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A probiotic treatment containing Lactobacillus, Bifidobacterium and Enterococcus improves IBS symptoms in an open label trial

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Abstract: Objective: To evaluate the efficacy and safety of live combined Bifidobacterium, Lactobacillus and Enterococcus capsules in treatment of irritable bowel syndrome. Methods: Eighty-five patients [male 32, female 53; age (45.31±11.72) years] were given live combined Bifidobacterium, Lactobacillus and Enterococcus capsules 1260 mg/d t.i.d.×4 weeks. Syndrome scales were used to evaluate the efficacy in gastrointestinal syndrome. Fecal flora was also measured before and after the treatment. Six bacteria were cultured and the colony forming units were counted in stool. SPSS was used for data analysis. Results: Seventy-four patients finished the follow-up. No side-effect was found. For treatment of irritable bowel syndrome, the effective rate of live combined Bifidobacterium, Lactobacillus and Enterococcus capsules was 56.8% in the second week, 74.3% in the fourth week and 73.0% in the sixth week. Single symptom was improved, especially in abdominal pain and stool character. The probiotica containing live combined Bifidobacterium, Lactobacillus and Enterococcus could increase bifidobacterium count (P<0.01) and lactobacillus count (P<0.05); decrease bacteroides count (P<0.05) and enterococci count (P<0.01); No obvious changes were observed in clostridium difficile colonitis and enterobacteriaceae (P>0.05). Conclusion: The result of the study indicated that the administration of live combined Bifidobacterium, Lactobacillus and Enterococcus improved the symptom of irritable bowel syndrome and that there was a gradual increase of this effect. Thereafter conditions remained stable for 2 weeks. That improvement may be associated with alterations in gastrointestinal flora.

Key words: Irritable bowel syndrome (IBS), Intestinal flora, Probiotic agents
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INTRODUCTION

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder. The typical symptoms are abdominal pain, flatulence, and irregular bowel movement. Prevalence rates are reported to be as high as 30% in the general population (Drossman *et al*., 1982), corresponding to about 25%~50% of all patients who need gastroenterologist's help (Everhart and Renault, 1991). Despite this high prevalence, the cause of IBS is unknown. Many factors are likely to give rise to symptoms like those of IBS e.g., food intake, disturbances in the intestinal bacterial flora, psychosomatic influences, changed motility in the colon (Maxwell *et al*., 1997). All these factors by themselves or in combination can exert an effect on the motor function of the gastrointestinal tract.

Using breath testing techniques, studies have demonstrated an association between small intestinal bacterial overgrowth (SIBO) and IBS (Pimentel *et al*., 2000). Some IBS blamed all their bowel symptoms on an acute bout of gastroenteritis. Nayak *et al*.(1997) demonstrated that IBS subjects treated with metronidazole were significantly better than placebo-treated patients, suggesting that bacteria may play a role in the symptoms of IBS.

The purposes of this study are to test whether

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intestinal flora is associated with irritable bowel syndrome and whether treatment by probiotics improves symptoms of IBS.

MATERIALS AND METHODS

Over one year period from 2002 through 2003, eighty-five patients with IBS satisfying the Rome II criteria were recruited from outpatients and inpatients in Sir Run Run Shaw Hospital. Of the total, thirty-two individuals were male and fifty-three were female. Patients ranged in age from seventeen to seventy-one years, mean (45.31±11.72) years. Colonscopy examination for each patient was normal. Exclusion criteria included: (1) severe systemic disease or diabetes mellitus or hyperthyreosis; (2) had taken antibiotics, antidiarrhea agents, laxative agents, spasmolytics or probiotics during the last two weeks; (3) failed to follow up due to side effect or others.

The study was performed as an open-label trial; all patients were given live combined Bifidobacterium, Lactobacillus and Enterococcus capsules, 1260 mg/d t.i.d.×4 weeks. The amount of each type of bacteria in one capsule was 5×10^7 CFU/g. Symptoms were evaluated with a symptom score at the beginning, two weeks after treatment, four weeks after treatment and two weeks after stopping treatment. The symptom score included the six parameters of pain time, pain frequency, stool character, stool urgency, passage of mucous frequency and abdominal distension. Every symptom was scored from 0 to 3 according to severity and a total composite score thereby obtained from 0 to 18. Total symptom relief: (1) obvious relief: symptom disappeared or total score decreased >80%; (2) moderate relief: total score decreased 50%~80%; (3) no relief: total score decreased <50%. Single symptom relief: (1) obvious relief: two score decreased; (2) moderate relief: one score decreased; (3) no relief: no relief or became worse. If the score decreased from 1 to 0, we considered it as obvious relief. Total relief rate was the summation of obvious relief rate and moderate relief rate. Stool character was recorded by Bristol stool character picture. Side effect was recorded at the same time.

Twenty patients' fecal samples for microbiological analyses were taken before and after treatment. The specimens were collected in sterile plastic containers, stored at 5 °C and sent to laboratory immediately. Then one gram of each fecal sample was homogenized in 1 ml dilution, serially diluted to 10^{-8}. The dilutions of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-3} and 10^{-1} were spread onto plates containing special agar media: EG (Eiken Chemical Co., Ltd., Japan) for anaerobes; LBS (Clinic Laboratory of the First Affiliated Hospital of Zhejiang University, China) for lactobacillus; BS (Clinic Laboratory of the First Affiliated Hospital of Zhejiang University, China) for bifidobacterium; NBGT (Clinic Laboratory of the First Affiliated Hospital of Zhejiang University, China) for bacteroides; TSN (bioMerieux, China) for clostridium perfringens; TS (bioMerieux, China) for aerobe; EMB (Microbial Factory in Hangzhou, China) for enterobacter; EC (Clinic Laboratory of the First Affiliated Hospital of Zhejiang University, China) for enterococcus. The first five media were anaerobically incubated for 48 h, the last three media were aerated incubated at 37 °C for 24 h. Bacterial concentrations were expressed as CFU per gram of wet feces. Representative colonies of each selective medium were identified to genus level by standard bacteriological procedures, such as Gram stain reaction, colonial morphology.

Data analysis

All data were analyzed by SPSS. Relief rate was counted as percentage, the colony forming units were counted as mean±*SD*, and statistical analysis was performed using *t*-test. Value of $P<0.05$ was considered statistically significant.

RESULTS

Seventy-four of the original 85 patients finished the follow-up. All tolerated the products well, and no adverse events were reported during the period of intake.

1. Total effective rate was 56.8% in the second week, 74.3% in the fourth week and 73.0% in the sixth week. The result indicated that the administration of live combined Bifidobacterium, Lactobacillus and Enterococcus improved the symptom of IBS and that there was a gradual increase of this effect. Thereafter conditions remained stable for 2 weeks (Table 1).

2. Single symptom was improved, especially in abdominal pain and stool character (Table 2).

Table 1 Total symptom relief rates [*n* (%)]

Weeks	Obvious relief	Moderate relief	No relief	Total symptom relief	Bristol stool character
2 weeks after treatment	4 (5.4)	38 (51.4)	32 (43.2)	42 (56.8)	4.33±1.31
4 weeks after treatment	4 (5.4)	51 (68.9)	19 (25.7)	55 (74.3)	4.00±0.74
2 weeks after stopping treatment	4 (5.4)	50 (67.6)	20 (27.0)	54 (73.0)	4.13±0.86

Table 2 Single symptom score and relief rate [*n* (%)]

Symptom	2 weeks after treatment			4 weeks after treatment			2 weeks after stopping treatment		
	Obviously relief	Moderate relief	Total relief	Obviously relief	Moderate relief	Total relief	Obviously relief	Moderate relief	Total relief
Pain time	5 (6.8)	34 (45.9)	39 (52.7)	16 (21.6)	44 (59.5)	60 (81.1)	24 (32.4)	35 (47.3)	59 (79.7)
Pain frequency	10 (13.5)	34 (45.9)	44 (59.5)	16 (21.6)	44 (59.5)	60 (81.1)	27 (36.5)	32 (43.2)	59 (79.6)
Stool character	17 (23.0)	32 (43.2)	49 (66.2)	19 (25.7)	39 (52.7)	58 (78.4)	21 (28.4)	38 (51.4)	59 (79.7)
Stool urgency	17 (23.0)	28 (37.8)	45 (60.8)	30 (40.5)	36 (48.6)	66 (89.2)	33 (44.6)	32 (43.2)	65 (87.8)
Mucous	10 (13.5)	22 (29.7)	32 (43.2)	14 (18.9)	30 (40.5)	44 (59.5)	15 (20.3)	21 (28.4)	36 (48.6)
Distension	10 (13.5)	22 (29.7)	32 (43.2)	11 (14.9)	23 (31.1)	34 (45.9)	12 (16.2)	24 (32.4)	36 (48.6)

3. No special pathovar bacteria were found in IBS group. The probiotica containing live combined Bifidobacterium, Lactobacillus and Enterococcus could increase bifidobacterium count ($P<0.01$) and lactobacillus count ($P<0.05$); decrease bacteroides count ($P<0.05$) and enterococci count ($P<0.01$); no obvious changes in clostridium difficile colonitis and enterobacteriaceae ($P>0.05$) (Table 3).

Table 3 Intestinal flora alteration after treated in IBS (CFU/g)

	Before treatment	After treatment
Lactobacillus	7.55±1.16	8.85±0.54*
Bifidobacterium	9.04±0.99	10.51±0.45**
Bacteroides	10.89±0.84	10.12±0.74*
Clostridium	9.34±0.91	8.97±0.97
Enterococci	9.43±0.97	7.20±1.15**
Enterobacteriaceae	9.33±0.81	9.30±0.77

* $P<0.05$; ** $P<0.01$

DISCUSSION

Irritable bowel symptom is a common disease and seriously affects life-quality. The cause of IBS is unknown and no treatment is definite. Recent studies indicated that IBS might be caused by intestinal flora alteration. King *et al*.(1998) found that colonic-gas production, particularly of hydrogen was greater in patients with IBS than in controls, and that both symptoms and gas production were reduced by an exclusion diet. This reduction may be associated with alterations in the activity of hydrogen-consuming bacteria. Balsari *et al*.(1982) showed in a study of 20 patients with IBS that there was great homogeneity in the fecal flora and that there was a decrease of coliforms, lactobacilli, and bifidobacteria in patients, compared with healthy individuals. Our research found that after receiving live combined Bifidobacterium, Lactobacillus and Enterococcus capsules 1260 mg/d, the effective rate was 56.8% in the second week, 74.3% in the fourth week. All suggested that intestinal flora imbalances play an important role in IBS patients.

Normal intestinal flora is important for health and is kept in balance. As protection barrier, it prevents foreign microorganism, provides essential microelement and vitamin, regulate metabolism. So the balance of intestinal flora is the foundation of normal gastrointestinal function, altered flora by any cause could lead to gastrointestinal symptom.

IBS has many risk factors, all which by themselves or in combination could exert effect on the motor function of the gastrointestinal tract, which resulted in entry of bacteria into the intestines and altered intestinal flora. Pimentel *et al*.(2000)'s study of 200 IBS patients showed 157 (78%) had small intestinal bacterial overgrowth. Of these, 47 had follow-up antibiotics-treatment testing. Twenty-five subjects had eradication of SIBO and improvement in IBS symptoms. Furthermore, 48% of SIBO eradicated subjects

no longer met Rome criteria (Pimentel *et al*., 2000). Stress, diet, infection, taking of excessive antibiotics could also cause decrease of the dominant flora and overgrowth of foreign flora or non-pathovar flora. Rodriguez and Ruigomez (1999) found that, after an episode of gastroenteritis, patients were 10 times more likely to have IBS than those in the general population. They concluded that bacterial gastroenteritis is a major independent risk factor for IBS.

Altered intestinal flora could result in excessive gas in the intestinal path. Using a radiological technique, Koide *et al*.(2000) found a considerable proportion of patients with IBS had excessive intestinal gas. King *et al*.(1998)'s study found that although total gas production in IBS patients was not greater than that in controls, the maximum rate of gas excretion was significantly greater. The exclusion diet reduced symptoms and produced a fall in maximum gas excretion. After taking lactulose, breath hydrogen was greater on the standard than on the exclusion diet. This reduction may be associated with alterations in the activity of hydrogen-consuming bacteria. Pimentel *et al*.(2003) found methane excretion was 100% associated with constipation-predominant IBS.

Diet regulation is the main method for IBS treatment but difficult to perform persistently. The belief in the beneficial effects of probiotic agents is based on the knowledge that live microbial feed supplements could beneficially affect the host animal by improving its microbial balance. The mechanism is unknown, maybe due to its promotion of chemical reaction, nutrition competition, immunity, receptor, combination, etc. As bifidobacteria produce both acetic and lactic acid. The production of those acids reduces intestinal pH, which in turn restricts or prohibits the growth of many putrefactive bacteria. O'Mahony *et al*.(2005)'s research found that B infantis 35624 could alleviate symptoms in IBS and that this symptomatic response was associated with normalization of the ratio of an anti-inflammatory cytokine to a proinflammatory cytokine, suggesting an immune-modulating role for this organism in IBS. In fact, a number of clinical studies showed that probiotic agents could improve the symptom (Kajander *et al*., 2005). Such as administration of VSL-3 improved the clinical picture and changed the composition and biochemistry of fecal microbiota. At the same time, a significant increase in lactobacilli, bifidobacteria and streptococcus thermophilus was observed as a consequence of probiotic treatment, while enterococci, coliforms, bacteroides and clostridium perfringens did not change significantly (Brigidi *et al*., 2001).

In sum, our research, which accorded with Nobaek *et al*.(2000)'s conclusion, indicated that the administration of live combined Bifidobacterium, Lactobacillus and Enterococcus improved the IBS symptom. That improvement may be associated with alterations in gastrointestinal flora. The puzzling result was that enterococcus levels went down even though enterococcus was in the capsule. We speculate that some intestinal flora balance mechanism or other unknown reasons may play a role. A large-scale, double-blind and placebo-controlled clinical study is needed in future research.

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