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Active components alignment of *Gegenqinlian* decoction protects ulcerative colitis by attenuating inflammatory and oxidative stress

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ABSTRACT

Ethnopharmacological relevance: Gegenqinlian Decoction (GQD) has been used as a folk remedy for gastrointestinal diseases in China over thousands of years. It has significant treatment efficacy for patients with inflammatory bowel disease (IBD). We analyzed and showed that the active components alignment of *Gegenqinlian* Decoction (ACAG) possesses broad pharmacological effects including analgesic, antipyretic, anti-inflammatory, antibacterial, antiviral and antidiarrhea, as well as the effect of adjusting gastrointestinal function in our preliminary experiments. However, the exact molecular mechanisms on how ACAG exerts these pharmacological effects still remain elusive. In the present study, the plausible pharmacological effects of ACAG on 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis were investigated.

Materials and methods: Male Sprague-Dawley (SD) rats with TNBS/ethanol-induced colitis were used. The colonic wet weight, macroscopic and histological colon injury, superoxide dismutase (SOD), malonyldialdehyde (MDA), and inducible nitric oxide synthase (iNOS) activity were observed. Pro-inflammation cytokines were determined by ELISA methods, semi-quantitative RT-PCR and Immuno-histochemistry.

Results: We showed administration of ACAG was able to improve colitis. This was manifested by a decreased in the score of macroscopic and histological colonic injury, by lowered colonic wet weight, accompanied by significant increased of SOD activity, and decreased of MDA and iNOS activities. The treatment also significantly reduced tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) levels in colon and serum as well as the colonic mRNA levels for several inflammatory cytokines such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), macrophage inflammatory protein-2 (MIP-2), intercellular adhesion molecule-1 (ICAM-1) and toll-like receptor 2, 4 (TLR2, TLR4). In addition, we also showed that ACAG was able to inhibit the activation and translocation of transcription factors, nuclear factor kappaBp65 (NF-κBp65) in colon.

Conclusions: Our results suggest that ACAG exhibits protective effect in TNBS-induced ulcerative colitis. We postulate that this might be due to its modulation of oxidant/anti-oxidant balance, downregulation of productions, expressions of pro-inflammatory cytokines and inhibition of NF-κBp65 signal transduction pathways.

1. Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD), primarily affecting the colon and rectum that features a recurrent, debilitating chronic with relapsing condition in the intestine (Xavier and Podolsky, 2007; Sakthivel and Guruvayoorappan, 2013). The clinical manifestations include diarrhea, blood in the stool, abdominal pain, and weight loss (Liu et al., 2009). Molecular mechanisms underlying the pathogenesis of IBD are currently not fully understood, but it has been reported that immune dysfunction plays a decisive role in the development of UC. There are convincing evidences indicating that imbalances between proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1beta (IL-1β), IL-6 and IL-12, and anti-inflammatory cytokines, such as IL-4, IL-10, IL-11 and expression of inflammatory proteins which include cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) collectively to play a central role in modulating pathologic inflammation (Sakthivel and Guruvayoorappan, 2013; Sklyarov et al., 2011).

Currently available treatments for IBD are only effective for temporary symptomatic relief while having many concomitant disadvantages. Furthermore, immunosuppressant and anti-inflammatory drugs have many undesirable side effects including

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diarrhea, cramps, abdominal pain accompanied by fever and high blood pressure. Besides, antibiotics administration could adversely change the environmental conditions of microflora (Sakthivel and Guruvayoorappan, 2013; Marwa et al., 2013). All these adverse events have limited their use particularly for long-term therapy.

According to the diversified etiology and clinical manifestations of IBD, it is possible that a combination of more than one type of drugs with complement pharmacological modes is more effective to treat this disease. Remedy with traditional Chinese medicinal (TCM) formulae, combination therapy, is a unique ancient Chinese medical science in treating various diseases (Wang et al., 2008). Although more effective, the underlying mechanisms of these TCM is unclear and so limits their broad clinical application and development (Liu et al., 2008).

Gegenqinlian Decoction (GQD) is a classic prescription which has been used to treat chronic diarrhea and lingering dysentery and has shown significant clinical efficacy (Yu et al., 2005). Active components alignment of *Gegenqinlian* Decoction (ACAG), an oral Chinese medicine compound, is derived from this traditional Chinese empirical formula. Our previous study had confirmed that ACAG possesses a variety of pharmacological effects such as analgesic, antipyretic, anti-inflammatory, antibacterial, antiviral and antidiarrhea, as well as the effect of adjusting gastrointestinal function (Xu et al., 2011; Xu et al., 2013). Although ACAG is an effective formula for these effects, the possible mechanism in the treatment of UC remains unknown. We hope findings in current study provide an insight in filling the gap to understand pharmacological mechanisms of ACAG in treating TNBS-induced colitis and the possibility for its application in the treatment of clinic UC.

2. Materials and methods

2.1. Experimental animals

Specific pathogen free male Sprague-Dawley (SD, 200 ± 20 g) rats were obtained from Weitonglihua Laboratory Animal Services Center (Changping, Beijing, China). All animals were fed with standard diet pellets and given free access to tap water, and kept in a controlled room temperature (22 ± 1 °C) and humidity (65–70%). Experiments performed here were based on the Institutional guidelines and approved by Beijing University of Chinese Medicine. Rats were randomly divided into four groups (eight or ten animals per group): Control (Normal) group, TNBS group, TNBS plus ACAG group (TNBS+ACAG), TNBS plus Sulfasalazine group (TNBS+SASP). All rats were deprived of food for 24 h prior to the induced colitis, but were allowed for free access to tap water throughout.

2.2. Drugs and reagents

ACAG consists of components: puerarin, baicalin, berberine, glycyrrhizic acid and palmatine (Baoji, Runyu biological Co., Ltd), daidzin (Tianjin, Zhongxin Pharmacal Co., Ltd), liquiritin (Dalian, Fusheng Pharmacal Co., Ltd), jatrorrhizine (self-made). TNBS was purcured from Sigma Chemical Co. (USA). SOD, MDA, and iNOS kits were obtained from Jiancheng Bioengineering Institute (Nanjing). TNF-α, IL-1β ELISA kits and NF-κBp65 antibody were purchased from Senxiong Biotech Co., Ltd (Shanghai). RNA kits and primers were purchased from SUNBIO Co., Ltd (Beijing).

2.3. ACAG combination preparation

GQD consists of four Chinese herbs. The names and the dosage of each herb in GQD are listed in Table S1. According to main pharmacologic content of *Gegenqinlian* prescription, puerarin, daidzin, baicalin, berberine, palmatine, jatrorrhizine, glycyrrhizic acid and liquiritin were considered as the major components of the formula. The content of these components in GQD were determined by HPLC, and chromatographic conditions and HPLC chromatograms of components in GQD are also shown in Table S1. For example, components labeled "superscript a" (puerarin and daidzin) were detected by HPLC using "chromatographic condition a". Correspondingly, "chromatogram a" was the HPLC chromatogram of these components (puerarin and daidzin) in GQD. The ratio of these components was always puerarin(52): daidzin(4): baicalin(11): berberine(3): palmatine(1): jatrorrhizine(2): glycyrrhizic acid(4): liquiritin(5) in each of the *Gegenqinlian* formulations, namely ACAG combination.

In the present study, the determination of ACAG and its main active ingredients was performed by HPLC and FT-IR as the following method. HPLC analyses were performed on Agilent 1100 equipped with DAD detector. An analytical column, Agela Venusil MP C18 Column (250 mm × 4.6 mm, 5 μm) was used for chromatographic separation. The mobile phase was a mixture of acetonitrile-water containing 0.1% (v/v) formic acid employing gradient elution (from 20:80 to 80:20, v/v) at a flow rate of 0.8 mL/min. The injection volume was 10 μL. Detection was set at 250nm, 280nm and 346nm at 25 °C. The HPLC chromatogram of the main constituents in ACAG preparation is shown in Fig. 1A. Spectrum GX FT-IR system (Perkin Elmer) equipped with a DTGS detector was employed for IR spectrum. The ACAG powder was blended with KBr power and then was ground again and pressed into a tablet. The IR spectrum of ACAG was obtained from the accumulation of a total of 32 scans in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. The IR spectrum of ACAG combination is shown in Fig.1B.

2.4. Induction of experimental colitis and treatment

The experimental colitis was induced in SD rats according to the well-established inflammatory bowel disease model as described previously (Zhang et al., 2011). Briefly, the rat was lightly anesthetized with 3% pentobarbital sodium (1 mL/kg) following 24 h fast. Then, the flat lavage apparatus (length 8cm, diameter 2mm) was gently inserted into the lumen of the colon through the anus with the tip positioned approximately 8cm proximal to the anus. The solution containing 2.5%TNBS (w/v) dissolved in 50% ethanol (1:1) was slowly infused into the colon (at a dose about 4 mL/kg). Following the instillation of the hapten, the animals were maintained in a head down position for about 30 s and then were kept flat for a few minutes to prevent leakage of the intracolonic instillation. The same procedure was used with the control group but the rats were administered with normal saline instead of TNBS. The rats were inspected daily for behavior, body weight, and stool situation. The rats were administered with saline only (2.0 mL/d, i.g.) in the control group and model group; ACAG (0.12 g/kg/d, i.g.) in treatment group (TNBS+ACAG); SASP (0.27 g/kg/d, i.g.) in positive control group (TNBS+SASP) by oral gavage once daily respectively.

2.5. Collection of samples and assessment of colitis

Stools of the rats were evaluated using DAI scoring system described in previous report (Koetzner et al., 2009). The scores for stool consistency and occult blood for each rat were added and then given a DAI score for each rat. Each score was determined as follows: stool consistency (0 and 1: normal, 2 and 3: loose stool, 4: diarrhea) and stool blood (0: negative, 1: ±, 2: +, 3: ++, 4: gross) (Table S2).

At the end of seven days of administration, all rats were anaesthetized, blood samples were collected from the abdominal aorta, and colons were excised. The distal 8cm of the colon were excised, opened longitudinally, and rinsed with saline solution.