

Effects of pentasa-combined probiotics on the microflora structure and prognosis of patients with inflammatory bowel disease

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ABSTRACT

Background/Aims: The aim of the present study was to investigate the effects of the combination treatment of pentasa and probiotics on the microflora composition and prognosis in patients with inflammatory bowel disease (IBD).

Materials and Methods: A total of 40 patients with IBD (19 control group and 21 observation group) were randomized. Patients in the control group were given pentasa, and patients in the observation group were given probiotics along with pentasa. The microflora composition, biochemical indices, inflammatory markers, and activity scores of the two groups were analyzed.

Results: After treatment, the number of enterobacteria, enterococci, saccharomyces, and bacteroides; the levels of fecal lactoferrin, 1-antitrypsin, β 2-microglobulin, high-sensitivity C-reactive protein, and interleukin (IL)-6; activity scores; and recurrence rate in the observation group were significantly lower than those in the control group. Bifidobacterium and lactobacillus counts and IL-4 levels were significantly higher in the observation group than in the control group.

Conclusion: The combination of probiotics and pentasa can improve microflora composition in patients with IBD and reduce the level of inflammatory cytokines; therefore, it is worthy of further clinical validation.

Keywords: Pentasa, probiotics, inflammatory bowel disease, microflora structure, prognosis

INTRODUCTION

Inflammatory bowel disease (IBD) refers to diseases involved in chronic gut inflammation. The two main types of IBD are ulcerative colitis (UC) and Crohn's disease (CD), which are similar in etiology, pathogenesis, and clinical manifestations (1). Epidemiological studies show that the incidence of IBD is on the rise, mainly in Europe and North America, and a large number of patients are young and often with a lifelong risk of recurrence; there have been a few reports of IBD incidences in China (2). In recent years, researches on IBD have increasingly focused on the role of the intestinal flora (3,4). The main treatment strategy for IBD is immunosuppression via corticosteroids and biological agents, such as monoclonal antibodies; however, despite the short-term alleviation of symptoms, immunosuppressive treatments show poor long-term maintenance of intestinal integrity and considerable adverse effects that affect disease progression (5). Pentasa, a controlled-release form of the anti-inflammatory drug mesalazine, is recommended for patients with mild to moderate UC. It inhibits the synthesis and release

of prostaglandin, blocks the inflammatory cascade, and relieves intestinal mucosal inflammation; however, its long-term maintenance is still poor (6). Moreover, there are many reports on probiotic therapy for IBD, some of which have good function, and mainly contain lactobacillus, bifidobacterium, and saccharomyces (7). Compared with immunosuppressive agents, probiotics have good effect on repairing the intestinal mucosa but do not work well on extensive mucosal inflammation (8). Therefore, patients with IBD were treated with a combination of the anti-inflammatory pentasa and restorative probiotics, and then the clinical outcomes were evaluated to provide stronger evidence for clinical application.

MATERIALS AND METHODS

General data

A total of 40 patients with IBD who visited The First Affiliated Hospital of Fujian Medical University from January 2015 to June 2016 were selected and randomized into the control group (n=19) and observation group (n=21).

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The study was approved by the ethics committee of The First Affiliated Hospital of Fujian Medical University.

Inclusion criteria were (1) confirmed IBD diagnosis with mild and moderate symptoms as per the current standards in China, (2) no previous probiotic treatment, (3) no allergy to drugs used in the present study, and (4) cognizance of the purpose of the present study and willingness to sign an informed consent.

Exclusion criteria were (1) severe heart, liver, kidney, and other systemic diseases; (2) pregnancy or lactation; (3) unresponsive to medical treatment and with complications; and (4) immune system disorders.

Treatment regimens

Both groups were given the basic treatment for improving electrolyte balance and proper diet. The control group was given 1–2 pentasa (mesalazine extended action tablet) tablets once and three times a day and a maintenance dose of 1 tablet once and three times a day. The observation group was given 2 probiotics tablets (Bifico) once and three times a day and a largely liquid-based high nutrition diet, in addition to the pentasa regimen.

Observation indices

In the colony counts of gut microbes, a 0.5 g fresh feces was obtained from the subjects and serially diluted to 10^{-8} . Specific medium was inoculated with 10 μ L of the fecal diluent and cultured for 48 h at 37°C. The morphology of the resulting colonies was noted, the colonies were counted, and the bacteria were identified by Gram staining and biochemical tests. The number of bacteria in each gram of feces (CFU/g) was calculated, and the results were expressed in logarithmic form.

In biochemical tests, a 10 mL fasting venous blood was collected from each subject early in the morning into heparinized tubes. The blood samples were centrifuged at 5000 rpm for 5 min, and the supernatant was separated for immediate analysis or stored at -80°C for later use. The levels of 1-antitrypsin (ml027560), β 2-microglobulin (ml027518), high-sensitivity C-reactive protein (hs-CRP) (ml027874), IL-6 (ml038115), and IL-4 (ml027384) were detected by enzyme-linked immunosorbent assay (ELISA; Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai, China) according to the manufacturer's instructions. To measure fecal lactoferrin levels, 50–120 mg of sample was mixed with an extraction solution (1:100 w/v). After shaking vigorously, 1 mL of the solution was centrifuged, and then 0.5 mL supernatant was obtained for ELISA (ml024507). The activity scores of the two groups were calculated, and the incidence of adverse reactions and the recurrence rate after 1 year were recorded (Table 1).

Statistical analysis

Statistical Package for Social Sciences version 21.0 software (IBM Corp.; Armonk, NY, USA) was used for statistical analysis. Measurement data were expressed as mean $\pm$ SD. Count data were expressed as percentage and compared by the χ^2 test and Fisher's exact probability test as expressed by chi-square. t-Test was used to compare data with normal distribution, and the rank sum test was used for data with non-normal distribution. A p value <0.05 was considered statistically significant.

RESULTS

Patient data

There were no differences between the two groups with respect to gender, age, duration of disease, and the number of disease types (all $p>0.05$; Table 2).

Table 1. Activity scores of patients with IBD (score).

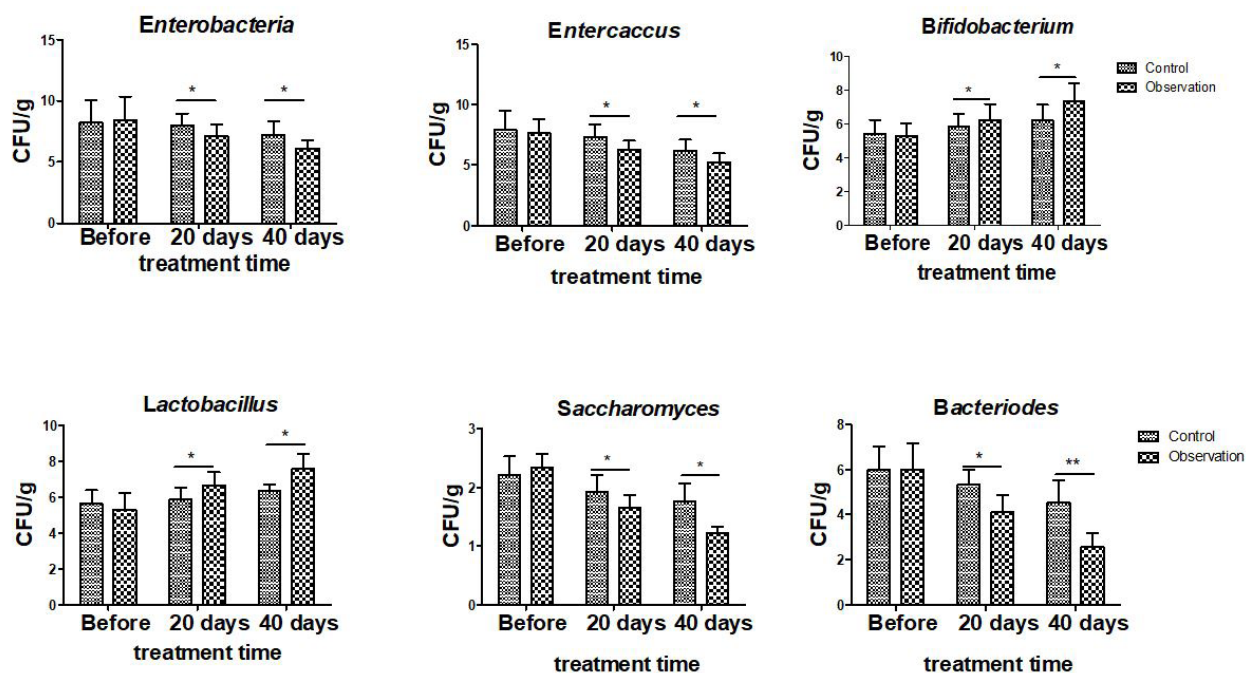
Items	UCAI	CDAI
Stomachache	No, 0; slight, 1; medium, 2; severe, 3	Same with UCAI
Diarrhea	0–2 times, 0; 3–4 times, 1; 5–6 times, 2; 7–9 times, 3; >10 times, 4	Once daily loose stool counts for 1
Others	Gross blood stool: no, 0; <50%, 1; >50%, 2; 100%, 3 Fecal incontinence: no, 0; yes, 1 Abdominal haphalgnesia: no, 0; slight, 1; medium resistance, 2; severe or rebound pain, 3	Abdominal mass: no, 0; doubtful, 1; confirmed, 2; with haphalgnesia, 3 Complication: every one count for 1 score

IBD: inflammatory bowel disease; CDAI: Crohn's disease activity index; UCAI: ulcerative colitis activity index.

Table 2. Comparison of general information ($\pm$ SD; n, %).

Group	Control group (n=19)	Observation group (n=21)	t/ χ^2	p
Gender (male/female)	10/9	10/11	0.1003	0.7515
Age (year)	39.97 $\pm$ 8.68	42.56 $\pm$ 7.58	1.0512	0.1495
Course of disease (year)	4.87 $\pm$ 1.58	5.03 $\pm$ 1.64	0.3333	0.3703
Types of disease			0.0435	0.8348
UC	15 (78.95)	16 (76.19)		
CD	4 (21.05)	5 (23.81)		

UC: ulcerative colitis; CD: Crohn's disease.

**Figure 1.** Changes in microflora composition at different stages in the two groups. The control group was given pentasa (1 tablet once and three times a day), and the observation group was given probiotics (2 tablets once and three times a day) along with pentasa. *p<0.05, **p<0.01.**Microflora composition in the two groups**

Before treatment, there was no difference in the fecal bacterial counts between the two groups (all p>0.05). After treatment, the number of enterobacteria, enterococci, saccharomyces, and bacteroides decreased significantly in the patients' feces of both groups, and the observation group had significantly lower counts than the control group. In contrast, the number of bifidobacteria and lactobacilli was significantly increased after treatment and was higher in the observation group than in the control group (all p<0.05; Figure 1).

Biochemical indices of both groups

Both groups showed similar pretreatment levels of fecal lactoferrin, serum 1-antitrypsin, and β 2-microglobulin (all p>0.05). After treatment, the above indices were significantly lower in observation group than in the control group (all p<0.05; Figure 2).

Inflammatory markers in both groups

The levels of inflammatory markers, such as hs-CRP, IL-6, and IL-4 levels, were similar in both groups prior to treatment (all p>0.05). After treatment during the same

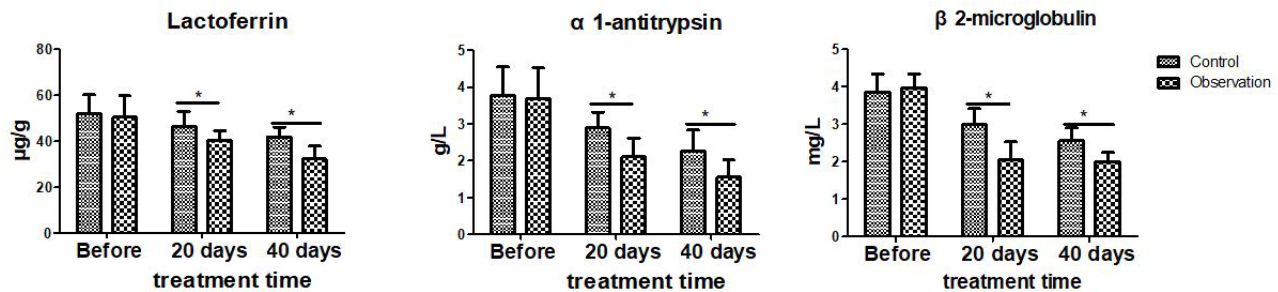


Figure 2. Comparison of biochemical indices of both groups. The control group was given pentasa (1 tablet once and three times a day), and the observation group was given probiotics (2 tablets once and three times a day) along with pentasa. * $p < 0.05$.

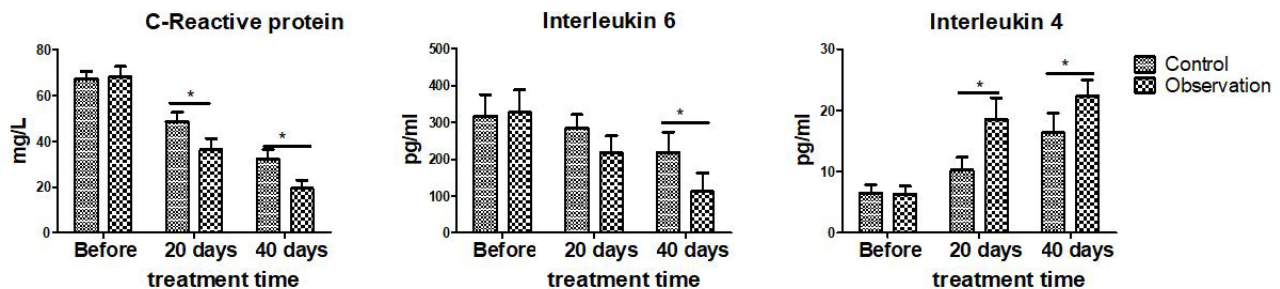


Figure 3. Comparison of inflammatory markers before and after treatment in the two groups. The control group was given pentasa (1 tablet once and three times a day), and the observation group was given probiotics (2 tablets once and three times a day) along with pentasa. * $p < 0.05$.

period, the levels of hs-CRP and IL-6 were significantly lower, and the level of IL-4 was significantly higher in the observation group than in the control group (all $p < 0.05$; Figure 3).

Prognosis of the two groups

After treatment, the Crohn's disease activity index (CDAI) and UC activity index (UCAI) scores, as well as the recurrence rate, in the observation group were significantly lower than those in the control group (all $p < 0.05$). There was no difference in the cases with adverse reactions between the two groups ($p > 0.05$; Table 3).

DISCUSSION

IBD is a common intestinal disease characterized by the loss of intestinal mucosa, congestion, and ulcers (9-11). The etiological factors of IBD mainly include infection, environmental stress, autoimmunity, and genetic factors, in addition to the breakdown of the mucosal immune system that causes immune failure of intestinal tract antigen and microflora imbalance (12-14). When the in-

testinal tract is colonized by conditioned pathogen and stimulated by exogenous stimuli, the intestinal tract multiplies and releases toxins that trigger an inflammatory reaction and damage the mucosal lining (15,16). However, the gut microflora or probiotics, which compete with these pathogens for space and nutrition, can inhibit their proliferation and maintain a balanced intestinal flora, thereby reducing intestinal tissue damage (17,18). In addition, the prebiotics synthesized by these probiotics can promote their growth, accelerate mucosal repair, restore immune response, and inhibit intestinal inflammation (19). The intestinal microflora is known to change under different physiological conditions. Bjerrum et al. reported that compared with healthy people, the intestinal microflora in patients with IBD is imbalanced, but at the same time, the number of probiotics is also higher in the patients, indicating that probiotics are a defense mechanism against intestinal inflammation (20-23).

Since the intestinal microflora is highly diverse, a single probiotic cannot restore its balance. Current probiotic

Table 3. Comparison of prognosis of the two groups ($\pm$ SD; n, %).

Group	Control group (n=19)	Observation group (n=21)	t/ χ^2	p
Activity scores				
CDAI	5.29 $\pm$ 2.48	3.86 $\pm$ 2.16	2.0587	0.0233
UCAI	8.54 $\pm$ 3.28	5.38 $\pm$ 2.76	3.4889	0.0006
Recurrence rate	6 (31.58)	1 (4.76)	3.2848	0.0395
Adverse reaction			0.8087	0.3284
Nausea	3 (15.79)	3 (14.29)		
Emesis	2 (10.53)	1 (4.76)		
Others	4 (21.05)	2 (9.52)		

CDAI: Crohn's disease activity index; UCAI, ulcerative colitis activity index.

treatments comprise composite probiotic preparations, such as the Bifico capsule, which is used to regulate the intestinal pH, improve enzyme activity, and antagonize pathogens. In the present study, probiotics treatment was found to reduce the number of *Escherichia coli*, enterococci, saccharomyces, bacteroides, and other conditional pathogens in the patients' feces and increase the number of probiotics, such as bifidobacteria and lactobacilli (all $p < 0.05$), thereby restoring the intestinal microflora. This is consistent with the observation that the intestinal flora is correlated with the onset of IBD; the intestinal flora only differs between healthy people and patients with active IBD with respect to species and quantity but has no difference between the active state and the remission state in patients.

The levels of fecal lactoferrin and blood α 1-antitrypsin and β 2-microglobulin were significantly lower in the observation group than in the control group (all $p < 0.05$), indicating that the local tissue damage has been effectively repaired upon probiotics administration. IL-6 can induce T cell apoptosis, result in abnormal accumulation of intestinal mucosal cells, and aggravate the inflammatory response (24). The levels of proinflammatory hs-CRP and IL-6 were significantly lower, and the level of anti-inflammatory IL-4 was significantly higher in the observation group than in the control group (all $p < 0.05$). The CDAI and UCAI scores in the observation group were significantly lower than those in the control group, whereas the recurrence rate of enteritis in the observation group was significantly lower than that in the control group (all $p < 0.05$). There was no difference in the number of adverse reactions between the two groups ($p > 0.05$).

In summary, the combination treatment of pentasa and probiotics for IBD can effectively re-adjust the composition of the intestinal microflora; reduce intestinal lactoferrin, blood 1-antitrypsin, and β 2-microglobulin levels; inhibit inflammatory factors; improve the activity score; and reduce the recurrence rate of enteritis in patients with IBD. The results indicate that our treatment regimen is worthy of further clinical application. However, our study has several limitations. The limitations of the present study include small cohort, short follow-up time, and doubtful patient compliance. In addition, since the specific pathogen of IBD enteritis has not been confirmed, there is uncertainty regarding the correlation between the intestinal microflora balance and IBD. Therefore, the IBD treatment with probiotics needs to be further studied with larger patient cohorts and more controlled conditions.

Ethics Committee Approval: This study was approved by the Ethics Committee of The First Affiliated Hospital of Fujian Medical University ((2018)077).

Informed Consent: N/A.

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Conflicts of Interest: The authors declare that there are no conflicts of interest.

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