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Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome

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SUMMARY

Background
The intestinal permeability is increased in patients with diarrhoea-predominant irritable bowel syndrome (D-IBS).

Aim
To determine the possible efficacy of lactic acid bacteria on the increased intestinal permeability in D-IBS.

Methods
Treatment was employed for 4 weeks in a randomized single blind placebo controlled study with 30 D-IBS patients. Patients were given either probiotic fermented milk (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium Longum*) or milk beverage containing no bacteria. The clinical symptoms were scored and intestinal permeability was measured by a triple sugar test before and after treatment.

Results
Small bowel permeability was measured as the ratio of lactulose and mannitol recovery and colonic permeability was measured as the total mass of sucralose excretion (mg). After probiotics treatment, small bowel permeability decreased significantly from 0.038 (0.024) at baseline to 0.023 (0.020) ($P = 0.004$), the proportion of patients with increased small bowel permeability was lower than baseline (28.6% vs. 64.3%, $P = 0.023$). However, colonic permeability improved neither in the probiotics group nor in the placebo group at week 4. Treatment with probiotics significantly decreased the mean global IBS scores compared with the baseline scores (9.62 ± 1.05 vs. 7.64 ± 1.24, $P < 0.001$).

Conclusion
Short-term active lactic acid bacteria treatment for D-IBS improved mucosal barrier function.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by chronically recurring abdominal pain or discomfort relieved after defecation and altered bowel habits. While largely considered to be a multifactorial disease involving abnormal gastrointestinal motility, visceral hypersensitivity, psychosocial factors and immune activation, the concise aetiology and pathophysiology of IBS remain unknown. Recently, studies from several research groups support a view that the impaired intestinal mucosal barrier function, as measured by an increase in the intestinal permeability, may be implicated in the pathogenesis of some patients with diarrhoea-predominant IBS (D-IBS).[1–4] The increased intestinal permeability in D-IBS has been shown to be accompanied by persistent low-grade immune activation in the intestine[1, 5–9] presented as increased numbers of T lymphocytes, mast cells and enterochromaffin cells. In its normal condition, gut epithelial lining forms a relatively impermeable barrier between luminal contents and submucosa required for intestinal homeostasis. This barrier is determined by complexes of proteins composing the junctional complexes. Tight junctions (TJs) are the most apical organelle of the epithelial junctional complexes and are crucial for the formation and function of epithelial barriers. TJs comprise numerous proteins, with the best characterized being zonula occludens (ZO)-1 and occludin.[10, 11]

Attenuation of the inflammation and preservation of the impaired mucosal barrier function may be an attractive therapy for D-IBS. One option is to use glucocorticoid to reduce the inflammation. However, glucocorticoid therapy failed to improve symptoms and rectal inflammation in D-IBS.[12] A promising alternative is to use probiotic bacteria that interact with the host epithelium to resolve inflammation and preserve the barrier function. Probiotics are defined as 'living microorganisms that (when ingested) have a beneficial effect in the prevention and treatment of specific pathological conditions'.[13] Probiotics have been proposed to exert beneficial effects by enhancing gut barrier function, maintaining a normal intestinal milieu, synthesizing antibacterial substances and stimulating local immunity etc.[14, 15] Currently, the most commonly studied probiotics are the lactic acid bacteria (LAB). Some strains of LAB including Bifidobacterium and Lactobacillus species have been shown to relieve symptoms[16–18] and improve health-related quality of life in IBS.[19] In addition, they have been proven to exert the beneficial effect in IBS by stabilizing intestinal microbiota[20] and normalizing abnormal interleukin (IL)-10/IL-12 ratio produced by peripheral blood mononuclear cell.[18] However, whether probiotics can improve the intestinal mucosal barrier function in D-IBS remains unknown. Therefore, we performed a single-blind randomized placebo-controlled study of probiotic fermented milk containing a multistrain of active LAB for patients with D-IBS and compared the intestinal permeability before and after treatment.

MATERIALS AND METHODS

Patients and normal controls

Patients were constitutively enrolled from the outpatient gastroenterology clinic at Qilu Hospital of Shandong University. The inclusion criteria were as follows: (i) the presence of Rome II criteria for D-IBS; (ii) negative screening examinations within 3 months including detailed history, warning symptoms, physical examination, colonoscopy and biopsy or barium enema examination, abdominal ultrasonography, whole blood count, stool routine, faecal occult blood test, stool culture, antiendomysial antibody, erythrocyte sedimentation rate, C-reactive protein, liver function tests, thyroid function tests, fasting plasma glucose, calcium, electrolytes and hydrogen breath test; (iii) having at least moderate IBS symptoms during 2 months before the study, defined as the scores of abdominal pain and diarrhoea subscales ≥4 on the seven-point Gastrointestinal Symptom Rating Scale (GSRS) respectively. Exclusion criteria were: (i) age <18 years; (ii) other organic gastrointestinal diseases, such as peptic ulcer, inflammatory bowel diseases (IBDs), coeliac disease, gastrointestinal infection and lactose intolerance; (iii) organic diseases: diabetes mellitus, hepatic, renal or cardiac dysfunction, thyroid disease or tumour etc.; (iv) the use of aspirin, nonsteroidal anti-inflammatory drugs, alcohol and other medications known or suspected of gut damage for the 2 weeks prior to the study; (v) pregnancy or lactation. Normal controls were subjects without any digestive complaints who underwent colonoscopy for polyp surveillance or regular routine health examination and their colonoscopic manifestation appeared normal.

In all, 30 patients with symptomatic D-IBS and 12 asymptomatic controls were investigated. There are six patients who had a history of acute gastroenteritis

before the onset of IBS symptoms. The gastrointestinal infection has been excluded by symptoms (no blood or yellow mucus in stool, no fever, no watery stool) and negative tests (complete blood count, stool routine and stool culture) before enrolment in all patients. Three colonoscopic biopsies were collected from the recto-sigmoid in each subject and were immediately stored in −80 °C for RNA extraction or fixed in glutaraldehyde followed by embedding in resin for transmission electron microscopy (TEM). Informed written consent was obtained from each subject. This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and the study protocol was approved by the ethics committees in Shandong University affiliated Qilu Hospital.

Clinical protocol

This trial was designed as a randomized single-blind placebo-controlled study. All patients were required to stop their ongoing treatment for IBS a week before enrolment and no other treatments except the study drinks were permitted during the study period. The eligible 30 patients were enrolled at 1 week after colonoscopic biopsies and randomized (in a 1:1 ratio) to take a commercially available probiotic fermented milk (AB100 Jianneng; Bright Dairy, Shanghai, China) or a formulated milk beverage (DuDu; Bright Dairy) for 4 weeks. The probiotic fermented milk contained *Streptococcus thermophilus* [1.0×10^8 colony forming units(cfu)/mL], *Lactobacillus bulgaricus* (1.0×10^7 cfu/mL), *Lactobacillus acidophilus* (1.0×10^7 cfu/mL) and *Bifidobacterium longum* (1.0×10^7 cfu/mL). One of the researchers performed general LAB counts on a sample of the probiotic fermented milk to confirm that they were active. Patients were blinded to this study and took the probiotic fermented milk 200 g or placebo drink 200 mL twice daily half an hour before meals. The milk beverage was chosen as a placebo because it has identical colour and consistency to AB100 while having no bacterial content. The tastes of the two products were also very similar making it difficult to distinguish the two products from their tastes. However, it is still possible that some patients might have recognized the difference in taste. Thus, we excluded patients who regularly took probiotic products from this study before enrolment. We removed the commercial labels of the bottles and applied study labels to identify the patients. The clinical symptoms and intestinal permeability were evaluated at weeks 0 and 4. Researchers checked patients' consumption and recorded missed or refused drinks to assess compliance. A patient who took more than 80% of the drinks was considered to be compliant.

Sample size

Our preliminary study measured the small intestinal permeability in 8 patients with D-IBS by using the lactulose–mannitol test and calculated the proportion of patients with increased small intestinal permeability (68.9%). An expected proportion of increased small intestinal permeability after treatment with placebo was 65%. On the basis of result of our preliminary study, we estimated that the required sample size of 30 patients (15 in each arm) has a power of 80% to detect a difference of 30% in the proportion of increased small bowel permeability between the placebo group and the probiotics group after treatment at the significance level of 5% with a low dropout rate (<5%).

Clinical symptoms

The IBS symptoms were assessed by the GSRS questionnaire, which has a good reliability and validity for evaluating common gastrointestinal symptoms.[21] The clinical symptoms were evaluated and agreed by at least two investigators to diminish observer bias. The GSRS consists of 15 items, each rated on a seven-point Likert scale ranging from 'no discomfort at all' to 'very severe discomfort', with higher scores indicating more severity of symptoms. The scores for abdominal pain, reflux, diarrhoea, indigestion and constipation subscales are calculated by averaging the scores of the items completed within an individual subscale. The global IBS scores in this study were calculated by the sum of scores of two subscales of abdominal pain and diarrhoea. Patients were also asked to complete a daily diary to evaluate symptoms (abdominal pain, abdominal bloating and sensation of flatulence) on 100 mm visual analogue scale (VAS) scales.

Measurement of intestinal permeability

The intestinal permeability, which has the ability to assess the gut mucosa integrity,[22] was measured by a triple sugar test. After an overnight fast, patients and healthy volunteers were asked to empty their bladders

and then were given a test solution by one of the investigators, containing 10 g lactulose (obtained as 15 mL of Duphalac syrup; Solvay Pharmaceuticals, Marietta, GA, USA), 5 g mannitol (Baxter Healthcare Co., Ltd, Shanghai, China) and 5 g sucralose (Shanghai Plucky International Trade Co., Ltd, Shanghai, China) in 100 mL tap water. Urine was then collected for the following 24 h. After the first 2 h, an intake of water and food was allowed. Urine passed during the first 5 h and the last 19 h were collected into two separate containers with 1 mL 10% sodium merthiolate as preservative. The total volumes of urine collected during the first 5 h and the 24 h were measured by the first author and urinary sugar concentrations were determined by gas chromatography as previously described.[23, 24] A chemist in Shandong University performed the gas chromatography with no knowledge of this study. The ratio of lactulose and mannitol (L/M) recovery during the first 5 h was used as an index of small intestinal permeability and the total mass of sucralose excretion (mg) during the 24 h was used as an index of colonic permeability.[22]

RNA isolation and cDNA synthesis

Total RNA was extracted from two colonic samples using Trizol reagent (Invitrogen, Diego, CA, USA) according to the manufacturer's instruction by investigators. All samples were treated twice with RNase-free DNase I. RNA quality was determined by gel electrophoresis and RNA quantity was determined by photometry. For reverse transcription, 1 μg of the total RNA was reversed transcribed to complementary DNA with oligo-dT primers and 50^{U} M-MLV (Promega, Madison, WI, USA) in a total volume of 20 μL according to the routine procedure. Synthesized cDNA was stored in −20 °C.

Real-time quantitative polymerase chain reaction

Real-time quantitative polymerase chain reaction (qPCR) assays were performed in a fluorescence temperature cycler (LightCycler; Roche Diagnostics GMBH, Mannheim, Germany) by investigators. Briefly, 1.0 μL cDNA was used as a template in a 20 μL reaction containing 1.5 μM of each primer and 10 μL 1 × SYBR Green PCR Mix (Takara, Japan). Specific primers were designed as follows: ZO-1 sense: 5′-TACCTCTTGAG CCTTGAACTT-3′; anti-sense 5′-CGTGCTGATGTGCCAT AATA-3′, 259 bp; occludin[25] sense: 5′-TGCATGTTC GACCAATGC-3′; anti-sense: 5′-AAGCCACTTCCTCCA TAAGG-3′, 235 bp. Amplification cycles included initial denaturation at 95 °C for 10 s followed by 40 cycles, each cycle consisting of 95 °C for 10 s, 60 °C for 10 s and 72 °C for 15 s. Ten microlitres of each PCR reaction was electrophoresed on a 2% agarose gel in 1 × Tris–acetate–ethylenediaminetetraacetic acid buffer (EDTA). The point (designated as C_t value) is when the fluorescence intensity exceeds 10 standard deviation (s.d.) above the mean baseline fluorescence. All quantifications were normalized to the housekeeping-gene β-actin. Relative expression is calculated using the formula $2^{(R_t-E_t)}/2^{(R_n-E_n)}$ as described previously,[26] where R_t is the C_t observed in the experimental sample for β-actin, E_t is the C_t observed in the experimental sample for a specific gene, R_n is the average C_t observed in the normal control samples for β-actin and E_n is the average C_t observed in the normal control samples for a specific gene.

Transmission electron microscopy

One colonoscopic biopsy from each subject was immediately fixed in 2.5% glutaraldehyde for at least 24 h at 4 °C and postfixed in 2% osmium tetroxide prepared in 0.1 M phosphate buffer (pH 7.4) for 1 h. Samples were subsequently dehydrated by graded ethanol and embedded in EM bed 812 resins. Sections were cut and examined in a Hitachi H-600 electron microscope (Hitachi Ltd, Tokyo, Japan). The sample preparation and assessment were performed by Fengyi Scientific Center of Shandong University, unaware of the identity and clinical condition of the subjects.

Data analysis

The primary endpoint was the improvement in proportions of patients with abnormal intestinal permeability after 4 weeks treatment. The proportions in two treatment groups were compared with baseline. The secondary endpoint was the improvement in IBS symptoms.

Statistics

Categorical data were presented as proportions and analysed by chi-squared testing. Parametric data were expressed as means ± s.d., while nonparametric data were reported as medians with interquartile ranges. Normality of all data sets was determined using the

Kolmogorov–Smirnov test. Parametric data were analysed by two-tailed, paired or nonpaired *t*-test. Non-parametric data were analysed by Mann–Whitney *U*-test or Wilcoxon signed-rank test. SPSS Windows (version 12.0; SPSS Inc., Chicago, IL, USA) was used for data statistics. Statistical significance was determined at $P < 0.05$ and highly significant values at $P < 0.01$.

RESULTS AND DISCUSSION

Study population and adverse events

A total of 29 consecutive patients completed this study. One patient in the probiotics group discontinued participation during the course of the study because of rejection of the triple sugar test after oral administration of probiotics. The compliance was satisfactory (>95%). There were no reported adverse events related to the study drinks. Mean age, gender distribution, duration of disease and the mean global IBS scores were similar in the two treatment groups as listed in Table 1. The 12 normal controls consisted of nine females, three males and the mean age ± s.d. was 44.4 ± 12.8 years.

Intestinal permeability

The increased small bowel permeability was defined as the ratio of the L/M > 0.025.[3, 20] The upper limit of normal colonic permeability was 42.1 mg, defined as the P_{95} of total urinary sucralose excretion in 12 healthy volunteers. Before treatment, both small bowel permeability and colonic permeability of the 30 patients with D-IBS were increased when compared with normal controls [0.038 (0.026) vs. 0.018 (0.006), $P = 0.002$; 44.3 (43.7) vs. 31.4 (10.7), $P = 0.028$ respectively].

The proportions of patients with increased small bowel and colonic permeability were similar between the two treatment groups at baseline. After treatment with probiotics, the proportion of patients with increased small bowel permeability decreased significantly compared with the baseline value (28.6% vs. 64.3%, $P = 0.023$), while the proportion of patients with increased colonic permeability did not change (57.1% vs. 50.0%, $P = 0.705$). There were no significant changes in proportions of increased small bowel and colonic permeability in the placebo group (as shown in Table 2). A further numerical data analysis showed that the small bowel permeability in the probiotics group decreased significantly from 0.038 (0.024) at baseline to 0.023 (0.020) at week 4 ($P = 0.004$), but significant decrease was not achieved in the placebo group at week 4 compared with baseline values [0.029 (0.017) vs. 0.029 (0.023), $P = 0.156$]. At week 4, the colonic permeability improved neither in the probiotics group [50.0 (31.5)] nor in the placebo group [39.4 (24.8)] when compared with baseline values [47.1 (51.2), $P = 0.140$; 45.6 (26.6), $P = 0.256$ respectively]. The actual data of small bowel and colonic permeability at weeks 0 and 4 in the two treatment groups are shown in Figure 1.

Clinical symptoms

After 4 weeks of treatment, the mean global IBS score in the probiotics group improved compared with baseline values ($P < 0.001$); however, patients receiving placebo treatment reported no significant improvement. After treatment with probiotics, analysis of the VAS data showed that the mean scores of abdominal

Table 1. Demographic characteristics of the patient population

	Probiotics	Placebo
Number of patients	14	15
Age (years) (mean ± s.d.)	44.6 ± 12.4	45.8 ± 9.2
Gender (M/F)	10/4	9/6
Duration of disease (years) (mean ± s.d.)	6.0 ± 5.4	5.3 ± 5.0
Global IBS scores in GSRS (mean ± s.d.)	9.62 ± 1.05	9.60 ± 1.16

No significant differences were found between the two groups.
GSRS, Gastrointestinal Symptom Rating Scale.

Table 2. Intestinal permeability in two treatment groups before and after treatment

	Week 0	Week 4	*P*-value
Increased small bowel permeability			
Probiotics	64.3% (10/14)	28.6% (4/14)	0.023*
Placebo	53.3% (8/15)	60.0% (9/15)	0.464
Increased colonic permeability			
Probiotics	50.0% (7/14)	57.1% (8/14)	0.705
Placebo	53.3% (8/15)	40.0% (6/15)	0.464

The *P*-values were determined when compared with week 0.
* $P < 0.05$.

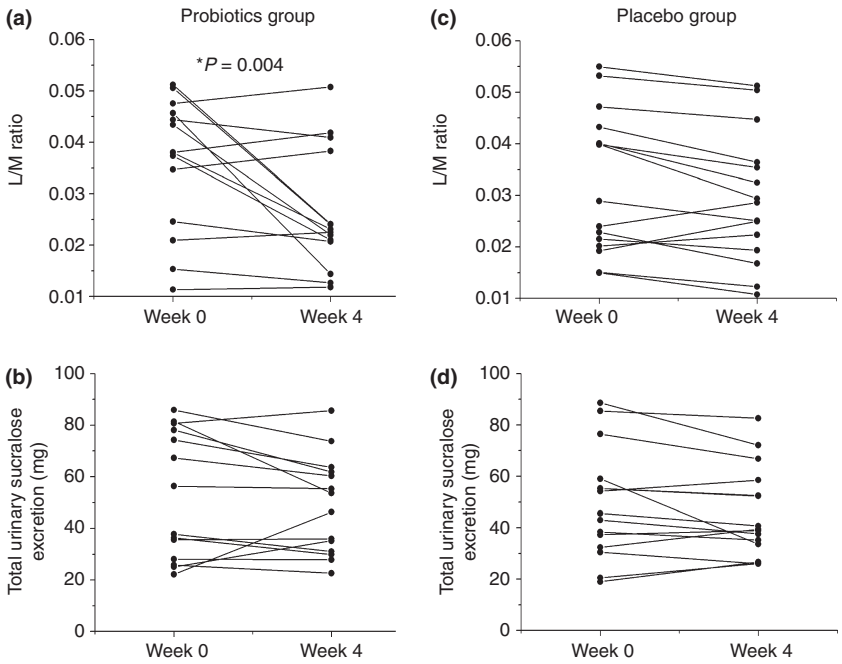

Figure 1. The intestinal permeability in two treatment groups before and after treatment. The *P* value was determined when compared with week 0. * indicates the *P* values reach statistical significance ($P < 0.05$). (a) and (b) are data in the probiotics group; (c) and (d) are data in the placebo group.

pain and flatulence were significantly lower than baseline ($P < 0.001$; $P = 0.010$ respectively) and the sensation of bloating failed to show any improvement (as shown in Table 3).

Table 3. The global IBS scores in GSRS and the weekly symptoms VAS scores at weeks 0 and 4 in two treatment groups (mean ± s.d.)

	Week 0	Week 4	*P*-value
The global IBS scores in GSRS			
Probiotics	9.62 ± 1.05	7.64 ± 1.24	<0.001
Placebo	9.60 ± 1.16	9.18 ± 1.48	N.S.
VAS – abdominal pain			
Probiotics	37.76 ± 5.87	30.11 ± 7.71	<0.001
Placebo	40.40 ± 4.87	39.42 ± 5.92	N.S.
VAS – sensation of flatulence			
Probiotics	36.61 ± 6.04	32.50 ± 8.11	0.010
Placebo	38.77 ± 6.71	37.62 ± 7.76	N.S.
VAS – bloating			
Probiotics	31.67 ± 4.65	32.10 ± 4.53	N.S.
Placebo	29.23 ± 3.51	29.67 ± 3.91	N.S.

The *P*-values were determined when compared with week 0. GSRS, Gastrointestinal Symptom Rating Scale; VAS, visual analogue scale; N.S., no significance.

Alterations in junctional complex in D-IBS

Real-time qPCR analysis revealed that the relative transcription levels of ZO-1 and occludin in patients with D-IBS decreased compared with normal controls [0.573 (0.734) vs. 1.016 (0.47), $P = 0.038$; 0.393 (1.32) vs. 0.856 (1.46), $P = 0.027$ respectively; Figure 2a]. The staining of junctional complex among colonic enterocytes visualized by TEM was strong and continuous in normal controls; however, the staining was faint and discontinuous in 33.3% of 30 patients with D-IBS (representative images were shown in Figure 2b), while it appeared continuous and strong in the other 66.7% patients.

DISCUSSION

Several agents have been proposed to have a beneficial effect on the prevention and treatment of impaired gut barrier function including probiotics. In this study, we reported a single-blind randomized-controlled study of fermented milk containing multistrain of viable LAB vs. placebo milk for increased intestinal permeability in D-IBS for 4 weeks. We found that the multistrain of viable LAB was associated with the improvement in intestinal barrier function as measured

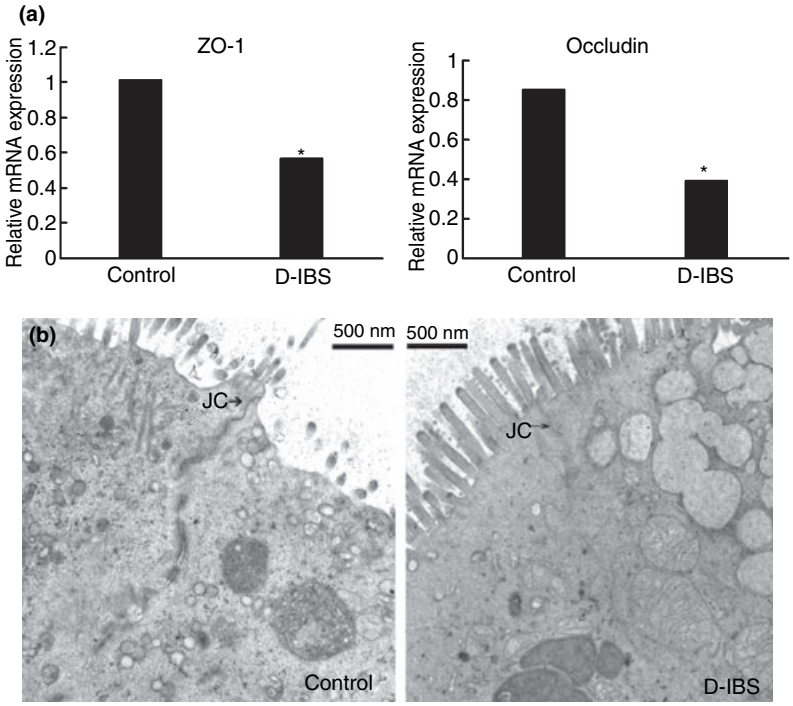

Figure 2. Alterations of junctional complex (JC) in D-IBS. (a) Relative mRNA expressions of ZO-1 and occludin were significantly reduced in D-IBS (n = 30). *P < 0.05 versus controls. (b) The staining of JC between colonic epithelium visualized by TEM was faint and discontinuous in 33% of patients with D-IBS (n = 10). Representative images are shown. Original magnification, 2000x.

by a reduction in small bowel permeability. Other strains of LAB, such as *Lactobacillus plantarum* species 299, also have been shown to play a protective role of the gut mucosal barrier disruption in patients with IBD[27] or in IL-10 knockout mouse model of colitis.[28] This study chose fermented milk containing 4 strains of LAB, but not a single probiotic strain because probiotics exert their beneficial effect on multifactorial diseases with a variety of probiotic properties and such properties may be strain-specific.[29, 30] When in combination of strains, they may complement each other and thus have synergistic probiotic effects. However, other studies failed to demonstrate that a variety of probiotic species improved intestinal permeability in critically ill patients despite favourable alterations in the microbial composition of the upper gastrointestinal tract and the systemic inflammatory response.[31, 32]

The mechanisms by which probiotics improve barrier function remain to be elucidated. It has been proven than probiotics also have the ability to counteract the disruptive effects of inflammation on barrier function. Feeding VSL#3, a mixture of eight bacteria strains, in addition to decreasing colonic inflammation, restores the barrier integrity in IL-10 knockout mice model of colitis.[28] It is also known that certain LAB can adhere to mucosal surfaces, inhibit adherence of enteropathogens and enhance secretion of mucins.[33, 34] These properties may be instrumental in improving mucosal barrier function. Moreover, probiotics may have effects on epithelial barrier function via cellular molecular mechanisms. Two recent studies found that the probiotic *Escherichia coli* Nissle, 1917 restored the barrier disruption by silencing protein kinase C zeta[35] and altering the redistribution and expression of TJ proteins such as ZO-1 and ZO-2, which are important to maintain the integrity of TJ.[34, 36]

Consistent with previous studies,[2, 3] our results yield experimental evidence that the small intestinal permeability was significantly increased in D-IBS patients compared with normal controls. Dunlop *et al.* demonstrated that the small intestinal permeability assessed by ^{15}Cr-EDTA increased more in D-IBS without an infectious onset than in diarrhoea-predominant postinfectious IBS, which may indicate that mechanisms of increased intestinal permeability in postinfectious-IBS and nonpostinfectious D-IBS may be different from each other.[2] In addition, in contrast to previous studies,[2, 37] the measurement of the urinary sucralose concentration

suggested that the colonic permeability was increased in D-IBS. Despite the different results, differences among the studies may account for the discrepancies. Sucralose, a nonmetabolized sweetener, is considered a suitable probe for assessment of colonic permeability.[22] However, using a single probe to measure the intestinal permeability has limitations because some factors could affect the urinary concentration of sugar probes.[24] In addition, values of the intestinal permeability in asymptomatic controls using ^{15}Cr-EDTA technique varied.[2] Furthermore, the ethinicity of patients between the two studies were different, which may affect the permeability data. The patients from the Spiller research group were life-long Caucasian residents in the UK. However, the IBS patients with predominant diarrhoea symptoms in our study were all resident Chinese.

This study also found that the decrease in small intestinal permeability by probiotics was accompanied by the relief of IBS symptoms, which suggests that increased intestinal permeability may partially contribute to the pathogenesis of IBS symptoms. The mechanisms of the increased intestinal permeability in D-IBS were lacking. Gut mucosal barrier disruption has been proven to be associated with various inflammatory conditions, such as gastroenteritis and allergic reaction. Spiller *et al*. reported that IBS patients with atopy had a higher intestinal permeability than patients without atopy, which indicates that the inflammation has a detrimental effect on intestinal mucosal barrier function in IBS.[2]A further research in this study found that the location of junctional complex was discontinuous in some D-IBS and that expressions of ZO-1 and occludin were decreased at transcriptional level. As the colonoscopic manifestation appeared normal in patients with IBS, colonoscopic biopsies cannot be extracted from the inflamed and the non-inflamed sites as a related research did in patients with IBD.[38] So, we cannot identify whether the alterations of junctional complexes were associated with the gut inflammation in D-IBS.

There are several limitations of this study. First, as the colonoscopic manifestation and the histological assessment of biopsies by conventional criteria were normal in IBS, many Chinese patients refused to repeat biopsies after treatment. Whether the probiotics have a potential beneficial effect on junctional complex proteins expressions in D-IBS is not evaluated in this study and this issue deserves the further studies in detail. Secondly, three colonoscopic biopsies of each patient were taken and sent off for RNA extraction and TEM study as described above. So, we solely reported the discontinuous distribution of junctional complex visualized by TEM in some D-IBS patients; however, the alterations in expressions and locations of various junctional proteins in D-IBS should be further confirmed by properly designed studies. Thirdly, the study was single blinded. The observer bias was possible but unlikely because the clinical symptoms were evaluated and agreed to by at least two investigators and the urinary sugar concentrations were determined by a chemist in Shandong University who was blind to this study.

In conclusion, our data suggest that short-term fermented milk containing multistrain of active LAB treatment is effective and safe at improving both intestinal mucosal barrier function and bowel symptoms in patients with D-IBS. Data from this trial justify further investigations into the potential utility of active LAB in patients with D-IBS.

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