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Effect of Astragalus polysaccharides on expression of TNF-α, IL-1β and NFATc4 in a rat model of experimental colitis

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ABSTRACT

Aim: Astragalus membranaceus is a Chinese medicinal herb and has been shown to improve hapten-induced experimental colitis. One of its major components is polysaccharides. We investigated the effect of Astragalus polysaccharides (APS) on expression of TNF-α, IL-1β and NFATc4 in a rat model of experimental colitis.

Methods: The experimental colitis model was induced by TNBS. Forty five rats were divided into five groups ($n = 9$): Normal control group, receiving ethanol vehicle with no TNBS during induction and IP saline injection during treatment; TNBS colitis model group (TNBS + IP saline), receiving only IP saline vehicle treatment; APS low dose group (TNBS + L-APS), receiving APS 100 mg/kg; APS high dose group (TNBS + H-APS), receiving APS 200 mg/kg; and positive control group (TNBS + Dexm), receiving dexamethasone 0.3 mg/kg. The clinical features, macroscopic and microscopic scores were assessed. The expressions of TNF-α, IL-1β and NFATc4 were measured by real-time PCR and ELISA assays.

Results: Compared to normal control rats, TNBS + IP saline had significant weight loss, increased macroscopic and microscopic scores, higher disease activity index (DAI) up-regulation of TNF-α, IL-1β and NFATc4 mRNA expression and up-regulation of TNF-α and IL-1β protein expression. Compared to TNBS + IP saline, treatment with APS or dexamethasone significantly reduced DAI, partially but significantly prevented TNBS colitis-induced weight loss and improved both macroscopic and microscopic scores; high dose APS or dexamethasone significantly down-regulated TNF-α and IL-1β expressions (both mRNA and protein) and up-regulated NFATc4 mRNA and protein expression. The effect of high dose APS and dexamethasone is comparable.

Conclusions: APS significantly improved experimental TNBS-induced colitis in rats through regulation of TNF-α, IL-1β and NFATc4 expression.

1. Introduction

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a chronic idiopathic inflammatory disorder that affects the gastrointestinal tract of children and adults. The precise etiology of IBD remains unclear. The current hypothesis states that complex interactions among various factors, including genetic factors, the host immune system and environmental factors, cause disruption of intestinal homeostasis, leading to dysregulated inflammatory responses of the gut [1–3]. Basic and translational research [4,5] has led to a better understanding of the role of inflammatory mediators including cytokines in the gut of patients with IBD. Cytokines carry signals between immune, epithelial and mesenchymal cells and play a pivotal role in the development of IBD. The inflammatory cytokines are the most logical targets for IBD treatment. Among various cytokines, tumor necrosis factor-α (TNF-α) is the cytokine that has been widely studied. Currently, several TNF-α blockers (infliximab, adalimumab and certolizumab) have been approved for IBD therapy in clinical practice [6]. The choice of treatment depends on severity, localization and the course of the disease [7]. Although these available agents have shown clinical benefits to some degree, they are not entirely effective and have multiple adverse effects.

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Furthermore, IBD management requires long-term treatment that often leads to drug refractoriness or intolerance [8]. Therefore, it is necessary to develop novel therapeutic approaches.

Various natural products have been shown to safely suppress the pro-inflammatory pathway and control IBD. In vivo and/or in vitro studies suggest [9–11] that the anti-IBD effects exhibited by natural products are mainly caused by their ability to modulate cytokine production such as TNFα, IL-1β, IL-6 and IL-17. Traditional Chinese medicine (TCM) has received great interest in recent years. *Astragalus membranaceus* is a Chinese herb and has been used in China for more than 2,000 years to strengthen human immunity. Ko et al. [12] demonstrated that root extract of *Astragalus membranaceus* administered orally and locally can protect rats against hapten-induced colitis through attenuation of TNF-α and IL-1β and up-regulation of IL-10. However, it is still unknown what active component is mainly responsible for the effect of *Astragalus membranaceus* on experimental colitis.

Astragalus membranaceus contains different active components, including polysaccharides, flavonoids, astragalosides I–VII (saponins), amino acids and trace elements [12–14]. Our previous studies have demonstrated that Astragalus polysaccharides (APS) has a potent effect on erythroid lineage differentiation and increases expression of NFATc4 mRNA by gene expression profile analysis in K562 cells [15]. NFATc4 is a key modulator of intestinal cell proliferation and differentiation. It is one of the crucial transcription factors that tightly control pro-inflammatory cytokine expression for adaptive immunity in T and B lymphocytes [16]. We hypothesize that APS is the main active component of *Astragalus membranaceus* that would improve experimental colitis. We therefore investigated the effect of APS on expression of TNF-α, IL-1β and NFATc4 in a rat model of experimental colitis.

2. Methods

2.1. Animals

45 pathogen- free male Wistar rats, weighting 180–220 g, were obtained from the animal facility of Nanfang Medical University (Guangzhou, China). All animals were allowed free access to tap water and standard chow diet. They were adapted to laboratory conditions for 7 days, with 12 h day/night cycles at 23 °C and 60% humidity. All animal protocols were reviewed and approved by Guangzhou Women and Children's Medical Center Institutional Review Committee on Laboratory Animal Care and all animals were handled in accordance with institutional guidelines.

2.2. Animal model of colitis

Colitis was induced in the rats by rectal administration of Trinitrobenzene sulfonic acid (TNBS) (Sigma) into the colons in a dose of 150 mg/kg, dissolved in 50% solution of ethanol as described by others [17]. Briefly, the animals were anaesthetized with 10% Chloral hydrate (300 mg/kg), and 3 ml/kg of TNBS – ethanol solution (50 mg/ml) was administered into the colon at the depth of 8 cm from the rectum with the use of a soft polyethylene catheter. The rats were positioned in the Trendelenburg position for one minute in order to avoid loss of TNBS solution *via* the rectum. Normal control animals received rectal administration of 50% ethanol solution at 3 ml/kg without TNBS during induction.

2.3. Experimental protocol and sample preparation

72 h after induction of colitis, animals were treated daily by intraperitoneal injection (IP) for 7 days. Animals in the normal control group (Normal control) received ethanol vehicle with no TNBS during induction and received IP saline injection during treatment. TNBS-induced colitis animals were randomly divided into 4 treatment groups: TNBS colitis control group, receiving only saline vehicle treatment (TNBS + IP saline); APS low dose treatment group, receiving APS 100 mg/kg (TNBS + L-APS); APS high dose treatment group, receiving APS 200 mg/kg (TNBS + H-APS); and dexamethasone treatment group, receiving dexamethasone 0.3 mg/kg (TNBS + Dexm).

Animals were then allowed to recover and observed daily throughout the duration of the study. Clinical symptoms, including the amount of food consumed, consistency and frequency of stools and the change of body weight were monitored until tissue harvest.

24 h after the last treatment, all animals were sacrificed after being deeply anesthetized with ether. The distal 8 cm of colon from rectum was removed, dissected along the longitudinal mesentery, rinsed with isotonic saline and assessed for extent of colonic mucosa injuries. Multiple tissue specimens about 0.3 cm × 0.5 cm were prepared for microscopic examination, real-time PCR and ELISA assays.

2.4. Disease activity index

Disease activity index (DAI) was based on weight loss, stool consistency and blood in stools. Briefly, score was assigned for each item to calculate DAI as follows: (i) Percentage of weight loss: 0, none; 1, 1–5%; 2, 6–10%; 3, 11–15%; 4, >15%. (ii) Stool consistency: 0, normal; 2, loose stool; 4, diarrhea. (iii) Blood in stools: 0, hemoccult (−); 1, hemoccult (±); 2, hemoccult (+); 3, hemoccult (++); 4, gross bleeding. All animals were monitored daily for the duration of the study to assess DAI changes in response to treatment.

2.5. Macroscopic and microscopic assessment

The removed colon was excised free of adherent adipose tissue, rinsed with ice-cold saline and dissected longitudinally. It was examined visually immediately and damage was scored on a scale of 0–5 by the pathologists who were blinded to the group, as previously described by others [18]. Briefly, scoring of macroscopic colon damage was as follows: 0, no colonic damage; 1, hyperaemia and no ulcer; 2, linear ulcer and no colonic wall thickening; 3, linear ulcer and colonic wall thickening in one area; 4, colonic ulcer at multiple areas; and 5, major ulcer and perforation.

The samples of colonic tissue were routinely fixed and stained for microscopic examination. Four samples for each group were selected randomly and their paraffin blocks were prepared. Four paraffin blocks were investigated. The colonic pathological changes were observed and evaluated by two trained independent researchers using a modified histopathological score formula [19]: (i) infiltration of acute inflammatory cells: 0 none, 1 mild, 2 severe; (ii) infiltration of chronic inflammatory cells: 0 none, 1 mild, 2 severe; (iii) fibrin deposition: 0 negative, 1 positive; (iv) submucosal oedema: 0 none, 1 focal, 2 diffuse; (v) necrosis of epithelial cells: 0 none, 1 focal, 2 diffuse; and (vi) mucosal ulcer: 0 negative, 1 positive.

2.6. Real-time PCR analysis

Total RNA was isolated from colonic tissue using the TRIzol reagent. Reverse transcription of the isolated RNA was performed in a solution containing 4 μg of total RNA, 1 μg of Oligo (dT), 10 μL of 5× buffer, 2.5 μL of 10 mM dNTPs, and 400 U of MMLV reverse transcriptase. The final reaction volume was 50 μL. The reverse transcription was performed at 42 °C for 1 h. Real-time PCR was conducted according to the manual of SYBR ExScript™ RTPCR kit using a Stratagene Mx3000P QPCR system. The primers