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Celastrol ameliorates murine colitis via modulating oxidative stress, inflammatory cytokines and intestinal homeostasis

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ABSTRACT

Therapeutic agents that block the nuclear factor-kappa B (NF-κB) pathway might be beneficial for incurable inflammatory diseases, such as ulcerative colitis. Here, we investigated the effect of the novel NF-κB inhibitor celastrol on murine colitis. Colitis was induced in male mice by administration of 5% (w/v) dextran sulfate sodium (DSS) in drinking water for a period of 5 days, followed by a 2 day recovery period. Celastrol (2 mg/kg, oral) was administered daily over the 1 week of the study. Our results indicated that treatment with celastrol attenuated DSS-induced colon shortening and neutrophil infiltration. Besides, celastrol ameliorated DSS-induced colon injury and inflammatory signs as visualized by histopathology. The mechanisms behind these beneficial effects of celastrol were also elucidated. These include (i) counteracting DSS-induced oxidative stress in the colon via decreasing lipid peroxidation products (malondialdehyde and 4-hydroxynonenal) and increasing the antioxidant levels (reduced glutathione, glutathione-S-transferase and superoxide dismutase); (ii) inhibiting DSS-induced activation of the NLRP3-inflammasome, as evidenced by decreased production of IL-1β and IFN-γ as indirect measure of IL-18 in the colon; (iii) targeting DSS-induced activation of the IL-23/IL-17 pathway by abating the elevation of IL-23 and IL-17A levels in the colon; (iv) augmenting the anti-inflammatory defense mechanisms via increasing IL-10 and TNF-α levels in the colon; (v) and more importantly, maintaining intestinal epithelial reconstitution and homeostasis via attenuating the overexpression of CD98 in colonic epithelial cells. In conclusion, our study provides novel insights into the beneficial effects of celastrol as a promising candidate for the treatment of ulcerative colitis in humans.

1. Introduction

Ulcerative colitis is one of the inflammatory bowel diseases affecting the distal colon and rectum that is generally recognized as a dysregulated immune response to the intestinal microbiota [1,2]. Of the various signaling pathways involved in the colonic inflammation, the Toll-like receptor 4 (TLR4) pathway is considered the most important. TLR4 recognizes lipopolysaccharide (LPS), which is present in the cell wall of gram-negative bacteria. Interaction of LPS with TLR4 triggers signaling cascades that lead to the activation of the transcription factor nuclear factor-kappa B (NF-κB) and the production of proinflammatory cytokines and proteins, such as IL-1β, IL-6, IL-8, IL-12, TNF-α and IFN-γ [3]. Therefore, understanding the molecular mechanisms involved in this pathway is an important step in countering the damaging effects of these proinflammatory mediators in ulcerative colitis.

Unfortunately, current treatments for ulcerative colitis, like corticosteroids, sulfasalazine, classical immunosuppressives and antibiotics, focus only on the suppression of inflammation, and their use is associated with numerous and serious side effects. Emerging therapies that block the TNF-α pathway, such as infliximab and adalimumab, might be effective in the treatment of ulcerative colitis [4]. However, a large proportion of patients failed to respond to the therapy, and others were subjected to anaphylactic reactions and infections [5]. Hence, the use of active compounds from medicinal plants represents an attractive approach for ulcerative colitis treatment. Celastrol, a triterpene extracted from the root bark of the Chinese medicinal plant Tripterygium wilfordii (also known as Thunder of God Vine), has recently gained much interest as a natural remedy for inflammation, cancer and autoimmune diseases [6,7]. Molecular studies have identified several targets for celastrol which are mostly dependent on the inhibition of NF-κB signaling [8,9].

In the present study, we hypothesized that the natural NF-κB inhibitor celastrol might be a promising candidate for the treatment of ulcerative colitis in humans. To evaluate this idea,

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the effect of celastrol was investigated on the dextran sulfate sodium (DSS)-induced colitis model in mice. This model has the advantage of mimicking human inflammatory bowel disease and is considered the most suitable for investigating potential therapeutics [10]. Here, we report for the first time that the natural triterpene celastrol ameliorates DSS-induced colitis in mice via modulating colonic oxidative stress, inflammatory cytokines and intestinal epithelial homeostasis. Thus, our study provides novel insights regarding the potential usefulness of celastrol in the treatment of ulcerative colitis in humans.

2. Materials and methods

2.1. Drugs and chemicals

Celastrol (purity 98%) was purchased from Cayman (Ann Arbor, MI, USA). DSS (molecular weight 40 kDa) was purchased from TdB Consultancy (Uppsala, Sweden). 1-Methyl-2-phenylindole, 1,1,3,3-tetramethoxypropane, 5,5′-dithiobis(2-nitrobenzoic acid), L-glutathione reduced and vanadium III chloride (VCl_3) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanesulfonic acid was purchased from Merck (Darmstadt, Germany). 3,3′,5,5′-Tetramethylbenzidine and hexadecyltrimethylammonium bromide were purchased from MP Biomedicals (Irvine, CA, USA). 1-Chloro-2,4-dinitrobenzene (CDNB) and AEBSF was purchased from Acros Organics (Morris Plains, NJ, USA). Pyrogallol was purchased from Fluka (Buchs SG, Switzerland).

2.2. Animals

Adult male BALB/c mice (28–32 g) were purchased from Nile Pharmaceuticals (Cairo, Egypt). The animals were allowed access to food and tap water *ad libitum* throughout the acclimatization and experimental periods. All animals received humane care in compliance with the National Institutes of Health and the Research Ethics Committee Criteria for Care of Laboratory Animals at Mansoura University.

2.3. Induction of colitis by dextran sulfate sodium (DSS) and treatment protocol

Acute colitis in mice was induced by adding DSS to the drinking water at a concentration of 5% (w/v) for a period of 5 days. Thereafter, the DSS solution was replaced with drinking water for additional 2 days to allow some colonic epithelial recovery. From the first day of adding DSS to the drinking water, celastrol treatment was administered to mice daily over the 1 week. Celastrol was freshly prepared by predissolving 2 mg in 0.05 ml dimethylsulfoxide due its limited aqueous solubility, followed by dilution with normal saline to 10 ml.

2.4. Experimental design

The mice were divided into four groups as follows: (1) Control: allowed to drink tap water and received only the vehicle (0.3 ml/30 g/day, oral); (2) DSS: allowed to drink tap water containing 5% (w/v) of DSS over 5 days and received only the vehicle (0.3 ml/30 g/day, oral) over the 1 week; (3) DSS + Celastrol (2 mg/kg): allowed to drink tap water containing 5% (w/v) of DSS over 5 days and received celastrol at a dose of 2 mg/kg/day (0.3 ml/30 g, oral) over the 1 week; (4) Celastrol (2 mg/kg): allowed to drink tap water and received celastrol at a dose of 2 mg/kg/day (0.3 ml/30 g, oral) over the 1 week.

At the end of the week, blood samples were withdrawn from thiopental-anesthetized animals *via* cardiac puncture and centrifuged at 2000*g* for 10 min at 4 °C for serum preparation, followed by storage at −80 °C. After measuring the length with digital caliber, and several washings with normal saline, portions of colon tissues were stored at −80 °C for the enzyme-linked immunosorbent assay (ELISA) and oxidative stress/antioxidant assays. Portions of colons were also fixed in 10% (v/v) neutral-buffered formalin solutions for 24 h for histopathological and immunohistochemical analysis.

2.5. Colonic histopathological and immunohistochemical evaluations

Standard histopathological techniques were followed for processing portions of distal colon, preparation of paraffin blocks and staining of the slides with hematoxylin–eosin to evaluate DSS-induced colitis. Histological scoring was based on 3 parameters as previously described [11]. Severity of inflammation was scored as follows: 0, rare inflammatory cells in the lamina propria; 1, increased numbers of granulocytes in the lamina propria; 2, confluence of inflammatory cells extending into the submucosa; 3, transmural extension of the inflammatory infiltrate. Crypt damage was scored as follows: 0, intact crypts; 1, loss of the basal one-third; 2, loss of the basal two-thirds; 3, entire crypt loss; 4, change of epithelial surface with erosion; 5, confluent erosion. Ulceration was scored as follows: 0, absence of ulcer; 1, 1 or 2 foci of ulcerations; 2, 3 or 4 foci of ulcerations; 3, confluent or extensive ulceration. Values were added to give a maximal histological score of 11. For immunohistochemical analysis, anti-CD68 and anti-CD98 (Santa Cruz, CA, USA) were used as primary antibodies to detect their targeted proteins using standard immunohistochemical methods.

2.6. Enzyme-linked immunosorbent assay (ELISA) for cytokines

Portions of colons were homogenized (10% w/v) in ice-cold lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 0.5% Triton X-100, 2 mM AEBSF). The lysates were centrifuged at 8000*g* for 10 min at 4 °C, and the supernatants were transferred for ELISA. Mouse IL-1β, IFN-γ, IL-17A, IL-23(p19/40), IL-10 and TNF-α concentrations were determined using the ELISA MAX™ Deluxe set (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. The protein content was assayed by the Bradford method [12]. IL-17A and TNF-α concentrations were also determined in serum.

2.7. Measurement of colonic myeloperoxidase (MPO) activity

The extent of neutrophil accumulation in the colon was measured by assaying myeloperoxidase (MPO) activity as previously described [13] with a slight modification. After processing the supernatant of colon homogenate, a part of the corresponding pellet (50 mg) was weighed, homogenized in 1 ml of the buffer (0.1 M NaCl, 0.02 M NaH_2PO_4, 0.015 M EDTA, pH 4.7) and centrifuged at 6000*g* for 20 min at 4 °C. The pellets were then resuspended in 0.5 ml of 0.05 M sodium phosphate buffer (pH 5.4) containing 0.5% (w/v) hexadecyltrimethylammonium bromide. The suspensions were freeze–thawed three times, heated for 2 h at 60 °C to increase myeloperoxidase recovery, and finally centrifuged at 6000*g* for 20 min at 4 °C to separate the supernatants for MPO assay. The reaction was started by mixing 0.2 ml of 1.6 mM 3,3′,5,5′-tetramethylbenzidine in dimethylsulfoxide with 0.8 ml of 0.05 M sodium phosphate buffer (pH 5.4) containing 0.006% (v/v) H_2O_2 and 0.2 ml of the 6000*g* supernatant from the colon tissue sample. MPO activity was assayed by measuring the change in optical density (OD) for 5 min at 650 nm. Results were expressed as change in OD per g of wet tissue.