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A Chinese medicinal formulation ameliorates dextran sulfate sodium-induced experimental colitis by suppressing the activity of nuclear factor-kappaB signaling

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ABSTRACT

Ethnopharmacological relevance: Inflammatory bowel disease (IBD) is generally associated with a set of debilitating symptoms including abdominal pain, tenesmus, diarrhea and bloody stool. The standard approaches for treating IBD, which are the application of pharmaceuticals, are often unsatisfactory. IBD patients may suffer from repeated relapses and even exacerbation after taking these medications. Thus, patients are increasingly seeking relief through the use of complementary and alternative medicines.
Aim of study: To provide scientific ground for the mode of actions of a Chinese medicinal formulation—modified ZenWu Decoction (MZWD) in ulcerative colitis.
Materials and methods: C57BL6 mice were fed with 3 cycles of 2% dextran sulfate sodium (DSS) in drinking water for the induction of chronic colitis and then given MZWD at 17.47 g/kg/day. Effects of MZWD were evaluated by histopathological and biochemical assays.
Results: When MZWD was given, inflammatory responses namely immune-cell infiltration, elevated serum levels of pro-inflammatory cytokines and mucosal lesions were notably suppressed. Further, MZWD treatment attenuated the activation of nuclear factor-kappaB (NF-κB), the vital regulator of inflammatory cascades, while lessening the degradation of I-kappaB-alpha and reducing the activity of protease-activated receptor 2 in DSS-induced colonic tissues. Consequently, diarrhea, bloody stool and colon shortening were reduced whilst mucosal integrity was improved in MZWD-treated colitis mice.
Conclusions: Our findings suggest that MZWD is a potential remedy for treating IBD, and the mechanism of its efficacy is an anti-inflammatory effect associated with the suppression of the NF-κB pathway.

1. Introduction

Inflammatory bowel disease (IBD), commonly refers to ulcerative colitis (UC) and Crohn's disease (CD), is ordinarily characterized by a set of complicated chronic inflammatory conditions of the intestines. In general, UC is solely restricted to the large intestine whereas CD may affect the whole gastrointestinal tract, literally from mouth to anus. IBD causes debilitating symptoms including abdominal pain, tenesmus, diarrhea and bloody stools; therefore, the quality of life of IBD sufferers is noticeably disturbed to varying degrees (Papadakis and Targan, 2000). According to recent reports, the incidence of IBD has been significantly increasing over the past two decades (Lakatos, 2006). However, the etiology of IBD is yet a mystery. Environmental, hereditary, infectious and autoimmune factors are considered as the plausible and intercalating causes (Xavier and Podolsky, 2007).

Anti-inflammatory drugs, immune system suppressors and antibiotics are the conventional medications for the treatment of IBD (Stone et al., 2003). Among the mainstream measures, biologic anti-tumor necrosis factor-alpha (TNF-α) remedies such as

Abbreviations: DSS, dextran sulfate sodium; IL-1β, interleukin-1beta; IFN-γ, interferon-gamma; IκB-α, I-kappaB-alpha; MZWD, modified ZenWu Decoction; NF-κB, nuclear factor-kappaB; PAR2, protease-activated receptor 2; TNF-α, tumor necrosis factor-alpha

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Infliximab and non-selective anti-inflammatory drug aminosalicylates are regarded as the mainstay of drug treatment (Magro, 2010); however, the anti-TNF-α protocol is not satisfactory as nearly 50% of the UC patients who received it suffered from repeated relapses or even exacerbations (Lok et al., 2007). Concerns about the safety of injecting anti-TNF-α antibodies have been expressed; these antibodies are associated with an increased risk of malignancy in the long term (Hudesman et al., 2013), and shorter-term risks of psoriasis (Guerra et al., 2012) and sarcoidosis (Fok et al., 2012). The immunomodulator Cyclosporine has been shown to be beneficial in severe flare-ups, though side effects including hypertension, nephrotoxicity and electrolyte imbalance are often associated with it (Mocciaro et al., 2012). If medications fail, colectomy, i.e. removing part of or the entire colon, becomes the last resort. As suggested by a number of medical reports, IBD patients are at a higher risk of developing colon cancer (Dobbins, 1984). Due to the unsatisfactory therapeutics of Western medicine, patients, physicians and researchers are turning to complementary and alternative medicine approaches such as Traditional Chinese Medicine (TCM) for a relief and a more effective way for treating IBD (Podolsky, 2002).

According to the Chinese medical theory, the stagnation of *Qi* and blood followed by combined dampness or cold-dampness in the stomach and intestine leads to the symptoms now defined as UC. The 5-herb formula ZenWu Decoction (ZWD) comprising Poria [sclerotium of *Poria cocos* (Schw.) Wolf, family: Polyporaceae], Paeoniae Radix Alba (root of *Paeonia lactiflora* Pall., family: Ranunculaceae), Macrocephalae Rhizoma (rhizome of *Atractylodes macrocephala* Koidz., family: Asteraceae), Zingiberis Rhizoma (rhizome of *Zingiber officinale* Rosc., family: Zingiberaceae) and Aconiti Lateralis Radix Praeparata (processed root of *Aconitum carmichaelii* Debx., family: Ranunculaceae) has been used as a folk medicine for the alleviation of chronic diarrhea for centuries. To this traditional formula, we replaced the relatively toxic component Aconiti Lateralis Radix Praeparata with Codonopsis Radix [root of *Codonopsis pilosula* (Franch.) Nannf., family: Campanulaceae] and Coptidis Rhizoma (rhizome of *Coptis chinensis* Franch., family: Ranunculaceae), and created modified ZenWu Decoction (MZWD). Unfortunately, no scientific evidence on the mechanism of the efficacy of both the original ZWD and MZWD has been provided. Therefore, we conducted this study to confirm the efficacy of MZWD in treating experimental IBD and to explore its underlying mechanism.

At the molecular level, inflammatory responses such as UC principally involve the activation of the pivotal inflammatory regulator nuclear factor-kappaB (NF-κB). In mammals, NF-κB plays important roles in a number of cellular and organismal processes (Hayden and Ghosh, 2008). The nuclear translocation of NF-κB dimmers implicates the transactivation of its target genes encoding inflammatory cytokines and mediators in response to tissue injury, repair and inflammation (Bonizzi and Karin, 2004). In IBD conditions, innate immune responses in the intestine are initiated by macrophages and dendritic cells for fighting against the luminal antigens and commensal bacteria conquering the mucosa (Platt and Mowat, 2008). Upon the activation of the immune cells, pro-inflammatory cytokines namely TNF-α, interleukin-1beta (IL-1β) and interferon-gamma (IFN-γ) are rapidly and massively produced. Transcription of the TNF-α gene in the activated immune cells results in consequent secretion of TNF-α in a positive feedback manner (Braegger et al., 1992). It is believed that TNF-α prolongs inflammatory conditions by activating NF-κB-dependent pathways by which ulceration and mucosal destruction is accelerated. Protease-activated receptor 2 (PAR2) is a pro-inflammatory mediator that expressed predominantly in epithelial cells of the internal milieu such as in the gastrointestinal tract (Bohm et al., 1996). It also largely contributes to NF-κB-mediated inflammatory processes and primes the activation of intracellular signaling networks since PAR2 is the common target of various proteolytic enzymes including beta-tryptase (β-tryptase), thrombin and matrix metalloproteases (Cenac et al., 2002). In firing pro-inflammatory responses, the nuclear translocation of NF-κB is undoubtedly the most pivotal initiative process. Increased activities of NF-κB are often observed in experimental colitis as well as in UC patients (Andresen et al., 2005; Dong et al., 2010). Therefore, agents that suppress the activation of this transcription factor have the potential for therapeutic intervention.

Among the several experimental animal models used for the investigation of IBD, oral administration of dextran sulfate sodium (DSS) has been widely acknowledged for accurately mimicking the UC situation in humans (Jurjus et al., 2004; Kim et al., 2012). In the current study, we examined the effectiveness of our modified 6-herb formula, MZWD, on DSS-induced colonic inflammation in mice by means of various histopathological and biochemical assays. Our results demonstrated that administration of MZWD significantly reduced the infiltration of immune cells and the secretion of pro-inflammatory cytokines. Most importantly, MZWD treatment suppressed the activation of NF-κB, the degradation of its cytosolic inhibitory complex and the activity of PAR2. As a result, the severity of colitis in the DSS-treated mice was reduced. Our findings not only elucidate the *in vivo* anti-inflammatory effects of MZWD, but also partially reveal the underlying mechanism of the pathogenesis of IBD. We suggest that MZWD is potentially an effective therapeutic approach for the treatment of IBD.

2. Materials and methods

2.1. Preparation of modified ZenWu Decoction (MZWD)

Top-grade herbal materials were obtained from GMP-certified pharmaceutical companies or certified suppliers. The herbal materials were authenticated by Ms Yu-ying Zong, School of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China. The site of origin and the name and address of the vendor for each acquired herbal material were documented. Individual herbs were assigned with unique voucher numbers for tracking, management and documentation purposes. Voucher samples (MZWD-01 to MZWD-06) have been deposited at the herbarium of the School of Chinese Medicine, The Chinese University of Hong Kong. Herbs were certified free of heavy metals, toxic elements and pesticide residues and within microbial limits. MZWD extracts were prepared by grinding and mixing of the 6 component dried raw herbs (Poria, Paeoniae Radix Alba, Macrocephalae Rhizoma, Zingiberis Rhizoma, Codonopsis Radix and Coptidis Rhizoma) in proportions as shown in Table 1. Herbs were decocted with boiling water at 100 g/L for 60 min twice. Decoctions were then filtered and pooled. For the purpose of oral feeding to mice in a smaller volume, the pooled decoction was further concentrated and freeze-dried. For quality control purposes, extracts were sonicated in methanol and subjected to high

Table 1
Composition of herbs in the formulation MZWD on a dry weight basis.

Chinese herbs	Alias	Composition (%)
Codonopsis Radix	Dangshen	17.6
Macrocephalae Rhizoma	Bai Zhu	17.6
Poria	Fu Ling	35.4
Zingiberis Rhizoma	Gan Jiang	11.8
Paeoniae Radix Alba	Bai Shao	11.8
Coptidis Rhizoma	Huanglian	5.9