

Original Article

Multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study

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Abstract: Aim: To investigate the effect of bifid triple viable capsule, a multistrain probiotic preparation on symptoms of irritable bowel syndrome (IBS), and the amount of fecal Bifidobacterium spp. and Lactobacillus spp. of IBS patients before and after treatment. Methods: A total of 60 IBS patients who met Rome III criteria were included in this double-blind, randomized, and placebo-controlled study. The patients were randomly assigned to receive composite probiotics or placebo for four weeks. The IBS symptoms of participants were surveyed using a questionnaire, and the amount of fecal Bifidobacterium spp. and Lactobacillus spp. was determined by quantitative Real-time PCR pre- and post-intervention. Results: During the four week intervention period, the patients receiving probiotic preparation showed a significantly greater improvement in the symptom severity score of IBS, severity and frequency of pain or discomfort, abdominal distention and satisfaction with bowel habits. The symptom subtypes revealed that low amounts of both Bifidobacterium spp. and Lactobacillus spp. were present in the samples of diarrhea-predominant IBS patients, while the alternating-predominant IBS patients had only low amounts of Bifidobacterium spp. Post-intervention for diarrhea-predominant IBS patients with lower symptom severity score showed even lower amounts of Bifidobacterium spp. and Lactobacillus spp. Conclusion: Bifid triple viable capsule supplement may benefit patients with IBS. Multistrain probiotic preparation may be a promising candidate for IBS therapy, although the mechanism needs to be further studied.

Keywords: Irritable bowel syndrome, probiotics, real-time PCR, gut microbiota

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder, characterized by recurring symptoms of abdominal pain, bloating, and altered bowel function in the absence of structural, inflammatory, or biochemical abnormalities [1]. IBS results in not only great inconvenience to patients, but also significant impact on their quality of life and social functioning. Although IBS has not been associated with the development of more serious diseases or excessive mortality, treating IBS is important because the symptom causes substantial impairment in life quality, resulting in increased use of health resources and reduced work productivity.

The pathogenesis of IBS is far from clearly defined. Multiple features may affect IBS etiology,

including stress, altered GI motility, visceral hypersensitivity, altered intraluminal milieu, and immune activation [2]. It has been proposed that IBS may result from a dysfunctional interaction between the indigenous flora and the intestinal mucosa, leading to immune activation in the colonic mucosa [3]. Changes in the colonic microbiota could result in the proliferation of gas-producing organisms, which facilitate deconjugation of bile acids, and thereby influence water and electrolyte transport in the colon.

Dysregulation of the microbiota is also linked to the growing evidence for the onset of IBS following an attack of acute gastroenteritis, which is associated with on-going inflammation induced by the infecting organisms.

Patients with IBS may have alterations in the

Table 1. Baseline characteristics of the study population.

	Controls	IBS patients
Age(yr): Mean±SD	48.45±14.08	44.38±15.08
Gender: M/F	6/14	18/42
BMI(kg/m ²): Mean±SD	20.77±2.66	21.62±3.07
Predominant bowel habit: n(%)		
Diarrhea		29 (48.3%)
Constipation		18 (30.0%)
Mix		7 (11.7%)
Un-typed		6 (10.0%)

intestinal microbiota. Thus, investigators logically consider targeting the intestinal microbiota for the treatment of this condition. It has been affirmed that use of probiotic-based products is beneficial for the control of IBS symptoms. Probiotic are live microorganisms, and can confer health benefits on the host when administered in an adequate amount [4].

The probiotic product used in this study comprises a consortium of *Bifidobacterium longum* and *Lactobacillus acidophilus* organisms. The aim of this randomized, double-blind placebo-controlled trial was to assess the potential of bifid triple viable capsule to attenuate the symptoms of IBS and evaluate the amount of fecal *Bifidobacterium* spp. and *Lactobacillus* spp. before and after receiving the bifid triple viable capsule for four weeks.

Materials and methods

Participants

Consecutive patients who met the Rome III criteria for IBS were selectively included in the study, which was conducted in the Department of Internal Medicine and Gastroenterology of the XinHua Hospital, Shanghai, China. The exclusion criteria were pregnant, a history of inflammatory bowel disease, diabetes, unstable thyroid disease, previous abdominal surgery (other than cholecystectomy or appendectomy), human immunodeficiency virus infection, and renal or hepatic disease, currently taking alosetron, tegaserod, lubiprostone, warfarin, or antipsychotic, antispasmodic, antidiarrheal, probiotic, narcotic drugs or antibiotics within the previous 14 days or rifaximin within 60 days. Smoking habits of healthy subjects or IBS patients were not recorded. Healthy subjects were recruited to form an age- and gender-matched control group

for those IBS patients (Table 1). All patients provided written informed consent before study-related procedures were initiated. The Human Ethics Committee of Gastroenterology approved the study protocol.

The participants were randomly divided into probiotic group and placebo group. During the trial, participants in probiotic group ingested two bifid triple viable capsules three times per day, and the placebo group ingested 200 mg placebo capsule three times per day (produced by Shanghai Sine Pharmaceutical Co. Ltd.). Thirty-seven patients received probiotic treatment and twenty-three patients received the placebo treatment. All patients in the clinical investigation underwent an endoscopy operation 0-1 year prior to the study.

Study design

This was a double-blind placebo-controlled study, which was conducted over a period of four weeks. In the study, subjects were asked to complete a validated questionnaire to assess IBS symptoms at the beginning of the study as the baseline and at the fourth week after treatment. Six gastrointestinal symptoms were recorded. The time of abdominal pain and abdominal distension using a rating scale (0: none; 1: < 1 h/d; 2: 1-8 h/d; 3: > 8 h/d), the frequency of abdominal pain, abdominal distension, satisfaction with bowel habits and satisfaction with defecation using the scale (0: none; 1: occasional; 2: often; 3: always). The assessment of compliance was based on the returned capsules.

During the four-week study, two fecal samples were obtained from the IBS patients at the beginning of the study and at the fourth week after treatment. One fecal sample was collected from

Table 2. Primer of Bifidobacterium and Lactobacillus for Real-time PCR.

Bacterium	Primer	Primer sequence (5'→3')	amplicaon size(bp)
Bifidobacterium	g-BifF	ctc ctg gaa acg ggt gg	520
	g-BifR	ggt gtt ctt ccc gat atc tac a	
Lactobacillus	L159-f	gga aac agr tgc taa tac cg	600
	L677-r	cac cgc tac aca tgg ag	

the healthy subjects at the beginning of the study. Fecal samples were stored anaerobically immediately after defecation, aliquoted and stored at -70°C within 4 h of delivery.

Bacterial strains and conditions/Control DNAs

The following bacteria were used to evaluate the specificity of PCR primer sets (**Table 2**), *Bifidobacterium longum* DSM 20219 and *Lactobacillus acidophilus* DSM 20079.

DNA extraction from feces

DNA was extracted from 200 mg of fecal by using a QIAGEN stool kit with a modified protocol for cell lysis analogous to the one described for bacterial cells. Briefly, cells were collected by centrifugation at 20,000 g (10 min) and were resuspended in 1.4 ml of ASL lysis buffer. Glass beads (350 mg) were added, and the slurries were bead beaten for 2 min at the maximum speed. After the suspension was heated for 5 min at 95°C, the bead-beating step was repeated, and the cell debris was removed by centrifuging at 5,000 g for 2 min. DNA-damaging substances and PCR inhibitors were removed from the DNA-containing supernatant by using the Inhibit EX tablets provided in the kit. Protein digestions and DNA purifications were done using QIAamp spin columns, according to the manufacturer's instructions.

Primers and PCR amplification

Primers used in this study (**Table 2**) were designed on the basis of 16S rRNA gene sequences available from the GenBank database. Quantitative PCR was performed with LightCycle (Roch USA). All PCRs were performed in duplicate in a volume of 20 µl. Amplification reactions were done with SYBR Premix Ex Taq (Takara) containing 3 mM MgCl₂, 20 mM Tris HCl (pH 8.4), 50 mM KCl, 200 mM dNTPs, SYBR Green I, 10 nM fluorescein, 0.625 U of Taq DNA polymerase mixed with the selected primers at

a concentration of 0.5 mM for each primer, and 1.6 µl of the respective template DNA or water. Amplifications were performed at one cycle at 95°C (3 min), 40 cycles of denaturation at 95°C (40 sec), primer annealing (75 sec), and one final cycle at 95°C (30 sec). Finally, melt curve analyses were made by slowly heating the PCR mixture from 55 to 95°C (1°C per cycle of 10 s) with simultaneous measurements of the SYBR Green I signal intensities. Quantitative analysis was done by using standard curves made from known concentrations of plasmid DNA containing the respective amplification for each set of primers.

Statistical analysis

The endpoints were analyzed by a Wilcoxon rank sum test, and the effective rate was analyzed by X² test. Two-sided *P* values < 0.05 were considered statistically significant, and the 95% confidence interval (CI) was calculated for the main outcomes. Logarithms of fecal 16S rRNA gene copy numbers were used to achieve normal distribution, and the mean ± standard deviation (SD) was calculated. All statistical analyses were carried out using SPSS 11.0.

Results

Table 3 shows the demographic and baseline characteristics of the subjects. The two groups of subjects were similar in terms of age, gender, type of bowel habit, and symptoms. Subjects receiving the probiotic preparation had the same IBS severity score at baseline as the subjects receiving placebo (**Table 4**).

After the four-week intervention period, significant difference was seen between the probiotic group and the placebo group on time and frequency of events of pain or discomfort, abdominal distention and dissatisfaction with bowel habits. No significant difference was found between these two groups in regard to dissatisfac-

Table 3. Baseline characteristics of the study population.

	Probiotic	Placebo
Gender (m/f)	11/26	7/16
Age (M $\pm$ SD)	42.92 $\pm$ 15.09	47.45 $\pm$ 15.36
BMI (kg/m ²)	22.08 $\pm$ 3.23	20.77 $\pm$ 2.65
Predominant bowel habit: n(%)		
Diarrhea	18(48.6%)	11(47.8%)
Constipation	11(29.7%)	7(30.4%)
Mix	4(10.8%)	3(13.0%)
Untype	4(10.8%)	2(8.7%)

Table 4. Baseline of the IBS patients.

	Probiotic	Placebo
Time of abdominal pain	2.00 $\pm$ 0.67	2.08 $\pm$ 0.73
Frequency of abdominal pain	2.11 $\pm$ 0.77	1.95 $\pm$ 0.56
Dissatisfaction with bowel habits	2.08 $\pm$ 0.54	2.17 $\pm$ 0.49
Dissatisfaction with defecation	1.78 $\pm$ 0.41	1.78 $\pm$ 0.60
Time of abdominal distension	1.79 $\pm$ 0.82	1.65 $\pm$ 0.49
Frequency of abdominal distension	1.91 $\pm$ 0.82	1.86 $\pm$ 0.69
Total scores	11.68 $\pm$ 1.82	11.35 $\pm$ 1.43

Table 5. Change in symptom severity in treatment and placebo group at week 4.

		effective	ineffective	worsen	P-value
Time of abdominal pain	Probiotic	31	5	1	P<0.01
	Placebo	7	14	2	
Frequency of abdominal pain	Probiotic	23	12	2	P<0.01
	Placebo	6	14	3	
Dissatisfaction with bowel habits	Probiotic	26	10	1	P<0.01
	Placebo	8	13	2	
Dissatisfaction with defecation	Probiotic	20	15	2	P>0.01
	Placebo	10	12	1	
Time of abdominal distension	Probiotic	27	10	0	P<0.01
	Placebo	9	13	1	
Frequency of abdominal distension	Probiotic	27	10	0	P<0.01
	Placebo	7	16	0	

tion with defecation (**Table 5**). The effective rate of the probiotic group was superior to the placebo group (**Table 6**).

Sorting of the IBS patients according to the symptom subtypes, a summary of all findings are described in **Table 7**. The 16S rRNA gene copy number of *Bifidobacterium* spp. was significantly reduced in both diarrhea-type IBS patients and mix-type IBS patients, compared with the healthy controls. Similarly, the 16S rRNA

gene copy number of *Lactobacillus* spp. was significantly lower in diarrhea-type IBS patients than that of the healthy controls.

After 4 week probiotic treatment, the gene copy numbers of 16S rRNA of *Bifidobacterium* spp. and *Lactobacillus* spp. were significantly greater in both diarrhea-type IBS patients and constipation-type IBS patients than those at the beginning of the study (**Table 8**). No significant differences were seen between IBS patients treated

Multistrain probiotic preparation for treating irritable bowel syndrome

Table 6. The effective rate of probiotic group and placebo group.

group	effective	ineffective	effective rate	p-value
Probiotic group	24	13	64.86%	<0.01
Placebo group	7	16	30.43%	

Table 7. Fecal Bifidobacterium spp. and Lactobacillus spp. of control versus IBS patients beginning of the study^a.

Group	Control	Diarrhea	Constipation	Mix	Untype
	(n=20)	(n=29)	(n=18)	(n=7)	(n=6)
Bifidobacterium spp.	9.12±0.24	7.52±0.76*	8.91±0.30	7.75±0.70**	8.71±0.44
Lactobacillus spp.	8.02±0.18	7.49±0.31*	8.10±0.17	8.01±0.33	7.78±0.40

account unit is Log₁₀ copies/g fecal, Data mode is M±SD. *p<0.001 compared with control group; **p<0.01 compared with control group.

Table 8. Bifidobacterium spp. and Lactobacillus spp. of IBS patients beginning of the study versus IBS patients treated by probiotic for 4 week.

	Type	Beginning	4-week later	p-value
Bifidobacterium spp.	Diarrhea (n=18)	7.09±0.83	10.14±0.74	p<0.01
	Constipation (n=11)	8.91±0.32	10.52±0.41	p<0.01
Lactobacillus spp.	Diarrhea (n=18)	7.41±0.23	9.15±0.23	p<0.01
	Constipation (n=11)	8.08±0.18	9.84±0.32	p<0.01

Table 9. Bifidobacterium spp. and Lactobacillus spp. of IBS patients at the beginning of the study versus IBS patients treated by placebo for 4 week.

	Type	Beginning	4-week later	p-value
Bifidobacterium spp.	Diarrhea (n=11)	7.35±0.63	7.41±0.53	p>0.01
	Constipation (n=7)	8.91±0.13	8.76±0.16	p>0.01
Lactobacillus spp.	Diarrhea (n=11)	7.59±0.22	7.70±0.25	p>0.01
	Constipation (n=7)	8.13±0.15	8.20±0.19	p>0.01

by placebo and that group patients of the beginning of the study (**Table 9**).

In diarrhea-type IBS patients, the gene copy numbers of 16S rRNA of Bifidobacterium spp. and Lactobacillus spp. was significantly higher in the patients who were ineffective to the treatment of probiotic than the patients who were effective to the treatment. However, no significant difference was found in the constipation-type IBS patients (**Table 10**).

Discussion

Significant differences in the effective rate were

recorded between the probiotic and placebo groups. The overall placebo response rate observed in this study is comparable to that seen in many other IBS studies [5]. Several RCTs have been set up with IBS sufferers to assess the efficacy of multistrain probiotic preparations containing a variety of organisms at different doses and different study periods and the responses have been variable [6]. It was demonstrated that probiotics appear to be efficacious in IBS.

Pharmacologic therapy for IBS has primarily targeted individual symptoms by means of antidiarrheals, laxatives and antispasmodics

Table 10. Fecal Bifidobacterium spp. and Lactobacillus spp. of IBS patients treated by probiotic and treatment effect.

	type	effect	ineffective	p-value
Bifidobacterium spp	Diarrhea	9.75±0.60	10.75±0.49	p<0.01
	Constipation	10.60±0.30	10.42±0.54	p>0.01
Lactobacillus spp	Diarrhea	9.03±0.11	9.35±0.23	p<0.01
	Constipation	9.70±0.29	10.00±0.29	p>0.01

[7]. Some successes occur using antidepressants and serotonergic agents but the latter are associated with some safety issues. In general, success with these medications has been limited. Attention has therefore focused on probiotics because consumption of probiotics is substantial in this most populations regardless of medical indications.

Probiotics are defined as “live microorganisms and they can confer a health benefit on the host when administered in adequate amounts. Evidence of benefit is the strongest for the treatment of antibiotic associated diarrhoea and the resolution of infectious gastroenteritis. There is also reasonable evidence indicating a modest benefit on flatulence in IBS patients [8]. However, probiotic approaches are and will always be confounded by the diversity of the human microbiota and its plasticity in the face of varied human diets and genetic backgrounds.

In humans, the interaction between animal and bacterial cells is especially important in the gastrointestinal tract. Technical and conceptual advances have enabled rapid progress in characterizing the taxonomic composition, metabolic capacity, and immunomodulatory activity of the human gut microbiota, allowing us to establish its role in human health and disease [9, 10]. The human host coevolved with a normal microbiota over millennia, and it developed, deployed, and optimized complex immune mechanisms that monitor and control this microbial ecosystem. These cellular mechanisms have homeostatic roles beyond the traditional concept of defense against potential pathogens, suggesting that these pathways contribute directly to the well-being of the gut.

Finally, with a full understanding of the host-microbe interaction in the gut, perhaps the pathogenic side of this relationship can be suppressed, whereas the beneficial effects of the

gastrointestinal microbiota can be maintained and restored.

The present study shows that the potential benefit of bifid triple viable capsule probiotic supplement in the treatment of IBS. Future studies will aim to identify the mechanism of the potential beneficial effect of probiotics [11].

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