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Evaluation the effect on ulcerative colitis of traditional Chinese medicine formula huanglian-huangqin herbal pair

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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. Huanglian-huangqin herbal pair(LQ), a traditional Chinese medicine formula, composed of coptidis rhizoma(huang lian) and scutellariae radix(huang qin). Xie xin tang (symopsis of prescriptions of the Golden chamber, or jin gui yao lue) containing this herbal pair. LQ is widely used as a clinically medication formula for its efficiency in inflammatory diseases. But the underlying mechanisms by which it exerts therapeutic function of UC has 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, LQ (0.9g/kg body weight) decreased disease activity index and improved colon length at a certain degree, but compared with positive group(sulfasalazine) has no statistical significance. These results indicated that LQ can hold protective effect on UC mice, but can not be used as a pharmaceutical preparations for UC treatments pontentionally.**

Keywords: Huanglian-huangqin herbal pair; Coptidis rhizoma; Scutellariae radix; Ulcerative colitis

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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.

Traditional Chinese medicine is one of alternative treatment options and has been increasingly recognized worldwide. Huanglian-huangqin herbal pair(LQ), a traditional Chinese medicine formula, composed of coptidis rhizoma(huang lian) and scutellariae radix(huang qin). Coptidis rhizoma（huanglian） is the dry rhizome of Coptis chinensis branch, Coptis deltoidea c.y.cheng et Hsiao or Coptis teeta wall[8]. Scutellariae radix （huangqin） is the dry root of Scutellaria baicalensis Georgi, a Labiatae plant. Coptis is bitter and cold in nature. It has the effect of purgative fire and detoxification. Scutellaria is bitter and cold in nature. It has the effect of drying dampness, clearing heat and clearing fire[8]. The combination of the two drugs can give full play to the effect of relieving pathogenic heat, clearing away heat and dampness, purging fire and detoxifying, clearing away heat and calming down the fetus[9]. LQ is widely used as a clinically medication formula for its efficiency in inflammatory diseases. But the underlying mechanisms by which it exerts therapeutic function of UC has not

been thoroughly studied. This study was designed to investigate the anti-inflammatory and protective effect on dextran sulfate sodium (DSS)-induced colitis mice of LQ.

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).

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.

2.5 Statistical analysis

The results were expressed as mean ± standard deviation (SD) of three replicates. Statistical significance of data were analyzed by two-tailed Student's t test or one-way analysis of variance (ANOVA) followed by Dunnett's t-test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 The chemical components of LQ

Table 1 showed that Huanglian-huangqin herbal pair(LQ) was composed of coptidis rhizoma(huang lian) and scutellariae radix (huang qin) [10].

Table 1. The chemical components of LQ

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

3.2 Effect of LQ 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 LQ 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 LQ and positive control treatments. No significant differences in water and food intake were observed among all the DSS-treated groups in this study.

The colon length is believed to be inversely associated with the severity of DSS-induced colitis. To determine whether LQ had a beneficial effect on DSS-induced colonic shortening, we measured and compared the colon lengths of mice from different groups. Figure 2 showed the photographs of the intestine in each group, and the colons of LQ scarcely showed redness and swelling. However, the colons of mice in DSS group showed serious redness and bloody stools when compared with the normal group. The results indicated that LQ treatment, BOH in particular, could retain the colon length of DSS-induced mice.

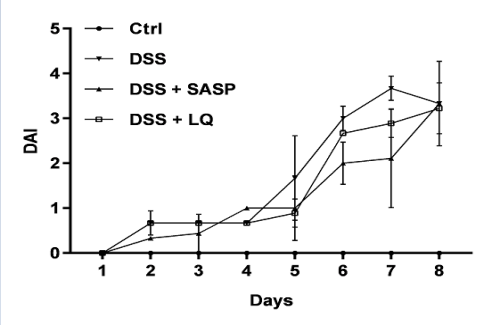

Figure 1. The disease activity index (DAI) in mice. Data are compared between groups on the 8th day of the experiment and expressed as the means±S.E.M. of 15 mice in each group. #P<0.05, ##P<0.01 vs. normal control; *P<0.05, **P<0.01 vs. DSS group.

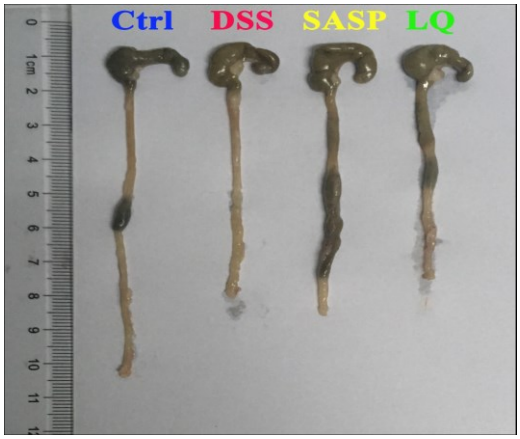

Figure 2. Representative photographs of colon lengths. Data are compared the groups on the 8th day of the experiment and expressed of 15 mice in each group.

4. 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 LQ in the main traditional applications such as dysentery. Based on the ethnopharmacological use of LQ, in the present work, we investigated the anti-inflammatory effect of LQ on DSS-induced UC mice and elucidated the potential mechanism of action.

Huanglian-huangqin herbal pair (LQ), a traditional Chinese medicine formula, composed of coptidis rhizome (huang lian) and scutellariae radix(huang qin). Coptdis rhizome (huang lian) contains berberine, coptisine, worenine and other alkaloids; it also contains phellodone, obakulactone and phenolic components, which mainly treats dysentery (as ulcerative colitis, UC), diarrhea and other diseases. Modern research has revealed that it has the functions of antibacterial, inhibiting gastric secretion, anti diarrhea, anti acute inflammation, anti-cancer, inhibiting tissue metabolism, anti ulcer, antipyretic, sedative, bacteriostatic, anticoagulant and lowering blood lipid[11]. Scutellaria radix (huang qin) mainly containing flavonoids: Baicalin, Baicalein, Wogonoside, Wogonin, Skullcapflavone, etc. It has the functions of antiviral microorganism, antipyretic, anti-inflammatory, anti allergy and detoxification[12].

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[13]. This model is simple and easy to operate, with high success rate and good repeatability[14]. 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[15]. Studies established that DSS-induced colitis was more representative of UC than TNBS-induced colitis, whose characteristics resemble human Crohn's disease (CD)[16]. 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[17]. Hence, in the present work, DSS-induced colitis mice model was employed to investigate the potential anti-inflammatory effect and underlying mechanism of LQ in the treatment of UC.

In this established model, we assessed DAI, colon length in DSS-induced Balb/c mice. 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[18]. It was found that DSS-treated colitis mice exhibited higher DAI and shorter colon length as compared with the control. In contrast to the DSS group, mice treated with LQ and SASP exhibited significantly attenuated a markedly reduced DAI after colitis induction. Colon length is an indirect index inversely related to the severity of DSS-induced UC. In this work, the colon

length was observed to be remarkably recovered in mice of LQ group. All the results above suggested that LQ abrogated established colonic inflammation and had a noticeably protective eff ect against DSS-induced colitis in mice.

In the present work, to more comprehensively assess the effcacy of LQ, 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[19] because of its safety profile, ease of administration, and low cost. It delivered a high concentration of 5-ASA to the colon[20]. In the present study, we found that SASP was effective in the treatment of UC. Furthermore, the therapeutic effect of LQ was also proved to be pronounced.

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

5. Conclusion

In summary, our results indicated that LQ 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 LQ.

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References

[1] Nanini HF, Bernardazzi C, Castro F, et al. Damage-associated molecular patterns in inflammatory bowel disease: From biomarkers to therapeutic targets[J]. World journal of gastroenterology, 2018, 24(41): 4622.

[2] Weisshof R, El Jurdi K, Zmeter N, et al. Emerging Therapies for Inflammatory Bowel Disease[J]. Advances in therapy, 2018: 1-17.

[3] Han SZ, 2014. The Progress of Traditional Chinese Medicine Treatment in Ulcerative Colitis. Beijing University of Chinese Medicine.

[4] Reynolds PD, Hunter JO, 1993. Pharmacotherapy of inflammatory bowel disease. Dig. Dis. 11 (6), 334–342.

[5] Turner D, Levine A, Escher JC, et al. Management of pediatric ulcerative colitis: joint ECCO and ESPGHAN evidence-based consensus guidelines[J]. J. Pediatr. Gastroenterol. Nutr. 2012, 55 (3), 340–361.

[6] Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or down?[J]. World J. Gastroenterol. 2006, 12 (38), 6102–6108.

[7] Ukil A, Maity S, Das PK. Protection from experimental colitis by the aflavin3,3-digallate correlates with inhibition of IKK and NF- κ B activation[J]. Br. J., 2006

[8] National Pharmacopoeia Committee. Pharmacopoeia of the People's Republic of China[M]. Part 1. Beijing: Chemical Industry Pres s, 2015.

[9] Zhang XL, Zhou MM, Zhao AH, et al. Analysis of the Main Components of Coptis-Scute Herbal Pair by UPLC-PDA-MS[J]. natural product research and development, 2012, 24(11):1502-1507.

[10] Wang SF, Qian ZZ. Reference Handbook for Chinese Pharmacopeia (Volume I)-Modern Analysis Technology For Evaluating The Quality Of Traditional Chinese Medicine, 2010.

[11] Jinxi Zhao, Chengwei Li. New use of diabetes clinical drugs [M], 2006.

[12] Zhou DS, Li ZB. Interpretation of the combination of Chinese and Western medicine in practical clinical practice [M], 2016:7.

[13] Chassaing B, Aitken JD, Malleshappa M, et al. Dextran sulfate sodium (DSS)-induced colitis in mice[J]. Curr Protoc Immunol, 2014.

[14] Shuang L, Xia L, Qi L, et al. Effect and Mechanism of Rhein on DSS-induced Ulcerative Colitis in Mice[J]. chinese journal of experimental traditional medical formulae, 2017,11(23),109-113.

[15] Seril DN, Liao J, Yang GY, et al. Oxidative stress and ulcerative colitisassociated carcinogenesis: studies in humans and animal models[J]. Carcinogenesis, 2003, 24(3), 353-362.

[16] Alex P, Zachos NC, Nguyen T, et al. Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis[J]. Inflamm. Bowel Dis, 2009, 15(3), 341-352.

[17] Waldner MJ, Neurath MF. Chemically Induced

Mouse Models of Colitis. John Wiley & Sons, Inc,2009.
[18] Murano M, Maemura K, Hirata I, et al. Therapeutic eff ect of intracolonically administered nuclear factor κ B (p65) antisense oligonucleotide on mouse dextran sulphate sodium (DSS)-induced colitis[J]. Clin. Exp.Immunol, 2000, 120 (1), 51-58.
[19] Narayan N, Rigby S. Carlucci, F. Sulfasalazine induced immune thrombocytopenia in a patient with rheumatoid arthritis[J]. Clin. Rheu. 2017, 36, 477-479.
[20] Christensen LA, Fallingborg J, Jacobsen BA, et al. Comparative bioavailability of 5aminosalicylic acid from a controlled release preparation and an azo-bond preparation[J]. Aliment. Pharmacol. Ther, 1994, 8 (3), 289–294.