

Study on changes of Th1/Th2 cytokines with the treatment of Huang-Qin-Tang in damp-heat type ulcerative colitis rats.

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Abstract

Objective: Explore the changes of Th1/Th2 cytokines with the treatment of Huang-Qin-Tang in damp-heat type ulcerative colitis rat.

Methods: A total of 90 SD rats were randomly divided into blank group, model group and Huang-Qin-Tang group. Blank group were not given any drug intervention, while Huang-Qin-Tang group and model group were given high-fat high-sugar diet and trinitro-benzene-sulfonic acid in artificial of hot humid climate to induce damp-heat type ulcerative colitis rat model. The model group did not receive any treatment measures, while the Huang-Qin-Tang group used Huang-Qin-Tang. Clinical activity, changes in body weight, colon length weight, spleen and thymus weight and myeloperoxidase (MPO) activity of three groups were observed. Serum interferon- γ (IFN- γ), interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-12 (IL-12) of rats was tested in the three groups.

Results: After treatment, the activity and the symptoms of Huang-Qin-Tang group were improved compared with model group. The expression of Th1 cytokines (IFN- γ , IL-12) decreased and Th2 cytokines (IL4, IL-10) increased in Huang-Qin-Tang group.

Conclusion: The Huang-Qin-Tang can effectively improve the inflammatory reaction of ulcerative colitis in rats and regulate the balance of Th1/Th2 cytokines, which provides a new idea and method for clinical treatment of damp-heat type ulcerative colitis.

Keywords: Damp-heat type, Ulcerative colitis, Huang-Qin-Tang, Th1/Th2 cytokines.

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Introduction

Inflammatory Bowel Disease (IBD) is a general term for chronic inflammation, which includes Ulcerative Colitis (UC) and Crohn's Disease (CD) [1]. At present, the pathogenesis of inflammatory bowel disease is not very clear, but studies have shown that it was associated with immune abnormalities [2]. CD4⁺T cells play an important role in acquired immune responses, especially inflammatory diseases. Since the 1980s, Mosmann et al. have found that CD4⁺T cells secrete Th1/Th2 cytokines that played an important role in the inflammatory

response [3,4]. The traditional medical science of China has unique advantages in the field of IBD treatment. Huang-Qin-Tang is the Chinese classical prescription. It has been proved by clinical and research section to have good treatment effects to damp-heat syndrome type ulcerative colitis. It not only has functions of anti-inflammation, analgesic and anti-diarrhoea effects to the rat model with damp-heat syndrome type ulcerative colitis, but also has the function of immune-regulation. Then, what functions does Huang-Qin-Tang have to IBD with immunity disease as main pathogenesis, and how will the immune system of rat model with IBD change

accordingly? On such basis, this study was designed to explore the changes of Th1/Th2 in damp-heat type ulcerative colitis with the treatment of Huang-Qin-Tang.

Materials and Methods

Materials

Ninety healthy male Sprague-Dawley rats (half male and half female, 200 ± 10 g) were randomly divided into blank group, model group and Huang-Qin-Tang group. Rats were provided by the Animal Experimental Center. Other materials and sources are listed in Table 1.

Table 1. Materials and sources.

Equipment, drugs, reagents	Sources
5% trinitro-benzene-sulfonic acid (TNBS)	Sigma
INF- γ , IL-12, IL-4 and IL-10 ELISA Kits	Nanjing Jiancheng
MPO activity detection kit	Nanjing Jiancheng
Huang-Qin-Tang	Dispensary TCM
Manual climatic box	Taihong Medical

Model preparation method

After the completion of adaptive feeding for 5 d, rats were weighed. The rats in blank group continued to receive high-fat high-protein diet (containing lard oil, protein powder and white sugar) at room temperature. Model group and Huang-Qin-Tang group: 1. hot humid climate simulation: the rats were placed in artificial climate simulation box at 36°C with level 5 light and humidity above 95% over 20 h a day for 4 weeks; 2. diet and drinking: same with the blank group; 3. colitis induced by TNBS: After fasting for solids and liquids for 36 h, the rats were injected intraperitoneally with 10% chloral hydrate (0.4 ml per 100 g body weight) until the complete anaesthesia. Then disposable premix of 100 mg/kg trinitrobenzene sulfonic acid and 0.25 ml ethanol was given by transanal cannula.

Treatment

Huang-Qin-Tang group: After preparation Huang-Qin-Tang (Scutellaria 9 g, Peony 6 g, Jujube 12, Licorice 6 g), every 1 g dose is equivalent to 10 g liquid medicine with concentration of 16.7%. The rats were given 1 ml/100 g dose, once in the morning and evening. The blank group and the model group were given high quality diet and water without treatment.

Specimen preparation

During the experiment, no death occurred. After the treatment, rats were fasted for 36 h. After anaesthesia with pentobarbital sodium, 5 ml blood was taken from the abdominal aorta for centrifugation at 1500 r/min. The length and weight of intestinal tract were measured and the anabrosis was observed. Then the weights of the rat thymus and spleen were measured.

Evaluation indexes

We observed the clinical activity, body weight, length of colon, weight of spleen, thymus and myeloperoxidase (MPO) activity in three groups. The levels of IFN- γ , IL-4, IL-10 and IL-12 were tested.

Evaluation criteria's of disease indexes mainly included clinical activity (body mass rate of decline, stool traits, bloody stool, recorded as 0-4 points according to condition) and lesions (inflammation and ulcerative colitis, recorded as 0~5 points according to ulcer area, mucosal congestion, edema and erosion).

Detection of cytokines used the corresponding kit in strict accordance with the instructions.

Statistical methods

All the data of this study were analysed by SPSS19.0 software. The data were measured by t-test. The chi-square X^2 test was used for comparison between groups. $P < 0.05$ was defined as a significant difference.

Results

Disease index of three groups

The disease indexes of the rats in the blank group, Huang-Qin-Tang group and model group were 0, 8 and 15, respectively. The comparison between groups was significantly different ($P < 0.05$).

MPO activity of three groups

The MPO activity of the rats in the blank group, Huang-Qin-Tang group and model group were 6, 18 and 12 μ /g, respectively. The comparison between groups was significantly different ($P < 0.05$).

Body weight and weight of colon, thymus, and spleen

The body weight and weight of colon, thymus, and spleen of three groups were shown in Table 2.

Table 2. Body weight changes, and weight/length ratio of colon, weight of thymus and spleen.

Groups	Cases (n)	Body weight (g)	Colon (kg/m)	Thymus (g)	Spleen (g)
Blank	30	28.5 ± 5.6 ^a	0.07 ± 0.01	0.54 ± 0.08	0.41 ± 0.21
Model	30	-18.26 ± 4.8 ^b	0.26 ± 0.07	1.14 ± 0.16	0.22 ± 0.15
Huang-Qin-Tang	30	-8.3 ± 2.4 ^c	0.12 ± 0.03	0.86 ± 0.12	0.36 ± 0.18

Note: ^aCompared to blank group and model group, the body weight and weight of colon, thymus and spleen were significant different, $P < 0.05$; ^bCompared to Huang-Qin-Tang group and model group, the body weight and weight of colon, thymus and spleen were significant different, $P < 0.05$; ^cCompared to blank group

and Huang-Qin-Tang group, the body weight and weight of colon, thymus and spleen were significant different, $P < 0.05$.

Th1/Th2 cytokines in three groups

In the experiment, the levels of Th1/Th2 cytokines in groups were compared (Table 3).

Table 3. Th1/Th2 cytokines in three groups after differentiated treatments.

Groups	IL4 (pg/ml)	IL-10 (pg/ml)	IL-12 (pg/ml)	IFN- γ (pg/ml)
Blank	183.36 15.78 ^a	\pm 48.95 5.72	\pm 327.47 27.81	\pm 987.62 \pm 46.01
Model	120.73 11.29 ^b	\pm 32.06 3.51	\pm 501.71 47.72	\pm 1389.46 82.92
Huang-Qin-Tang	155.68 14.61 ^c	\pm 43.82 5.28	\pm 398.98 46.11	\pm 1183.26 \pm 69.78

Note: ^aCompared to blank group and model group, the levels of Th1/Th2 cytokines were significant different, $P < 0.05$; ^bCompared to Huang-Qin-Tang group and model group, the levels of Th1/Th2 cytokines were significant different, $P < 0.05$; ^cCompared to blank group and Huang-Qin-Tang group, the levels of Th1/Th2 cytokines were significant different, $P < 0.05$.

Discussion

The pathogenesis of inflammatory bowel disease was not very clear, and the existing studies have shown that it was mainly associated with environmental factors, genetic susceptibility, infection and immune factors [5]. Because of its unknown etiology, symptomatic treatment was mainly adopted, including salicylic acid preparations, glucocorticoids, immunosuppressive agents, antibiotics, and even intestinal surgery resection [6]. The main mechanism of this treatment was to reduce the intestinal inflammatory response, restore normal intestinal absorption of nutrients and to prevent further deterioration of inflammatory bowel disease [7]. However, these drugs were intended to control symptoms, and it was difficult to achieve treatment for some intractable enteritis from the aspect of etiology. On the other hand, glucocorticoids and immunosuppressive agents had intolerable side effects, making inflammatory bowel disease easy to replace, and finally leading to high incidence of intestinal cancer. Therefore, it was particularly important to treat inflammatory bowel disease from the aspect of pathogenesis [8,9].

Recent foreign studies have found that both inflammatory bowel disease patients and animal models had different degrees of intestinal barrier dysfunction that increased intestinal permeability, causing intestinal endotoxin and bacteria passing into the blood circulation and leading to systemic inflammatory response [10]. The occurrence of inflammatory reactions undoubtedly caused the release of inflammatory factors that was divided into Th1 and Th2. The cytokines were involved in many inflammatory processes, such as asthma, rheumatoid arthritis, multiple sclerosis and so on. Therefore, it was supposed that regulation of T cells response could contribute to the control of inflammatory response in the treatment of

immune diseases [11]. Previous studies have confirmed that important feature of trinitrobenzene sulfonic acid-induced inflammatory bowel disease model was increased number of Th1 cells and decreased number of Th2 cells [12]. In this study, we have found that Th1 cytokines were significantly higher in the model group than those in the blank group, whereas Th2 cytokines were significantly lower ($P < 0.05$).

The traditional Chinese medicine has an irreplaceable advantage in the treatment of gastrointestinal diseases. The Huang-Qin-Tang was classic in commonly used prescriptions for the treatment of damp-heat gastrointestinal disease. In this study, simulated hot and humid environment combined with trinitrobenzene sulfonic acid, lard and high protein food were used in damp-heat rat ulcer model construction. The lard was greasy and likely to injury the spleen. Thus the damp heat constitution was easily formed by the hygrothermal environment plus lard [13]. As the role of trinitrobenzene sulfonic acid was clear in the construction of inflammatory bowel disease model, the model construction was considered to be successful in this study. As a result, the study showed that Huang-Qin-Tang could significantly reduce the expression of Th1 cytokines (INF- γ and IL-12) in model rats for damp-heat ulcerative colitis, and also improve the expression of Th2 cytokines (IL-4 and IL-10). Compared with the model rats in the model group, it was statistically significant ($P < 0.05$).

In summary, Huang-Qin-Tang could effectively improve the inflammatory response of ulcerative colitis rats. Besides, regulation of the Th1/Th2 cytokines balance could also be obtained for the clinical treatment of damp-heat ulcerative colitis. The therapy provided a new idea for further clinical treatment.

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