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The protective effect on ulcerative colitis of san huang shu'ai decoction by inhibited the IL-1β and telomerase

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Abstract: **Ulcerative colitis (UC) is a chronic inflammatory disease affecting the colon, and its incidence is rising worldwide. Patients with ulcerative colitis have mucosal inflammation starting in the rectum that can extend continuously to proximal segments of the colon. Ulcerative colitis usually presents with bloody diarrhoea and is diagnosed by colonoscopy and histological findings. San huang shu ai decoction (SH), take in 《lei zheng huo ren shu》 in Song Dynasty, composed of coptidis rhizoma(huang lian), scutellariae radix (huang qin), phellodendri chinensis cortex (huang bo), and artemisiae argyi folium (ai ye), is a traditional Chinese medicine formula and is widely used as a clinically medication formula for its efficiency in improving hot diarrhoea. But the underlying mechanisms by which it exerts therapeutic function have not been thoroughly studied. In this study, acute ulcerative colitis was induced by oral administration of 2.5% dextran sodium sulfate for 7 days in drinking water, SH (0.8, 1.6g/kg body weight respectively) decreased disease activity index and improved colon length at a certain degree, the expression of IL-1β, IL-6, TNF-α, MPO, and telomerase in colon of positive group (sulfasalazine) and SH (low and high doses) group decreased significantly ($P<0.05$). These results indicated that SH can hold protective effect on UC mice, which may be related to its inhibition of the expression of inflammatory mediators and oxidative stress in colon, and can be used as a pharmaceutical preparations for UC treatments.**

Keywords: San huang shu'ai decoction; Coptidis rhizoma;scutellariae radix; Phellodendri chinensis cortex; Artemisiae argyi folium; Ulcerative colitis;IL-1β; Telomerase

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1. Introduction

Ulcerative colitis (UC) is a chronic and relapsing inflammatory disorder of the colon and rectum with increasing morbidity in recent years, its symptoms include diarrhea and hematochezia[1,2]. In traditional Chinese medicine theory (TCM), UC is known as the"changpi" and chronic dysentery[3]. With the exception of patients who have a cecal patch, the inflammatory response usually begins in the rectum and extends proximally with a diffuse, continuous pattern. Characterized by chronic mucosal inflammation of the colon, UC presents with bloody diarrhea, tenesmus, abdominal pain, weight loss, fatigue, and even vomiting when symptoms become severe[4,5]. To date, UC remains one of the most challenging gastrointestinal diseases, impairing the quality of life and posing a high-risk threat of colorectal cancer in patients. The incidence and prevalence of UC have been reported to be on the rise over the past two decades[6]. Although the immunologic mechanism of UC has been postulated as an important participant in this disease, the etiology and pathophysiology are still unknown. Nowadays, the principal drugs for UC treatment are mainly consisted of four types: 5-aminosalicylic acid, steroid hormone, immunosuppressive agents and anti-tumor necrosis factor-α (anti-TNFα) drugs. However, serious side effects, such as easy to relapse, longterm medication side effects, refractory characteristics, limit their clinical application[7]. Therefore, it is of great significance to further understand the pathogenesis of UC and seek effective drugs for the treatment of UC. According to the recent reports, epithelial barrier function and innate and adaptive immunity play an important role in the pathogenesis of UC[8]. Oxidative stress and upregulation of some enzymes, such as inducible nitric oxide synthase (iNOS), and the secretion of various pro-inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor-α (TNF-α), play important roles in UC[9-11]. Nowadays, treatment of UC mainly relies on SASP, immune-suppressants or biologic therapies. Although chemical drugs treatment options are available, they have limitations in efficacy and safety, such as corticosteroids dependence and the risk of kidney injury[12,13]. Therefore, the

development of alternative prevention medicine is required for long-term management of UC.

Traditional Chinese medicine is one of alternative treatment options and has been increasingly recognized worldwide. San huang shu'ai decoction (SH), was taken in《lei zheng huo ren shu》) in Song Dynasty, composed of coptidis rhizome (huang lian), scutellariae radix (huang qin), phellodendri chinensis cortex (huang bo), and artemisiae argyi folium (ai ye), is a traditional Chinese medicine formula and is widely used as a clinically medication formula for its efficiency in improving hot diarrhoea[14]. But the underlying mechanisms by which it exerts therapeutic function have not been thoroughly studied. Coptidis rhizome (huang lian) is the dry rhizome of Coptis chinensis branch, Coptis deltoidea c.y.cheng et Hsiao or Coptis teeta wall[15]. Scutellariae radix (huang qin) is the dry root of Scutellaria baicalensis Georgi, a Labiatae plant. Phellodendri chinensis cortex (huang bo) is the dry bark of yellow bark tree of Rutaceae[15]. Artemisiae argyi folium(ai ye) is the dry leaf of Artemisia argyi levl.et vant[15]. This study was designed to investigate the anti-inflammatory and protective effect on dextran sulfate sodium (DSS)-induced colitis mice of SH.

2. Material and methods

2.1 Equipment

UV-visible spectrophotometer (UV2600, Shimadzu, kyoto, Japan); Ruler (SRL96080, M&G, Shanghai, China); Camera (ExmorRS, Sony, Tokyo, Japan).

2.2 Reagents

Dextran sulfate sodium salt (DSS) produced by MP Biomedicals LLC (California, USA); San huang shu ai decoction (SH) was provided by Baiyunshan Xingqun Pharmaceutical Co. Ltd. (Guangzhou, Guangdong, China).

Table 1. The chemical components of SH

name	Main chemical composition
huang lian	Berberine、Palmatine、Coptisine、Worenine、Jatrorrhizine、Magnoflorine、Ferulic、Chlorogenic acid
huang qin	Baicalin、Wogonoside、Wogonin、Baicalein-7-O-β-D glucopyranoside、Baicalein-7-O-β-D glucopyranosiduronic acid、Phenylacetic acid、4-O-β-D Glucopyranosyl-cis-cinnamic acid、Baicalein、Skullcapflavone Ⅰ、Skullcapflavone Ⅱ、Chrysin、Oroxylin A、(2S)-5,7,2',6'-Tetrahydroxy flavanone、(2R,3R)-3,5,7,2',6'-Pentahydroxy flavanone、β-Sitosterol、Campesterol
huang bo	Berberine、Ethyl caffeate、Isovanillin、Ferulic acid、 (±)-Lyoniresinol、β-Sitosterol、Stigmasterol、Hyperoside、Dihydrokaempferol、Phellochinin A、Obakulactone、Adenosine、Phellodendrine、Noroxyhydeastinine、Oxyberberine、Rugosinone、Jatrorrhizine、
ai ye	Eupatilin、Jaceosidin、Salylic acid、Chlorogenic acid、1,8-Cineole、Camphor、Apigenin、Isoartemisia ketone、Luteolin、Kaempferol、Borneol、Caryophyllene(β- Caryophyllene)、α-Cubebene、Octacosanoic acid、Linarin、Quercetin

2.3 Animals

Male Balb/c mice (6–8 weeks, 18–22 g) were obtained from the Laboratory Animal Center of Southern Medical University (Guangdong, China) and group-housed under controlled temperature (22 ± 2°C) and photoperiods (12 h: 12 h light–dark cycle). After the acclimation for 7 days, mice were matched by age and body weight. Care and experimentation of mice were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and the related ethical regulations of China Pharmaceutical University.

2.4 Treatment protocol for DSS-induced colitic mice

UC mice was induced with 3% (w/v) DSS dissolved in drinking water continuously for 7 days, while the control group mice drank water without DSS (n = 6 mice in each group). SH (1.0 g/kg/d) and SASP (0.6 g/kg/d) were gavaged once a day from day 1 to day 8.

Mice were observed once daily for weight, stool consistency, and the presence of gross blood in feces and at the anus. The DAI was calculated as previously described. On day 8, mice were executed, rapidly dissected, and the entire colons were quickly removed and took photos.

3.2 Effect of SH on clinical indices

In the animal model, 3% DSS in the drinking water induced marked symptoms as observed in human UC. No significant differences in body weight change were detected between groups at the beginning of the experiment. As shown in Figure 1, the DAI score for the DSS group was significantly higher as compared to the control group ($P<0.01$). However, the DAI scores for the positive groups (SASP and AZA) and SH groups (BO-L, -M, -H) were significantly lower as compared to the DSS group ($P<0.01$), suggesting that the experimental colitis was suppressed significantly by the SH and positive control treatments. No significant differences ($P<0.05$) in water and food intake were observed among all the DSS-treated groups in this study.

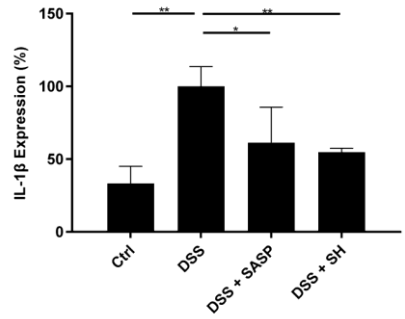

Figure 1. The disease activity index (DAI) in mice. Data are compared between groups on the 8th day of the experiment and expressed as the means±S.E.M. of 15 mice in each group. #P<0.05, ##P<0.01 vs. normal control; *P<0.05, **P<0.01 vs. DSS group.

3. Discussion

Although UC is generally treated with anti-inflammatory or immunosuppressive agents, these therapies proved to be inadequate. Hence, many options have shifted to alternative therapies including TCM. At present, there are few studies on SH in the main traditional applications such as dysentery. Based on the ethnopharmacological use of SH, in the present work, we investigated the anti-inflammatory effect of SH on DSS-induced UC in Balb/C mice and elucidated the potential mechanism of action.

San huang shu ai decoction (SH), take in《lei zheng huo ren shu》) in Song Dynasty, composed of coptidis rhizome(huang lian), phellodendri chinensis cortex(huang bo), scutellariae radix(huang qin), and artemisiae argyi folium(ai ye), is a traditional Chinese medicine formula. Coptdis rhizome(huang lian) contains epiberberine, berberine, palmatine, berberine, etc, which has the functions of clearing away heat, drying dampness, purging fire and detoxifying, and is used for damp heat, diarrhea, dysentery, etc[15]. Phellodendri chinensis cortex(huang bo) contains berberine, jatrorrhizine, phellodendrine and other alkaloids; in addition, phellodolone, obakulactone, stigmasterol and β-sitosterol. It is reported that phellodendri chinensis cortex has the functions of clearing away heat and drying dampness, purging fire and removing steam, detoxifying and treating sore and is used for damp heat, diarrhea, dysentery, eczema and other diseases[16]. Berberine, extracted from coptis chinensis, phellodendron amurense and other herbs, is a kind of alkaloid with significant antibacterial effects. It has been used in the treatment of bacterial gastroenteritis, dysentery and other gastrointestinal diseases for a long time. In recent years, other pharmacological activities of berberine have been graduallydiscovered[17]. Baicalin, wogonoside, baicalein and wogonin are the main effective components of scutellaria radix(huangqin)[18]. The report shows that Scutellaria radix and its active components have a wide range of pharmacological effects, such as anti-inflammatory, anti-oxidation, anti-tumor and immune regulation, especially anti bacteria /fungi[19]. Artemisiae argyi folium (ai ye), a well-known raditional Chinese materia medica commonly used in clinica, is widely distibuted in China. It is recorded to possess the effects of warming meridians for hemostaais and eliminating cold for analgesia The species is rich in terpenoids, flavonoids, phenylpropanoids, aromatic acids (aldehydes), stercids, and fatty acids, and exhibits pharmacological activities of analgesia; anti-inflammatory, antibacterial and antiviral, anti-tumor, immunoregulation activities and so on[20].

DSS is a sulfated polysaccharide synthesized from sucrose. Its mechanism of inducing UC model may be related to the destruction of intestinal mucosal barrier, the imbalance of intestinal flora and the inhibition of epithelial proliferation[21]. This model is simple and easy to operate, with high success rate and good repeatability[22]. Establishment of UC by oral administration of DSS in murine is a widely employed in vivo model for UC investigation, since the pathological alternations in this model closely resembles human UC[23]. Studies established that DSS-induced colitis was more representative of UC than TNBS-induced colitis, whose characteristics resemble human Crohn's disease (CD)[24]. Furthermore, DSS-induced colitis was characterized by focal crypt lesions, goblet cell loss and inflammatory

cell infiltration at the areas of lesions, which was more intimately associated with inflammation as compared to other colitis models such as TNBS or oxazolone induced colitis model[25]. Hence, in the present work, DSS-induced colitis mice model was employed to investigate the potential anti-inflammatory effect of SH in the treatment of UC.

In this established model, we assessed DAI. The DAI scores represented the severity of DSS-induced colitis. High DAI score represented discomfort conditions of mice, including weight loss, faecal bleeding and diarrhea[26]. It was found that DSS-treated colitis mice exhibited higher DAI as compared with the control. In contrast to the DSS group, mice treated with SH and SASP exhibited significantly attenuated a markedly reduced DAI after colitis induction. All the results above suggested that SH abrogated established colonic inflammation and had a noticeably protective effect against DSS-induced colitis in mice.

In the present work, to more comprehensively assess the effcacy of SH, we used positive drugs, namely sulfasalazine (SASP). SASP is a derivative of mesalazine (5-aminosalicylic acid) and has been used as an effective anti-inflammatory drug for the treatment of inflammatory bowel disease and rheumatoid arthritis[27] because of its safety profile, ease of administration, and low cost. It delivered a high concentration of 5-ASA to the colon[28]. In the present study, we found that SASP was effective in the treatment of UC. Furthermore, the therapeutic effect of SH was also proved to be pronounced.

To the best of our knowledge, this study was the first endeavor to demonstrate the therapeutic effect of SH against DSS-induced murine experimental colitis, and reveal the potential mechanism of SH in regulating inflammation-associated events. This study showed that SH possessed appreciable anti-inflammatory effect in treating murine experimental UC induced by DSS. This investigation provided experimental evidence for the traditional application of SH in the treatment of dysentery, and might add new therapeutic dimensions to its current clinical application of SH and also provided a foundation and justification for further research as a potential complementary therapeutic agent to the current conventional medications.

4. Conclusion

In summary, our results indicated that SH the potential of anti-inflammation to treat UC induced by DSS as a promising candidate. Our research provided experimental evidence for the traditional application of SH in the treatment of dysentery and might extend the clinical indications for SH.

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