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Gegen Qinlian decoction alleviates experimental colitis via suppressing TLR4/NF-κB signaling and enhancing antioxidant effect

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ABSTRACT

Background: Gegen Qinlian decoction (GQ), a Chinese medicinal herb decoction, has been widely used as efficient medicine for the treatment of acute colitis in clinics, but underlying molecular mechanisms have not been fully clarified.
Hypothesis/purpose: Inflammation and oxidative stress have been reported to constitute a crucial part in the pathogenesis of ulcerative colitis (UC). Hence, this study was designed to investigate the antiinflammatory activity and antioxidative effect of GQ.
Study design: Mice induced by 5% dextran sulfate sodium (DSS) and macrophage RAW264.7 cells stimulated by lipopolysaccharide (LPS) were used in this study.
Methods: Ethanol extracts of GQ were orally administered for 1 week on the dosage of 0.3, 1.5, or 7.5 g/kg/day and berberine (BBR, 100 mg/kg/d) was selected as a positive group in the animal experiments. In vitro, GQ (25, 50, 100 μg/ml) or BBR (20 μM) co-cultured with RAW264.7 for 2 h prior to LPS stimulation.
Results: The results showed that GQ oral administration alleviated the severity of colitis notably. It reduced toll-like receptor 4 (TLR4) expression and NF-κB activation in mucosa, which was accompanied with down regulation of several inflammatory cytokines in the colon, including tumor necrosis factor (TNF-α), interleukin (IL)-6, IL-1β and IL-4. Furthermore, GQ oral administration attenuated the oxidative stress in the colon of UC mice, evidenced by the decrease of myeloperoxidase (MPO) activity and malondialdehyde (MDA) level, and the elevation of glutathione (GSH) content. In parallel with the vivo experiment results, cell research indicated GQ dramatically reduced the production of TNF-α, IL-6, IL-1β and nitric oxide (NO), as well as that of reactive oxygen species (ROS) upon stimulation of LPS.
Conclusion: Together, our present study indicates that inhibition of TLR4/NF-κB signaling and enhancement of antioxidant effect might be the potential mechanisms for the therapeutic effect of GQ against UC.

Introduction

The prevalence of IBD is around one in a thousand of people in Europe, and the high incidence rate of the disease has been implicated to be closely associated with the increased risk of intestinal cancers (Loftus, 2004).The major forms of IBD, including UC and CD, have been empirically defined by clinical, pathological, endoscopic and radiological features (Xavier and Podolsky, 2007). Although CD and UC share many clinical and pathological characteristics, they also have some different marked immunological and histopathological features. Key features of UC include superficial mucosal inflammation that extends proximally from the rectum to a varying degree and depletion of goblet cell mucin, whereas CD is characterized by aggregation of macrophages that frequently from non-caseating granulomas and always has typical transmural inflammation (Lichtiger et al., 1994). The population of patients with UC has increased in Asia, similarly to western countries, and UC is more common than CD in clinics.

According to the recent reports, epithelial barrier function and innate and adaptive immunity play an important role in the

Abbreviations: GQ, Gegen Qinlian decoction; UC, acute ulcerative colitis; DSS, dextran sulfate sodium; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α; IL, interleukin; MPO, myeloperoxidase; MDA, malondialdehyde; GSH, glutathione; NO, nitric oxide; ROS, reactive oxygen species; LPS, lipopolysaccharide; IBD, inflammatory bowel disease; CD, Crohn's disease; GAPDH, glyceraldehyde phosphate dehydrogenase; DCFH-DA, H_2-2′,7′-dichlorodihydrofluorescein diacetate; BBR, berberine; BCA, bicinchoninic acid; DAI, disease activity index; HE, haematoxylin and eosin.

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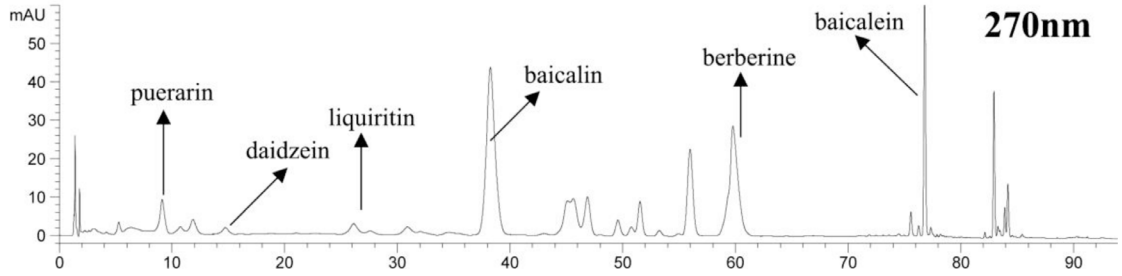

Fig. 1. A representative chromatogram and UV spectrum of GQ at 270 nm.

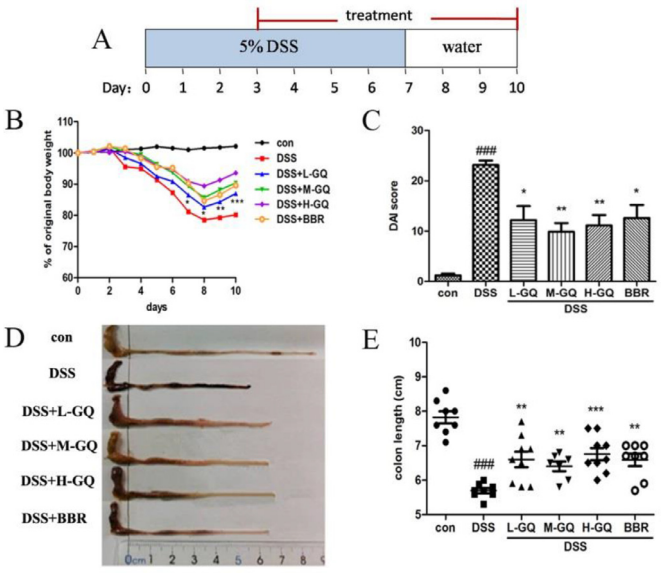

Fig. 2. Effect of GQ on the treatment of DSS-induced colitis mice. (A) The set of UC model and procedure of drug treatment. (B) Body weight changed with mice from day 1 to day 10 throughout the experiment. The data plotted as percentage of basal body weight. (C) The DAI score of mice in every group. (D) Photographs of the mice colon. (E) The colon length of mice in the groups. * Mean values were significantly different compared with DSS-treated group: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. ### Mean values were significantly different compared with control group ($p < 0.001$).

pathogenesis of UC (Xavier and Podolsky, 2007). The innate immune response represents the first line of defense against pathogens and its response is mediated by a large variety of different cells, including epithelial cells, neutrophils, dendritic cells, monocytes, macrophages and natural killer cells (Akira et al., 2006). ROS is implicated as mediators of intestinal inflammation and plays a vital role in innate immune response activation by regulating intracellular reduction-oxidation (redox) sensitive signaling (Mandalari et al., 2011). TLR4, a key pattern-recognition receptor for commensal recognition in gut innate immunity, is overexpressed on the surface of inflamed colon (Abreu et al., 2005). Activation of TLR4 triggers the formation of distinct signaling complexes NF-κB in cells. Thus, it is proposed an effective way to abrogate intestinal inflammation by transecting the TLR4 linked NF-κB signaling pathway (Spehlmann and Eckmann, 2009).

Today, the pharmacologic management of UC mainly relies on 5-aminosalicylates, corticosteroids and immune-suppressants and biologic therapies (Baumgart and Sandborn, 2007). Although pharmalogical treatment options are available, they have limitations in efficacy and safety, such as corticosteroids dependence, the risk of the colectomy and the development of colon cancer (Danese, 2012; Meier and Sturm, 2011). Accordingly, new therapies have to be explored and developed. In traditional Chinese medicine, combination therapy has been advocated for thousand years and it is a unique ancient Chinese medical science in treating various diseases (Wang et al., 2008). GQ, a Chinese medicinal herb decoction, including four quality assured herbs: *Scutellaria baicalensis* Georgi., *Coptis chinensis* Franch., *Pueraria lobata* (Willd.) Ohwi., *Glycyrrhiza uralensis* Fisch., has been used as an efficient acute diarrhea medicine from the Eastern Han dynasty for about 2000 years (Li et al., 2004). Nowadays, GQ has been clinically proven to be effective in the treatment of UC (WANG et al., 2012). However, the underlying mechanism is poorly understood.

Table 1
The components of GQ.

Crude drugs	Dry weight of crude drugs in GQ (g)	Dry weight of crude drugs in GQ (%)
Scutellaria baicalensis Georgi.	12	23.1
Coptis chinensis Franch.	12	23.1
Pueraria lobata (Willd) Ohwi.	20	38.5
Glycyrrhiza uralensis Fisch	8	15.3
Total	52	100

Active components of GQ, such as baicalin, glabridin and berberine, has been shown to alleviate inflammation and oxidative stress in vivo or in vitro (Cui et al., 2014; Kwon et al., 2008; Lee et al., 2010; Yan et al., 2012).Therefore, we hypothesized that GQ could exerted its effect against UC through anti-inflammation and anti-oxidation. In the present study, we explored the effects of GQ on UC mice induced by DSS and RAW264.7cells stimulated with LPS. The results indicate that the therapeutic effect of GQ on UC might be mediated through suppressing TLR4/NF-κB signaling and strengthening antioxidant effect.

Materials and methods

Materials

DSS (36–50 kDa) was purchased from MP Biomedicals (California, USA). Antibodies of p65(65 kDa), P-p65(65 kDa), TLR4(130 kDa), IκB(39 kDa) and P-IκB(41 kDa) were purchased from Cell Signaling Technology (Danvers, Colorado, USA) and GAPDH(36 kDa) antibody was obtained from ABclonal Biotech Co., Ltd (Wuhan, China). The HRP-conjugated Goat Anti-Rabbit/mouse IgG was purchased from Vazyme Biotech Co., Ltd (Nanjing, China). ELISA kits for murine IL-6, TNF-α, IL-4 and IL-1β were purchased from R&D Systems China Co. Ltd. (Shanghai, China). Kits for determining MPO, GSH, NO and MDA were purchased from Jiancheng Bioengineering Institute (Nanjing, China). LPS was purchased from Sigma-Aldrich (St. Louis, MO, USA). Berberine was obtained from Zelang Group (Nanjing, China). Hoechst 33258 (bisbenzimide) and DCFH-DA were