

Effect of Chunpi Decoction on Ulcerative Colitis Induced by Dextran Sulfate Sodium in Mice

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Abstract

Ulcerative colitis is a chronic disease with unclear pathogenesis and difficult to cure. Its drug treatment is highly dependent and has obvious side effects. In this study, dextran sulfate sodium (DSS) was used to establish a mouse model of ulcerative colitis, and Chunpi decoction (10 mL/d) were used for intervention. The body weight, disease activity index (DAI) and colon length of mice were detected, and the liver and kidney function indexes and oxidative stress levels of mice were detected by kits. Enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of inflammatory factors, and the colon tissue sections were observed. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the content of short-chain fatty acids (SCFAs) in the feces of mice. Among the findings: Compared with the DSS group, the body weight and colon length of the mice in the Chunpi decoction intervention group increased, DAI decreased, liver and kidney function improved, the expression of pro-inflammatory factors decreased significantly ($P < 0.01$), and the expression of anti-inflammatory factors increased significantly ($P < 0.01$). The level of inflammatory markers were significantly decreased ($P < 0.01$), the content of SCFAs in feces was up-regulated, and the structure of colon epithelium was improved. Chunpi decoction can improve intestinal mucosal injury and liver and kidney dysfunction in mice, and has a good therapeutic effect on DSS-induced ulcerative colitis in mice.

Keywords

Chunpi Decoction; Dextran Sulfate Sodium; Mice; Ulcerative Colitis; Short Chain Fatty Acids.

1. Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease caused by bacterial and fungal infection or genetic, immune and environmental factors, including ulcerative colitis (UC) and Crohn's disease [1]. Among them, UC is mainly manifested by abdominal pain, diarrhea, bloody stool and other symptoms, accompanied by strong oxidative stress and accumulation of cellular inflammatory factors, and has a long course of disease and is easy to relapse. Long-term chronic inflammatory state is easy to cause colon cancer and other related diseases [2]. Most studies believe that its pathogenesis is related to the proliferation of neutrophils, intestinal mucosal barrier damage, over-expression of pro-inflammatory factors and abnormal immune response of intestinal flora caused by the joint action of various factors [3-5]. At present, it is mainly treated by drugs such as biological agents and immunomodulators [6], but drug treatment often shows long-term dependence, temporary healing and unpredictable side effects. Traditional medicine, including traditional Chinese medicine, is highly respected for its unique efficacy on chronic non-infectious diseases, so it has become a mainstream method to find and verify drugs for the treatment of colitis from traditional Chinese medicine prescriptions.

Chunpi decoction is composed of Radix paeoniae alba, Rhizoma coptidis, Rhizoma coptifolia, Astragali radix, Codonopsis codonopsis, Poria codonopsis, Angelica sinensis, cinnamon and licorice. The rhizoma coptidis and Rhizoma coptidis are inserted into the large intestine meridian, which taste bitter and cold, dry dampness and clear heat, and can be used as a prescription to eliminate the cause of disease. Astragalus to saprophytic muscle, as an adjuvant. Licorice and Zhongtiaoyao, cinnamon heat heat, all kinds of medicine shared, can reconcile the blood gas, dampness and heat. To study the protective effect of Chunpi decoction on colitis induced by dextran sulfate sodium salt (DSS) in mice, and to provide a theoretical basis for the treatment of inflammatory bowel disease with Shaoqi-Chunpi decoction.

2. Materials and Methods:

2.1. Animal Grouping and Treatment Design

Thirty female Kunming mice were randomly divided into 5 groups after 7 days of adaptive feeding: control group (Normal group), model group (DSS group), mesalamine positive control group (5-ASA group), low dose Chunpi decoction group (LCD group) and high dose Chunpi decoction group (HCD group), with 6 mice in each group. Except for the control group, the other groups were given 3 g/100 mL DSS solution for 9 consecutive days, and fresh DSS solution was replaced every day. After the success of modeling, the 5-ASA group was given mesalazine enterocoated tablets (5-ASA, 130 mg/ (kg mb·d)) according to the dose relationship of human and animal equivalent body surface area. The LSCD and HSCD groups were fed with 30% and 100% Shaoqi Chunpi decoction 10 mL/d, respectively, while the control and DSS groups were fed with sterile water. After 14 days of intervention, the mice were dissected and the corresponding indexes were measured, and the experimental period was 23 days.

2.2. Disease Activity Index

The body weight of mice was recorded every 3 days, and the activity status, mental state, and fecal characteristics of mice were observed. The fecal occult blood was measured, and the disease activity index (DAI) was calculated.

2.3. Serum Biochemical Indexes and Inflammatory Factors were Determined

After the last feeding, the mice were fasted for 12 h and anesthetized by carbon dioxide asphyxia. Blood samples were collected from the abdominal aorta and centrifuged for 30 min at 3 000 r/min and 4 °C for 10 min to collect serum. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CR), and blood urea nitrogen (BUN) in the serum of mice were measured according to the instructions of the kit. The levels of inflammatory factors IL-1 β , IL-6, TNF- α , and IFN- γ were detected by enzyme-linked immunosorbent assay (ELISA).

2.4. Histopathological Observation

Fresh colon tissue was fixed with 4% paraformaldehyde solution for 24 hours, dehydrated by ethanol gradient and embedded in paraffin. After deparaffinizing, the sections were stained with hematoxylin-eosin (HE) and Alcian blue staining solution, respectively, and the sections were sealed.

2.5. Measurement of Fecal Short-chain Fatty Acid Content

SCFAs are one to six carbon saturated fatty acids, which play an important role in intestinal health. 0.1 g mouse fecal sample was vortexed and mixed with 1 mL phosphoric acid solution (0.1%), then centrifuged at 15 000 \times g at 4 °C for 10 min. The supernatant was removed and extracted with ethyl acetate at a volume ratio of 1:1. The ethyl acetate extraction phase was

passed through a 0.45 μm filter membrane. GC-MS analysis was performed on a DB-WAX capillary column (30 m \times 0.32 mm, 0.25 μm).

2.6. Data Processing and Analysis

SPSS 23.0 software was used for analysis of variance and Tukey multiple comparison test. Data were expressed as mean \pm standard deviation (n = 6). $P < 0.05$ was considered as significant difference, and $P < 0.01$ was considered as extremely significant difference. graphpad prism software was used for data visualization.

3. Results

3.1. Effects of Chunpi Decoction on Body Weight and DAI in Mice with Colitis

The body weight of the control group showed a stable rising trend during the experiment, and the body weight of the other groups continued to decrease within 9 days after drinking DSS solution. The symptoms of weight loss of the mice were relieved after the administration of mesalazine and Chunpi decoction, but there was no significant difference in the body weight of the mice in LCD and HCD groups compared with DSS group, which may be due to the inhibition of pancreatic lipase activity. It reduces the intestinal intake of food energy. Compared with the DSS group, the DAI of each intervention group was significantly decreased ($P < 0.01$), indicating that Chunpi decoction alleviated the symptoms of DSS-induced colitis in mice to a certain extent.

3.2. Effect on Colon Length in Mice with Colitis

The changes of colon length in mice can be used as an important indicator of colon inflammation. The colon length of mice in the DSS group was significantly shorter than that in the other groups ($P < 0.01$), and the colon length of mice in the 5-ASA, LCD and HCD groups was close to that of the control group. The colon length of mice in the HPJ group was longer, and the intestinal contents were in a healthy black state, indicating that drinking Chunpi decoction could alleviate DSS-induced colon length shortening in mice.

3.3. Effects on Liver and Kidney Function in Mice with Colitis

Ulcerative colitis and drugs used to treat ulcerative colitis can cause liver and kidney damage. ALT and AST can be secreted by hepatocytes. When liver cells are inflamed and injured, they can enter the serum with high sensitivity, so their levels can be used to indicate body damage. CR and BUN levels are closely related to renal function and the degree of damage, and their increase can indicate renal dysfunction. Compared with the control group, the activities of ALT and AST and the concentrations of CR and BUN in DSS group were significantly increased ($P < 0.01$), indicating that DSS could cause liver and kidney injury complications. Compared with the DSS group, the levels of the above indexes in the 5-ASA, LCD and HCD groups were better improved, and there was no significant difference between the LCD and HCD groups and the control group ($P > 0.05$), indicating that drinking Chunpi decoction had a certain protective effect on liver and kidney function.

3.4. Effects on the Expression of Serum Inflammatory Factors in Mice with Colitis

Increased levels of pro-inflammatory factors and decreased levels of anti-inflammatory factors are typical characteristics of colitis, which can reflect the development process and severity of colitis. Compared with the control group, the serum levels of TNF- α , IL-1 β , IL-6 and IFN- γ in DSS-treated mice were significantly increased ($P < 0.01$), and the anti-inflammatory factor IL-10 was significantly decreased ($P < 0.01$). After the intervention of positive drug (5-ASA group) and Chunpi decoction, the expression levels of inflammatory factors were improved, and the LCD and HCD groups showed the same trend as the 5-ASA group, indicating that Chunpi

decoction can alleviate DSS-induced colon inflammation by reducing the expression of pro-inflammatory factors and increasing the level of anti-inflammatory factors.

3.5. Effects on HE Staining and Goblet Cell Number in the Colon of Mice with Colitis

In the control group, the structure of colonic epithelial cells and mucosal crypts was complete and arranged neatly, and there was no inflammatory cell infiltration. In the DSS group, glandular atrophy, mucosal injury, epithelial cell exfoliation, inflammatory cell infiltration, and a large number of lymphocyte aggregation were observed in the colon tissue, and the intestinal villi became shorter. Compared with the DSS group, the crypt structure tended to be normal, the epithelial structure tended to be complete, the number of glands increased significantly, and the villi became longer in the 5-ASA group, LCD group, and HCD group. Goblet cells can secrete mucus, which can lubricate and protect the intestinal epithelium. The mucin in the mucus can be stained blue by alcian blue staining solution to judge the integrity of the colonic mucus layer. Compared with the control group, the goblet cells in the DSS group were significantly reduced, and the volume was different, indicating that DSS treatment damaged the structure of the intestinal mucosa. After the intervention of Chunpi decoction, the number of goblet cells in LCD and HCD groups increased significantly and arranged closely and orderly, indicating that Chunpi decoction can effectively alleviate the reduction of goblet cells caused by colitis and reduce the damage of intestinal mucosa

3.6. Effect on Fecal SCFAs Content in Mice with Colitis

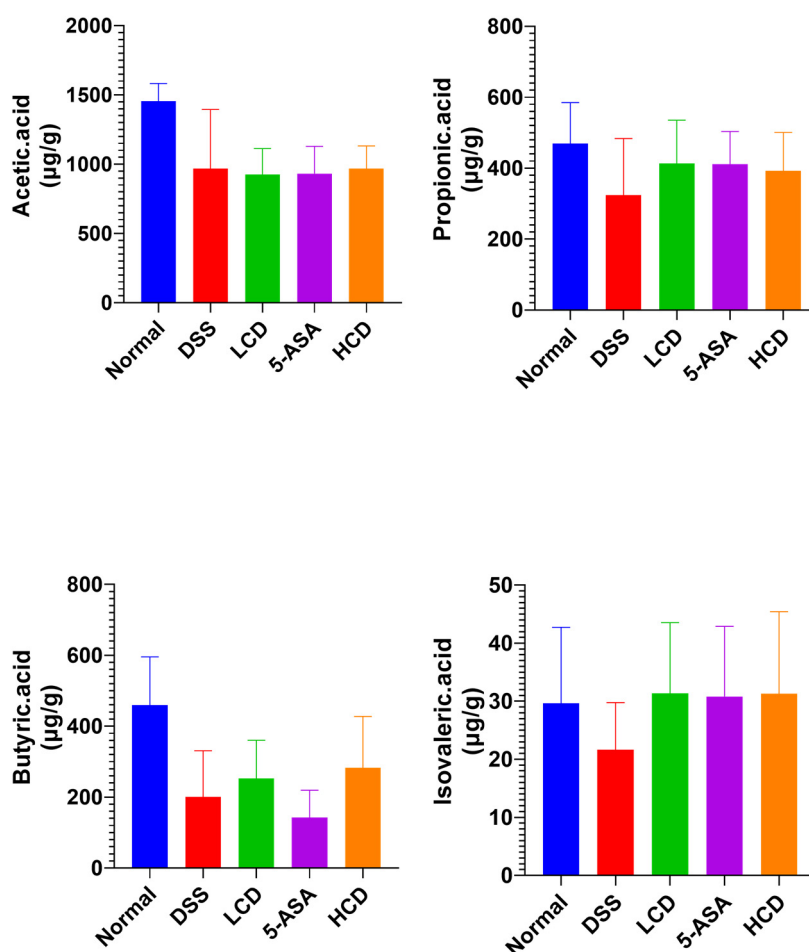


Figure 1. Fecal SCFAs content in mice with colitis

SCFAs are produced by related beneficial bacteria in the gut and play a positive role in maintaining intestinal homeostasis and normal function. Compared with the control group, the contents of the other five SCFAs except acetic acid and the total SCFAs in the feces of DSS group were significantly decreased ($P < 0.01$), indicating that DSS successfully induced intestinal environment disorder in mice. Except for acetic acid and isobutyric acid, the contents of the other four SCFAs in HCD group were higher than those in control group, indicating that Chunpi decoction intervention could not only alleviate the decrease of SCFAs content in DSS-induced colitis, but also increase the content of SCFAs in normal control group. Alleviating DSS-induced colitis by maintaining intestinal homeostasis. see figuer1z.

4. Conclusion

The aim of this study is to investigate the effect of Chunpi decoction on DSS-induced colitis in mice. After modeling, the DAI and the expression of inflammatory factors in mice were significantly increased, indicating that the mouse colitis model was successfully constructed. The intervention of Chunpi decoction in mice with colitis alleviated the symptoms of colitis, reduced the expression of inflammatory factors IL-1 β , IL-6, TNF- α and IFN- γ , and reduced the level of oxidative stress. The section observation showed that the structure of colon tissue epithelium and crypt was intact, the number of goblet cells and the content of SCFAs increased. In conclusion, Chunpi decoction can significantly alleviate and improve DSS-induced colitis in mice

Acknowledgments

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