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Shao Yao Decoction exerts a protective effect on ulcerative colitis by inhibiting inflammation mediated by hypercoagulability

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Author contributions

Cui HT carried out the experiments and manuscript writing. Wang YM provided experimental help and result interpretation. Wang N provided ideas and manuscript review & editing. All authors contributed to the article and approved the submitted version.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

SYD, Shaoyao decoction; UC, ulcerative colitis; DSS, dextran sulfate sodium; DAI, Disease Activity Index; PT, prothrombin time; FIB, fibrinogen; H&E, hematoxylin and eosin.

Citation

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Abstract

Background: Shaoyao decoction (SYD) has been found widespread clinical use in treating ulcerative colitis (UC). However, the mechanism underlying SYD impact on UC remains elusive. **Materials and methods:** We preliminarily evaluated the therapeutic effect of SYD intervention in a dextran sulfate sodium-induced UC mouse model by analyzing the body weight change, disease activity index score, colon length, and HE staining results of colon tissue in each group of mice. Subsequently, we determined pro-inflammatory cytokines level and blood coagulation markers in the colon tissues of mice in each group to evaluate the effect of SYD intervention on colonic inflammatory response and coagulation function in UC mice. **Results:** Our findings emphasize the significant therapeutic effect of SYD on UC, including slowed down body weight loss, reduced disease activity index score, increased colon length, and reduced inflammatory infiltration in colon tissue. Moreover, SYD intervention significantly downregulated the levels of pro-inflammatory cytokines IL-1β, IL-6, and IL-17A in the colon. Furthermore, SYD intervention reversed the coagulation-related indicators such as prothrombin time, fibrinogen, P-selectin, D-dimer, and platelet glycomembrane protein IIb/IIIa. **Conclusion:** Our results elucidate the substantial therapeutic impact of SYD on UC mice. Importantly, the therapeutic mechanism of SYD in addressing UC potentially involves the inhibiting of inflammatory response mediated by hypercoagulability.

Keywords: shaoyao decoction; ulcerative colitis; hypercoagulability; inflammation; traditional Chinese medicine

Background

Ulcerative Colitis (UC) is a common and challenging disease of the digestive system, often accompanied by complications such as colonic perforation and carcinogenesis, posing a serious threat to human health [1]. In recent years, the incidence of UC has rapidly increased, leading to significant social and medical burdens [2]. Although widely used first-line medications, such as 5-aminosalicylic acid derivatives, corticosteroids, and immunosuppressants, show significant efficacy, the relapse rate of UC is high after discontinuation of these drugs [2]. Furthermore, approximately one-third of patients will eventually undergo surgical procedures such as intestinal resection [3]. Therefore, there is an urgent need for safe, effective, and stable treatment strategies to combat UC.

Intestinal inflammatory response and hypercoagulability are the main pathological manifestations of UC and are crucial factors leading to mucosal damage in UC. Compared to the healthy population, patients with active UC have significantly increased levels of pro-inflammatory cytokines such as IL-6 and IL-1β and exhibit excessive coagulation function activation, including increased distribution width of platelets, increased secretion granules, decreased hematocrit, reduced mean platelet volume, and elevated levels of coagulation factors [4, 5]. Animal experiments have similarly shown that the levels of pro-inflammatory cytokines are significantly increased in the colonic tissues of UC mouse models induced by dextran sulfate sodium (DSS) [6]. Another animal study indicated that alleviating coagulation dysfunction by inhibiting platelet-activating factors can mitigate inflammatory damage to the UC intestinal mucosa [7].

Traditional Chinese Medicine has shown considerable promise in managing UC due to its notable therapeutic capabilities [8]. In a randomized controlled clinical study showed that Jianpi Qingchang Decoction could effectively improve the clinical symptoms of UC patients, reduce the Inflammatory Bowel Disease Questionnaire (IBDQ) scores, and the Sutherland Disease Activity Index (DAI), thereby improving patients' quality of life [9]. Another meta-analysis indicated that Gegen Qinlian Decoction might have potential benefits in the treatment of UC [10]. Clarifying the mechanisms of action of Traditional Chinese Medicine is significant for promoting its application in UC. Shaoyao decoction (SYD), composed of *Paeoniae Radix Alba*, *Angelicae Sinensis Radix*, *Coptidis Rhizoma*, *Scutellariae Radix*, *Rhei Radix Et Rhizoma*, *Arecae Semen*, *Eupatorii Herba*, *Glycyrrhizae Radix Et Rhizoma*, and *Cinnamomi Cortex*, has garnered widespread utilization in the clinical management of UC [11]. In vivo experiments have also confirmed the therapeutic effect of Shaoyao decoction on UC animal models [12, 13]. However, the mechanisms by which SYD improves UC remain unclear. This study initially induced a UC mouse model using DSS in order to assess the therapeutic efficacy of SYD on UC-afflicted mice. Secondly, it investigated the influence of SYD on inflammation-associated cytokines and coagulation-related factors in the colons of UC mice.

Methods

Animals and reagents

Animal: Healthy male C57BL/6 mice, were sourced from Beijing Huafukang Bioscience Co., Ltd. (Beijing, China) (SYXK (Jing) 2019-0030). The mice aged 6–8 weeks and weighing 20 ± 1 g. The experiments were conducted in an SPF-grade environment, with five mice per cage, fed standard feed, and provided with ad libitum access to food and water.This study was conducted by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by The Experimental Animal Ethics Committee in Yunnan University of Chinese Medicine (Approval No.: R-062023LH288).

Materials and kits: Dextran sulfate sodium (DSS) was purchased from MPBiomedicals Co., Ltd. (Shanghai, China). ELISA kits for mice IL-1β (ml098416), IL-6 (ml098430), IL-17A (ml037864), P-selectin (ml063072), D-dimer (ml038012), and GPIIb/IIIa (ml002086) were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China.

Preparation of Shaoyao decoction

According to the prescription of SYD, the following Chinese herbal slices were weighed: 30 g *Paeoniae Radix Alba*, 15 g each of *Angelicae Sinensis Radix*, *Coptidis Rhizoma*, and *Scutellariae Radix*, 9 g of *Rhei Radix Et Rhizoma*, 6 g each of *Arecae Semen*, *Eupatorii Herba*, and *Glycyrrhizae Radix Et Rhizoma*, and 5 g of *Cinnamomi Cortex*. The slices were acquired from the Chinese medicine pharmacy of the First Affiliated Hospital of Tianjin University of Traditional Chinese Medicine. All slices were mixed, soaked in 8 times their volume of purified water for 2 h, then brought to a boil on high heat and simmered on low heat for 0.5 h to obtain the decoction, which was then filtered and set aside. This process was repeated once more with 6 times the volume of purified water. The two batches of decoction were combined, centrifuged at 1,500 rpm for 10 min, filtered, and concentrated to a solid powder form, ground, and stored at 4 ˚C for future use. Subsequently, the concentration of the drug to be formulated was calculated based on the dose to be administered for the experiment and the number of mice so that each mouse was controlled to dilute at 0.2 mL of gavage volume.

Modeling, grouping, and drug intervention protocol

The mice were provided with a sterile 3% (w/v) DSS solution as their drinking water continuously for 7 days, after which they were given normal drinking water for the subsequent 3 days. A general decrease in body mass, along with the presence of loose stools or bloody feces, indicated successful model establishment [14].

In the investigationof SYD's the therapeutic effects on UC mice, 30 C57BL/6J mice underwent a 7-day acclimatization period before being randomly allocated into three groups: Control, DSS, and SYD groups. While the Control group received standard drinking water, whereas the remaining experimental groups were induced with UC using DSS.. At the start of modeling, the Control and DSS groups were administered 0.2 mL of saline by gavage daily, and the SYD group received SYD at 32 g/kg by gavage daily for 10 consecutive days. The dose of SYD administered was twice the human equivalent dose obtained by discounting based on body surface area. Since the effect of the high dose was observed to be the most obvious in the preliminary pre-test, only the high dose (twice the human equivalent dose) was selected for the study in this experiment.

Throughout the experiment, the mice's body weight was recorded daily, along with observations of fecal consistency and the degree of bloody stools. The severity of UC in mice was assessed using the Disease Activity Index (DAI) score, as previously reported, including the percentage of body weight loss (0, 0%; 1, 1–5%; 2, 6–10%; 3, 11–20%; 4, > 20%), stool consistency (0, normal; 2, loose; 4, diarrhea), and the degree of bloody stools (0, normal; 2, bleeding; 4, severe bleeding) [15].

On the 10th day of the experiment, subsequent to the final dosing, mice were anesthetized with sodium pentobarbital, blood samples were collected, and then euthanized by cervical dislocation. The colon tissues were rapidly dissected, the macroscopic state of the colon was observed, and the colon length was recorded. After measuring the colon length, the colon lumen was rinsed with ice-cold saline, and a segment of approximately 0.5 cm from the same position of the distal colon was fixed in 4% paraformaldehyde for 24 h. The remaining colon tissues were stored at −80 ˚C for future use.

Pathological staining

Colon tissues fixed in 4% paraformaldehyde were dehydrated with ethanol, embedded in paraffin, and then sectioned into 5 μm slices for hematoxylin and eosin (H&E) staining, following protocols from previous studies. The results of the H&E staining were observed under an optical microscope.

Pro-inflammatory cytokine assay

Colon tissues stored at −80 ˚C were homogenized in 9 times their

weight of saline to prepare a 10% tissue homogenate. The total protein of the tissue homogenate samples was normalized using a BCA assay kit. The levels of pro-inflammatory cytokines IL-1β, IL-6, and IL-17A in the colon tissue homogenate were detected using ELISA, with specific operating methods according to the instructions of the kit.

Coagulation function assay

After blood collection, fibrinogen (FIB), prothrombin time (PT), thrombin time, activated partial thromboplastin time were immediately measured using an automatic coagulation analyzer. The activation of platelet factors P-selectin, D-dimer, and platelet glycoprotein GPIIb/IIIa in colon tissue were detected using ELISA.

Statistical analysis

Statistical analysis was conducted using the SPSS Pro online data analysis platform, with results presented as mean ± standard deviation. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post-tests, with $P < 0.05$ considered statistically significant.

Results

SYD intervention demonstrated a significant therapeutic effect on UC mice

Initially, we analyzed the therapeutic effect of SYD on UC mice. The change in body weight showed that the Control group mice exhibited a steady increase in weight, while the Model group mice experienced a rapid decline in body weight starting from the 4th day of modeling. However, this decline in body weight was slowed after SYD intervention (Figure 1a). The Disease Activity Index (DAI) scores indicated that compared to the Control group, the DSS group mice's DAI scores rapidly increased from the 4th day of modeling, whereas after SYD intervention, the mice's DAI scores were lower than in the DSS group (Figure 1b). The colon length results showed that compared to the Control group, the DSS group mice had significantly shorter colons, while SYD intervention led to an increase in colon length compared to the DSS group (Figure 1c). H&E staining results revealed that compared to the Control group, the DSS group mice's colon tissues were congested and edematous with extensive inflammatory cell infiltration, while SYD intervention reduced congestion and edema and significantly alleviated inflammatory cell infiltration (Figure 1d).

SYD intervention significantly improved the colonic inflammatory response in UC mice

Following this, the levels of pro-inflammatory cytokines in the colon tissues of the mice were determined. The results showed that compared to the Control group, the DSS group mice had significantly elevated levels of IL-1β, IL-6, and IL-17A in their colon tissues, while the intervention with SYD notably decreased the levels of these pro-inflammatory cytokines (Figures 2a–2c).

SYD intervention effectively improved coagulation function in UC mice

Next, we assessed the coagulation function-related factors in the mice's peripheral blood and platelet activation-related factors in colon tissues to evaluate the impact of SYD on coagulation function in UC mice. Compared to the Control group, the DSS group mice showed shortened PT and elevated FIB (Table 1) and significantly increased levels of P-selectin, D-dimer, and GPIIb/IIIa (Figures 3a–3c), while SYD intervention notably reversed these indicators.

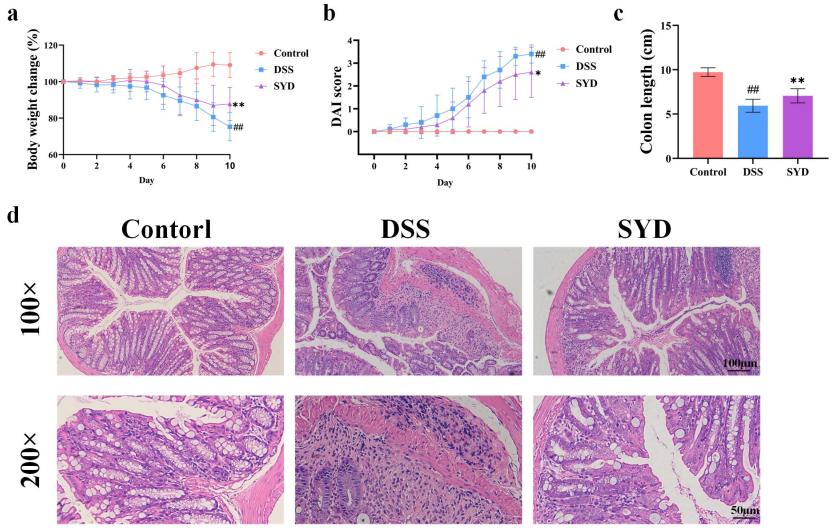
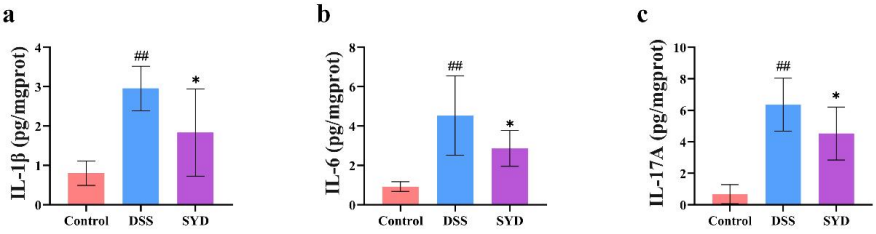


Figure 1 SYD intervention demonstrated a significant therapeutic effect on UC mice. (a) SYD can increase the body weight of DSS model mice. (b) Decrease the DAI score. (c) Increase the colon length of DSS model mice. (d) Improve the pathological changes in the colon tissue of DSS model mice. $^{\#\#}P < 0.01$, compared with control group; $^{*}P < 0.05$, compared with DSS group; $^{**}P < 0.01$, compared with DSS group. DSS, dextran sulfate sodium; DAI, disease activity index; SYD, Shaoyao decoction; UC, ulcerative colitis.

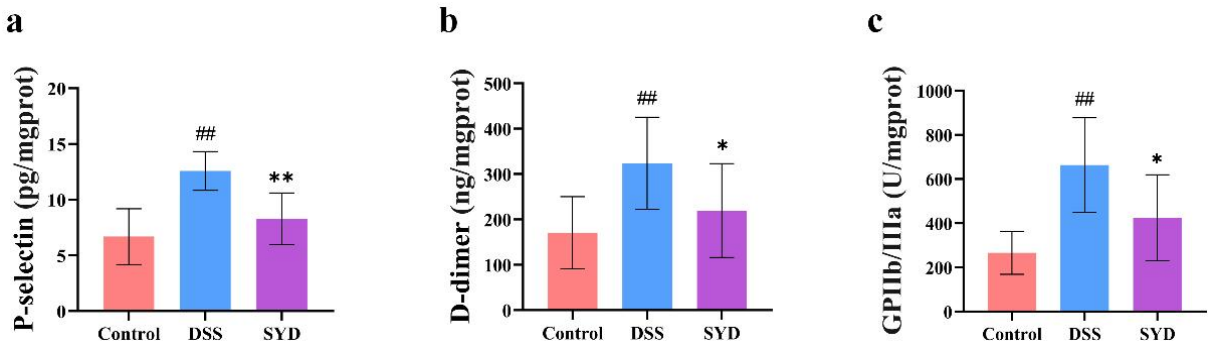


Figures 2 SYD intervention significantly improved the colonic inflammatory response in UC mice. ELISA results indicate that SYD can decrease the expression levels of (a) IL-1β, (b) IL-6, (c) IL-17A, in the colon tissues of DSS model mice. $^{\#\#}P < 0.01$, compared with control group; $^{*}P < 0.05$, compared with DSS group. SYD, Shaoyao decoction; UC, ulcerative colitis; DSS, dextran sulfate sodium.

Table 1 SYD intervention effectively improved coagulation function-related factors in UC mice

Group	PT (s)	APTT (s)	TT (s)	FIB (g/L)
Control	7.67 ± 0.77	25.03 ± 3.11	15.92 ± 1.73	1.46 ± 0.15
DSS	6.14 ± 0.65$^{\#\#}$	24.70 ± 2.43	15.29 ± 1.76	3.11 ± 0.29$^{\#\#}$
SYD	7.25 ± 0.40**	25.55 ± 2.79	14.70 ± 1.85	1.92 ± 0.17**

$^{\#\#}P < 0.01$, compared with control group; $^{**}P < 0.01$, compared with DSS group. SYD, Shaoyao decoction; UC, ulcerative colitis; DSS, dextran sulfate sodium; APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; PT, prothrombin time.



Figures 3 SYD intervention effectively improved coagulation function-related factors in UC mice. (a) P-selectin. (b) D-dimer. (c) GPIIb/III. $^{\#\#}P < 0.01$, compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with DSS group. SYD, Shaoyao decoction; UC, ulcerative colitis.

Discussion

The DSS-induced UC animal model is widely used in mechanism research and pharmacological efficacy verification due to its simplicity, stability, and closeness to the pathological manifestations of clinical patients [16, 17]. DSS, a sulfated polysaccharide with anticoagulant properties, can damage the intestinal mucosal barrier, leading to the infiltration of polysaccharides and other macromolecules into the mucosa, thereby triggering a series of inflammatory responses [18]. Mice freely drinking DSS rapidly exhibit symptoms such as weight loss, diarrhea, and severe colonic bleeding. The DAI score, based on the severity of these symptoms, assesses the success of the UC animal model [19]. In our study, DSS-induced UC mice showed significant weight loss, severe diarrhea, and colonic bleeding, with DAI scores rapidly increasing, indicating successful model establishment. SYD intervention alleviated these symptoms and improved colonic bleeding and inflammatory cell infiltration, suggesting SYD effectively improves DSS-induced UC.

Hypercoagulability plays a crucial role in the development of UC. Our results show that UC mice exhibit shortened PT, elevated FIB, and significantly increased levels of P-selectin [5]. D-dimer, and GPIIb/IIIa, indicating hypercoagulability and a significantly increased risk of thrombosis. PT levels are closely related to the coagulation state, with decreased PT indicating a hypercoagulable state. Furthermore, FIB levels are positively correlated with the risk of thrombosis. P-selectin, released by activated platelets, is a specific marker of platelet activation or thrombosis, reflecting the activation of platelets in a hypercoagulable state. D-dimer, a specific degradation product of fibrin, reflects the activation of the coagulation system. The conformational change of the platelet membrane glycoprotein GPIIb/IIIa complex from a resting to an activated state can expose fibrinogen binding sites, promoting platelet activation [20]. Studies have shown that levels of P-selectin, D-dimer, and GPIIb/IIIa are significantly elevated in patients with active inflammatory bowel disease [21–23]. Other studies have shown that blocking P-selectin can effectively alleviate tissue damage in UC [24]. Additionally, hypercoagulability can activate various pro-inflammatory cells and secrete multiple pro-inflammatory cytokines, such as IL-1β, IL-6, and IL-17A, amplifying the inflammatory response. The release of pro-inflammatory cytokines can directly interact with receptors on the surface of platelets, facilitating their activation and aggregation, and triggering coagulation pathways. This, in turn, enhances the activation of prothrombinase in the blood, exacerbating the hypercoagulable state and increasing the risk of thrombosis [25–27]. Blocking hypercoagulability can alleviate inflammatory damage in UC colons [7]. Our study results show that SYD intervention can effectively reverse these coagulation-related indicators and downregulate pro-inflammatory cytokines in UC mouse colon tissues. This suggests that SYD intervention effectively inhibits inflammation mediated by hypercoagulability, thereby alleviating UC.

Conclusion

This investigation validates the noteworthy curative impact of SYD on mice afflicted with UC, possibly related to SYD inhibition of inflammation mediated by hypercoagulability. However, this is only a preliminary exploration of the mechanism. In the future, we plan to delve deeper into the mechanisms and specific targets of SYD therapeutic effect on UC, combining single-cell sequencing and spatial transcriptomics technologies.

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