with free access to water. After light anesthesia with ether, colitis was induced by instillation of 2 ml acid acetic solution (3% in normal saline) using a polyethylene tube which was introduced into the anus to a distance of 8 cm. The rats were then maintained in the head-down position for 30 s to prevent leakage of the acetic acid [20]. Sham group received intracolonic normal saline, instead of acetic acid.

All experiments were done according to the protocol of the Ethical Committee of Kurdistan University of Medical Sciences. Body weight was measured each day during the experiment.

2.4. Hematocrit measurement and macroscopic evaluation of colon damage

Blood was sampled in a heparin coated capillary tube for hematocrit evaluation 3 days after acetic acid instillation. On the same day, the rat was sacrificed under ether anesthesia. The abdomen was opened *via* a midline incision and the distal 8 cm of the colon was removed, freed of adherent adipose tissue, opened longitudinally, washed with normal saline to remove any fecal material and was scored macroscopically as follow: 0: no damage; 1: mucosal erythema only; 2: mild mucosal edema, slight bleeding or small erosions; 3: moderate edema, bleeding, erosions or ulcers; 4: severe ulceration, erosions, edema or tissue necrosis [21].

The colon was photographed by a Canon camera (Powershot G9, 12 megapixel, Japan) and the ulcer area (%) was measured using Fiji-win 32 software (NIH Image for the Macintosh) [22]. The colon was then sectioned into 3 portions, frozen in liquid nitrogen and stored at −70 °C for further examinations (ELISA analysis, MPO assay, and histopathological study).

2.5. Measurement of proinflammatory cytokines

The concentrations of IL-1β, IL-6 and TNF-α in the colon of the rats with ulcerative colitis were measured using ELISA kits in accordance with the manufacturer's instructions (Boster Co., Pleasanton, CA, USA). ELISA was performed in triplicate.

2.6. Histopathological evaluation

Colon samples were fixed in formalin solution (10%). Then, tissues were sectioned, deparaffinized and stained with hematoxylin and eosin (H&E). Histopathological evaluation of colon was done as described previously [23] and by a pathologist, who was blinded to experimental groups. Total colitis index was the summation of inflammation extent, inflammation severity and crypt damage scores.

2.7. Myeloperoxidase (MPO) activity assay

One unit of MPO activity is defined as that required to degrade 1 μM of hydrogen peroxide at 25 °C. MPO activity was measured according to previous method with some changes [24]. Briefly, colon sample was homogenized in sodium phosphate buffer (50 mM) containing HTAB (0.5%) and EDTA (5 mM, pH 7.4) in an ice-water bath using a polytron homogenizer. More buffer was added to the homogenate to reach the concentration of 50 mg tissue per milliliter. Then, the homogenate was sonicated in an ice bath for 10 s, and freeze–thawed three times. Then homogenate was sonicated again for 10 s. After centrifugation at 10,000 × *g* for 15 min at 4 °C, a 100 μL of the resultant pellet was mixed with 2.9 ml of 50 mM phosphate buffer (pH = 6) containing 0.167 mg/ml O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The activity of MPO was measured using a UV/Vis spectrophotometer (LSI Model Alfa-1502) at 460 nm. MPO assay were performed in duplicate for each sample.

2.8. Statistical analysis

Data are presented as mean ± S.D. for parametric data and median (range) for non-parametric data. Non-parametric statistical analysis was done using Kruskal–Wallis test followed by Dunn's multiple-comparison or Mann–Whitney U test while, parametric data analysis was performed by one-way ANOVA followed by Tukey test. A $P < 0.05$ was considered statistically significant. Data were analyzed using GraphPad software (Prism ver. 6.01, San Diego, CA).

3. Results

3.1. The effect of theophylline on body weight loss

Body weight was significantly reduced after acetic acid instillation. This reduction was more severe in the colitis control group than in the theophylline (20 and 50 mg) and dexamethasone groups (at least $P < 0.05$) (Fig. 1).

3.2. The effect of theophylline on macroscopic changes

Intra-rectal administration of acetic acid induced severe colitis with extensive epithelial necrosis (Fig. 2B). Intraperitoneal administration of theophylline (20 and 50 mg/kg) produced a significant reduction in macroscopic colon damage score in comparison with colitis control group ($P < 0.05$) (Fig. 2C and D and Table 1).

The colitis control group presented significantly more extensive ulcer area and colon weight compared to Sham group ($P < 0.001$)