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Changtai granule, a traditional Chinese drug, protects hapten-induced colitis by attenuating inflammatory and immune dysfunctions

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Abstract

The study was aimed to investigate the effects and mechanism of action of Changtai granule (CT), a traditional compound Chinese medicinal formula, in rodent 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis. Rats with TNBS/ethanol-induced colitis were used. The colonic wet weight, myeloperoxidase (MPO) activity, macroscopic and histological colon injury was observed. Inflammation cytokines were determined by ELISA methods and semi-quantitative RT-PCR. When dosed orally once daily, CT markedly attenuated TNBS-induced colitis. CT significantly attenuated colonic wet weight, macroscopic and histological colon injury. CT decreased mucosal mRNA levels for several inflammatory mediators: inducible nitric oxide synthase, cyclooxygenase 2, and macrophage inflammatory protein 2. CT also decreased mucosal mRNA and protein levels of T effectors cytokines: tumor necrosis factor-alpha (TNF-alpha), interleukin-2 (IL-2) and interferon-gamma (IFN-gamma). Systemic levels of these cytokines were also dramatically attenuated. CD3/CD28-mediated costimulation of T helper 1 effector cytokines release in lamina propria mononuclear cells (LPMC) was markedly inhibited by CT ex vivo and in vitro. Also CT prevented cytokines production by nuclear factor-kappaB (NF-kappaB). The potential anti-inflammatory and immunomodulatory effect of CT in TNBS colitis suggests that CT may be an effective treatment approach for inflammatory bowel disease.

Keywords: Inflammatory bowel disease; Colitis; Nuclear factor-kappaB

1. Introduction

Crohn's disease (CD), together with ulcerative colitis as the primary constituent of inflammatory bowel disease (IBD), is characterized by chronic intestinal inflammation frequently relapsing with clinical manifestations including diarrhea, blood in the stool, abdominal pain, and weight loss (Hanauer, 2006). The exact pathogenesis of IBD is poorly understood. Infection, environmental factors, heredity, and immunological abnormalities have been proposed as causes (Podolsky, 2002). Several models of experimental colitis have been developed to investigate the molecular and cellular mechanisms of inflammation and immunological disorders (Feller et al., 1996). Currently, hapten-induced colitis, in which trinitrobenzene sulfonic acid (TNBS) shares important similarities with human Crohn's disease such as transmural inflammation, lymphocyte infiltration, Th_1-dominated cytokine profile, and stricture formation. Thus, this model is very suitable to study anti-inflammatory agents during the course of developing and resolving inflammation (Strober et al., 1998).

Most of the current therapies for IBD involve treatment with glucocorticosteroids and 5-aminosalicylic acid; however, they display limited beneficial action. Immunosuppressive drugs have also been used to control severe illness, regardless of the more serious complications and toxic side effects associated

Abbreviations: CD, Crohn's disease; IBD, inflammatory bowel disease; TNBS, 2,4,6-trinitrobenzene sulfonic acid; i.g., intragastric administration; IL-1, interleukin-1; MIP-2, macrophage inflammatory protein-2; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; TNF-alpha, tumor necrosis factor-alpha; LPMC, lamina propria mononuclear cells; TCM, traditional Chinese medicinal; MPO, myeloperoxidase; mRNA, messenger RNA; RT-PCR, reverse transcriptase polymerase chain reaction; NF-kappaB, nuclear factor-kappaB; IFN-gamma, interferon gamma.

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with them (Van Dieren et al., 2006). Although many other types of treatment for IBD have been proposed and used clinically, additional therapeutic approaches are needed because many patients either fail to respond to the currently available options or demonstrate significant side effects, thereby precluding their continued use. Remedy with traditional Chinese medicinal (TCM) formulae, on the other hand, basically safe, sustainable and practical has been implicated in the therapy of IBD. But the action mechanism of these TCM is unclear and so limits the application and development of these TCM (Kawashima et al., 2004).

Changtai granule (CT), an oral Chinese medicine compound, derived from traditional Chinese empirical formula comprised of *Phellodendro Chinense* Schneid., *Sanguisorba officinalis* L., *Euphorbia humifusa* Willd. and *Polygonum hydropiper* Linn., has long been used clinically in the treatment of IBD. Our previous study demonstrated that CT possesses a variety of pharmacological effects including analgesic-antipyretic, anti-inflammatory, antibacterial and anti-diarrhea actions, as well as the effect of adjusting gastrointestinal function (Cao et al., 2004a,b, 2005; Zhang et al., 2005). However, the exact molecule mechanism of CT granules for the treatment of IBD is unclear. This article describes the oral efficacy profile and action mechanism of CT in rodent TNBS colitis. Results of this study might provide an insight into the mechanism of CT in TNBS-induced colitis and the possibility for its application for the treatment of clinic IBD.

2. Materials and methods

2.1. Experimental animals

Specific pathogen free male Sprague–Dawley (225–275 g) rats were obtained from the Experimental Animal Department of Fu Dan University (Shanghai, China). The rats were fed with a standard laboratory diet and given free access to tap water, kept in a controlled room temperature ($22 \pm 1\,^{\circ}C$), humidity (65–70%), and a 12:12-h light:dark cycle. Rats were deprived of food for 24 h prior to the induced colitis, but were allowed for free access to tap water throughout. They were randomly assigned to four groups of 12 animals each: control group, TNBS group, TNBS plus Changtai granule group (TNBS + CT), TNBS plus dexamethasone group (TNBS + DT). All rats received humane care in compliance with the institutional animal care guidelines approved by the Experimental Animal Ethical Committee of Second Military Medical University.

2.2. Drugs and reagents

Changtai granule (CT) was purchased from Shanghai Changhai Pharmaceutical Co. Ltd. (No. 20040822, China). Changtai granule consists of four herbal components: Cortex Phellodendri (*Phellodendro Chinense* Schneid. in the family Rutaceae), Radix Sanguisorbae (*Sanguisorba officinalis* L. in the family Rosaceae), Herba Euphorbiae Humifusae (*Euphorbia humifusa* Willd. in the family Euphorbiaceae) and Herba Polygoni hydropiperis (*Polygonum hydropiper* Linn. in the family Polygonaceae) with the ratio of 4:3:3:3. Dexamethasone (DT) was from Hangzhou Sanofi-Synthelabo Minsheng Pharmaceutical Co. Ltd. (No. H32011541, China). TNBS was from Sigma (No. 095K5008, USA). The ELISA kit for cytokines and TransFactor extraction kit were from Jingmei Biotech Co. Ltd. (Shengzhen, China), RPMI 1640 was from GIBCO BRL, and new bovine serum was from Hangzhou Sijiqing Co. Ltd. (Hangzhou, China).

2.3. High performance liquid chromatography (HPLC) profiling

Changtai granule (1.0 g) was extracted with 70% ethanol (20 mL) under ultrasonication for 30 min. The solution was filtered and then submitted for HPLC analysis.

HPLC analysis was carried out using a Waters Alliance 2690 HPLC system equipped with diode array detector and automatic sample injector. The chromatographic conditions were: ZORBAX SB-C18 column (4.6 mm × 250 mm, 5 μm, America); sample injection volume, 20 μl; the temperature of column oven, 40 °C; flow rate, 1.0 ml/min; mobile phases, the acetonitrile–water with linear gradient elution (0–50 min, 5–95% acetonitrile, volume fraction); detection wavelength, 270 nm; reference standard, bererine hydrochloride was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

2.4. Induction of colitis

Colitis was induced in rats according to the model and method described by Morris et al. (Morris et al., 1989). Briefly, rats were lightly anesthetized with ether following 24-h fast, and then a medical-grade polyurethane cannula for enteral feeding (external diameter 2 mm) was inserted into the anus and the tip was advanced to 8 cm proximal to the anal verge. TNBS (Sigma) dissolved in 50% ethanol was instilled into the colon through the cannula (at a dose about 100 mg/kg). Following the instillation of the hapten, the animals were maintained in a head-down position for a few minutes to prevent leakage of the intracolonic instillation. The rats were checked daily for behavior, body weight, and stool consistency. Vehicle (pyrogen-free water), CT (11.4 g crude drug/kg, intragastric administration (i.g.) and dexamethasone (DT, 0.2 mg/kg, i.g.), were administered by oral gavage once daily (final volume, 1 ml) respectively.

2.5. Assessment of colitis

At day 7 of TNBS-induced colitis, all rats were decapitated, blood samples were collected by cardiac puncture, and colons were excised. The distal 8 cm of the colon was excised, opened longitudinally, and rinsed with saline solution. Then, the distal colon was weighed and the mucosal lesions were scored macroscopically using the criteria outlined in the previous report (Wallace et al., 1989). Samples were taken for histological assessment of the lesions after hematoxylin and eosin stain. His-