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ORIGINAL ARTICLE

Randomized Clinical Trial

Effectiveness of probiotic therapy for the prevention of relapse in patients with inactive ulcerative colitis

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Abstract

AIM: To evaluate the effectiveness of probiotic therapy for suppressing relapse in patients with inactive ulcerative colitis (UC).

METHODS: Bio-Three tablets, each containing 2 mg of lactomin (*Streptococcus faecalis* T-110), 10 mg of *Clostridium butyricum* TO-A, and 10 mg of *Bacillus mesentericus* TO-A, were used as probiotic therapy. Sixty outpatients with UC in remission were randomly assigned to receive 9 Bio-Three tablets/day (Bio-Three group) or 9 placebo tablets/day (placebo group) for 12 mo in addition to their ongoing medications. Clinical symptoms were evaluated monthly or on the exacerbation of symptoms or need for additional medication. Fecal samples were collected to analyze bacterial DNA at baseline and 3-mo intervals. Terminal restriction fragment length polymorphism and cluster analyses were done to examine bacterial components of the fecal microflora.

RESULTS: Forty-six patients, 23 in each group, completed the study, and 14 were excluded. The relapse rates in the Bio-Three and placebo groups were respectively 0.0% *vs* 17.4% at 3 mo (P = 0.036), 8.7% *vs* 26.1% at 6 mo (P = 0.119), and 21.7% *vs* 34.8% (P = 0.326) at 9 mo. At 12 mo, the remission rate was 69.5% in the Bio-Three group and 56.6% in the placebo group (P = 0.248). On cluster analysis of fecal flora, 7 patients belonged to cluster Ⅰ, 32 to cluster Ⅱ, and 7 to cluster Ⅲ.

CONCLUSION: Probiotics may be effective for

maintaining clinical remission in patients with quiescent UC, especially those who belong to cluster I on fecal bacterial analysis.

Key words: Ulcerative colitis; Probiotics; Inflammatory bowel disease; Cluster analysis

Core tip: We conducted a single-center, randomized, double-blind, placebo-controlled study to examine whether 12 mo of probiotic therapy was useful for preventing relapse of ulcerative colitis (UC) in patients who were already in remission. The relapse rates in the probiotic therapy group and placebo group were respectively 0.0% *vs* 17.4% at 3 mo (P = 0.036), 8.7% *vs* 26.1% at 6 mo (P = 0.119), and 21.7% *vs* 34.8% (P = 0.326) at 9 mo. At 12 mo, the remission rate was 69.5% in the probiotic therapy group and 56.6% in the placebo group (P = 0.248). Therefor probiotics may be effective for maintaining clinical remission in patients with quiescent UC.

Yoshimatsu Y, Yamada A, Furukawa R, Sono K, Osamura A, Nakamura K, Aoki H, Tsuda Y, Hosoe N, Takada N, Suzuki Y. Effectiveness of probiotic therapy for the prevention of relapse in patients with inactive ulcerative colitis. *World J Gastroenterol* 2015; 21(19): 5985-5994 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i19/5985.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i19.5985

INTRODUCTION

Ulcerative colitis (UC) is a chronic, idiopathic, refractory, inflammatory bowel disease (IBD) characterized by inflammatory mucosal injury of the colon, with repeated periods of remission and relapse. The cause and etiology of UC remain unclear. The mainstay of treatment for UC is sulfasalazine- or mesalazine-based therapy. In patients with moderate to severe UC, steroids are used concurrently to attempt to induce remission. However, a considerable number of cases are resistant to steroids. Patients with steroid-resistant disease are given immunosuppressants and newly developed biological preparations to promote remission induction. Although these new treatments have enhanced the remission induction rate as compared with conventional therapy, achievement of a high long-term rate of remission maintenance remains a largely unattained goal. Steroids are very effective for the induction of remission, but do not contribute to remission maintenance. In addition, long-term treatment with high doses of steroids is associated with high rates of various adverse effects, seriously impairing the quality of life of patients. Sulfasalazine, mesalazine, and immunomodulators promote remission maintenance, but are not adequately effective. Moreover, an appreciable number of patients cannot tolerate these drugs, and immunomodulators can cause serious adverse events, necessitating close follow-up. Therefore, the development of new remission maintenance treatments that are very effective and safe with good compliance when used on a long-term basis has been eagerly awaited.

Recently, probiotic therapy has been acknowledged to be potentially effective and safe in patients with UC. Probiotics are defined as a live microbial feed nutritional supplement that beneficially affects the host by improving the balance of the intestinal flora. Studies of animal models of colitis have suggested that the intestinal flora has an important role in the pathogenesis of colitis. In IBD-sensitive knockout or transgenic mice, colitis develops in the presence of a normal intestinal flora, but not in mice raised in a germ-free environment, strongly suggesting that the intestinal flora participates in the development of colitis[1,2]. Therefore, probiotic therapy designed to correct the intestinal flora is expected to be useful for preventing colitis.

Many studies have examined the effects of specific bacterial strains or species in active UC. However, very few studies have reported on the relation between the intestinal flora as a whole (including microorganisms that are difficult or impossible to culture) and the pathological characteristics of UC[3-7]. In a previous study, we therefore gave probiotic or synbiotic therapy for 4 wk to 20 patients with mild to moderate UC who did not respond to, or could not tolerate, standard therapy [oral mesalamine preparations, sulfasalazine, azathiopurine (AZA)/6-mercaptopurine (6-MP), and mesalamine enemas]. Our results confirmed that such therapy can improve clinical symptoms and endoscopic findings and provided evidence that remission induction is promoted by a certain improvement in the intestinal flora. We also reported that probiotic therapy might be effective for maintenance of remission[8]. On the basis of the results of our previous study, we conducted a single-center, randomized, double-blind, placebo-controlled study to examine whether 12 mo of probiotic therapy is useful for preventing relapse of UC in patients who were already in remission.

MATERIALS AND METHODS

Patients

The study group comprised patients with UC in remission who were receiving treatment on an outpatient basis at Sakura Medical Center, Toho University. UC was diagnosed in accordance with the diagnostic criteria proposed by the Survey Research Group of Intractable Inflammatory Intestinal Disorders/Specified Diseases, Japanese Ministry of Health, Labour and Welfare. Patients 13 years or older in whom the CAI was maintained at 5 or less while receiving drugs

such as mesalazine, salazosulfapyridine, or steroids, with no change in treatment regimens within 4 wk before study entry, were enrolled in this randomized, double-blind, placebo-controlled study.

Patients were excluded if they had serious cardiac disease, serious renal disease, hypotension (systolic blood pressure, ≤ 80 mmHg), a history of shock during extracorporeal circulation, serious infections such as sepsis or pneumonia, or a serum hemoglobin concentration of less than 10 g/dL. We also excluded patients who newly began treatments such as leukocytapheresis, granulocyte adsorptive apheresis, or immunosuppressant therapy with drugs such as 6-mercaptopurine, azathioprine, and cyclosporine to improve symptoms, as well as patients who had milk allergy or a CAI of 6 or higher. Pregnant women were also excluded. All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Study probiotic

Bio-Three tablets (Toa Pharmaceutical Co., Ltd., Toyama, Japan), a live microbial preparation, and matching placebo tablets (Toa Pharmaceutical Co., Ltd.) were used as the study preparations. Bio-Three tablets were granted manufacturing approval in 1963. Each tablet contains 2 mg of lactomin (*Streptococcus faecalis* T-110), 10 mg of *Clostridium* (*Clostridium butyricum* TO-A), and 10 mg of *Bacillus* (*Bacillus mesentericus* TO-A), combined with potato starch and lactose. This preparation is effective for resolving various symptoms caused by abnormal intestinal flora and mainly improves bowel movement disorders. Placebo tablets were prepared by substituting equivalent amounts of starch for the probiotic powder. Placebo tablets were identical to Bio-Three tablets and could not be distinguished from the active preparation on the basis of appearance.

Study design and treatment

At the start of the study, 30 outpatients were randomly assigned to the Bio-Three group and 30 to the placebo group by means of a computer-generated scheme. The study protocol was reviewed and approved by the Ethics Committee of Sakura Medical Center, Toho University.

In both the Bio-Three group and the placebo group, patients orally received 3 tablets 3 times daily. In principle, the duration of treatment was 12 mo. Fecal samples were collected immediately before and 3, 6, 9, and 12 mo after the start of treatment. Fecal samples for measurement of organic acids were preserved by freezing, without modification. Fecal samples used for DNA extraction were suspended in GTC solution (100 mmol/L Tris-HCl, pH 9.0; 40 mmol/L Tris-EDTA, pH 8.0; and 4 mol/L guanidine thiocyanate) and were preserved at 4 ℃. As for concomitant medication (therapy), the use of mesalazine and salazosulfapyridine was unrestricted, but steroids could not be used as remission maintenance therapy. The use of drugs with similar effects as the study drug, potentially affecting the evaluation of effectiveness (*i.e.*, other active live microbial preparations, laxatives, *etc.*) was prohibited from 1 wk before study entry to the completion of the study. In principle, the use of oral antibiotics was also prohibited, but the use of topical antibiotics other than oral preparations was not particularly restricted. If a patient received a new treatment in addition to their basic therapy with drugs such as mesalazine or salazosulfapyridine, relapse was diagnosed, and the study treatment and fecal sample collection were discontinued.

Analysis of intestinal microflora

DNA extraction: About 800 μL of the fecal sample suspension preserved at 4 ℃ was transferred to a tube containing zirconia beads (Nippon Gene Co., Ltd., Tokyo, Japan), and the cells were processed with the use of FastPrep FP120A cell disruptor (MP Biomedicals, Irvine, CA). After cooling on ice, the specimen was centrifuged at 5000 rpm for 1 min. DNA was automatically extracted from the processed supernatant with the use of a 12GC and GC series Magtration-MagaZorb DNA Common Kit 200N (Precision System Science, Chiba, Japan). The final concentration of the extracted DNA was adjusted to 10 ng/μL.

Terminal restriction fragment length polymorphism analysis: Terminal restriction fragment length polymorphism (T-RFLP) analysis was performed as described by Nagashima *et al*[9]. The 16S rRNA gene was amplified with the use of primer sets516F (5′-TGCCAGCAGCCGCGGTA-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The 5′-end of the forward primer 516F was labeled with 6′-carboxyfluorescein. Amplified polymerase chain reaction (PCR) products were refined with the use of MultiScreen® PCRμ96 filter plates (Millipore, Tokyo, Japan).

The refined PCR products (about 3 μL) were digested for 3 h at 55 ℃ with 10 U of *BslⅠ* restriction enzyme (New England Biolabs, Inc., Ipswich, MA, United States). The length of the separated fluorescent PCR fragment was determined with an ABI PRISM 3130xl genetic analyzer (Applied Biosystems, Tokyo, Japan), and the data were analyzed with GeneMapper® software. MapMarker® X-Rhodamine Labeled 50-1000bp (BioVentures, Inc., Murfreesboro, TN, United States) was used as a size standard marker.

Cluster analysis: To objectively interpret differences in T-RFLP patterns, NTSYSpc software (Exeter Software, Setauket, NY, United States) was used to perform cluster analysis. Each terminal restriction fragment (T-RF) was expressed as a percentage of the peak area of all T-RFs. Disparity in similarity

Table 1 Baseline characteristics of the study group

	Bio-three (n = 23)	Placebo (n = 23)
Male/female	16/7	12/11
Age (yr, mean ± SD)	44.8 ± 13.8	42.9 ± 15.9
Age of onset (yr)	37.1 ± 14.4	36.0 ± 14.2
Disease duration (yr, mean ± SD)	8.0 ± 6.3	6.7 ± 5.9
Left colon	6	9
Proctosigmoiditis	6	5
Total/subtotal	11	9
Concomitant drug		
Pentasa	11	13
Salazopyrin	10	9
Pentasa + salazopyrin	1	0
Nothing	1	1

among fecal samples in individual patients was calculated using a correlation matrix and was presented graphically on tree diagrams with the use of a weighted pair-group method with arithmetic mean (WPGMA) clustering[10].

High-performance liquid chromatography analysis of fecal organic acids: Organic acid concentrations in fecal samples were measured as follows. About 0.1 g of fecal sample was measured and combined with trans-crotonic acid as an internal standard substance, and extraction was performed twice with 0.6 mL of 0.25% ammonia solution. After adding a 0.3-fold dilution of 10% (w/v) perchloric acid, deproteinization was performed by centrifugation. The solution was filtered and analyzed with a post-column high-performance liquid chromatography (HPLC) system (Waters, Milford MA, United States). Organic acids in the sample were separated with an ion exchange column (organic acid column, 7.8 mm i.d. × 300 mm long; Waters). The reaction temperature in the column and post-column was 60 ℃. The mobile phase was 0.08% perchloric acid delivered at a flow rate of 0.8 mL/min. The solution eluted from the column was allowed to react with BTB solution (0.2 mmol/L bromothymol blue, 5.2 mmol/L sodium hydroxide, and 15 mmol/L disodium hydrogen phosphate), delivered at a flow rate of 0.8 mL/min. The absorbance was quantitatively measured at 445 nm using an ultraviolet visible spectrophotometer (2487 Dual λ UV/Vis Detector; Waters).

Statistical analysis

Clinical data were statistically analyzed with SAS software (version 8.2, SAS Institute Inc., Cary, NC, United States). For patients with UC in remission, the Kaplan-Meier method was used to compare the cumulative non-relapse rate over the course of 12 mo between the Bio-Three group and placebo group. The statistical significance of differences between groups was evaluated with the log-rank test and the generalized Wilcoxon test, and 95% confidence intervals were calculated.

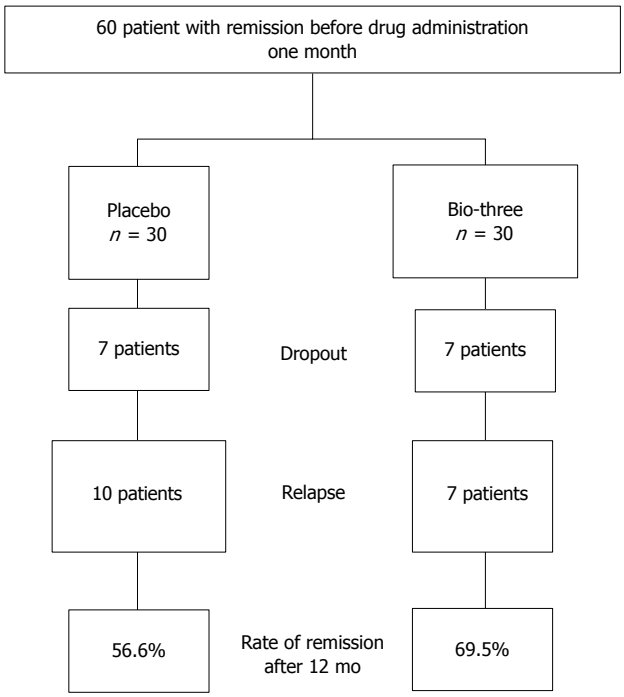


Figure 1 Clinical outcomes of patients according to treatment received.

RESULTS

Patient characteristics

At the start of the study, 30 patients each were randomly assigned to the Bio-Three group and the placebo group. After randomization, the baseline characteristics of sex, age, age at disease onset, disease duration, disease extent, and concomitant treatment did not differ between the groups (Table 1). Among the enrolled subjects, 7 patients in each group were excluded because they met the exclusion criteria specified in the protocol, such as the use of prohibited drugs or refusal to participate in the study. Treatment was actually begun in 23 patients in the Bio-Three group and 23 in the placebo group (Figure 1).

Clinical results

After the study began, the number of patients who had relapse was 2 at 6 mo, 5 at 9 mo, and 7 at 12 mo among the 23 patients in the Bio-Three group and 4 at 3 mo, 6 at 6 mo, 8 at 9 mo, and 10 at 12 mo among the 23 patients in the placebo group. Kaplan-Meier curves were plotted, and relapse rates at each time point were compared between the groups with the use of the χ^2 test (two-tailed, α = 0.05). The P value was 0.0363 at 3 mo, 0.1197 at 6 mo, and 0.3259 at 9 mo. The cumulative remission maintenance rate at 12 mo was 56.6% (n = 12) in the placebo group and 69.5% (n = 16) in the Bio-Three group (Figure 2). When remission maintenance rates were compared between the treatment groups with the use of the log-rank test, the P value was 0.302, with a hazard ratio of 0.607 (95%CI: 0.23-1.59). On the generalized Wilcoxon test, the P value was 0.248. During our study, no adverse

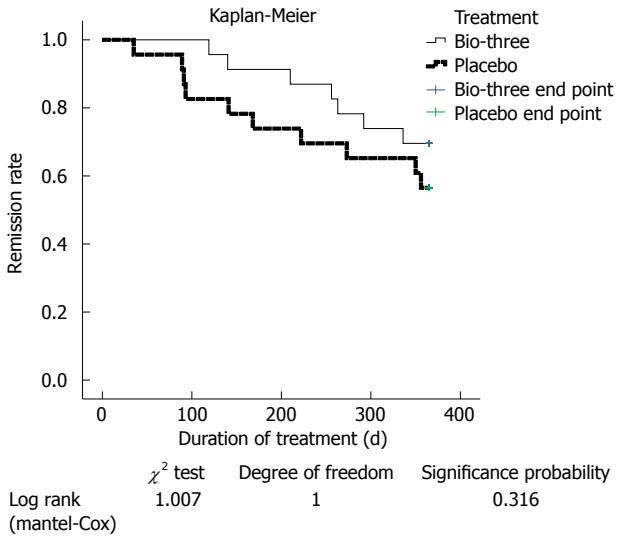


Figure 2 Cumulative remission maintenance rate in the Bio-Three group and placebo group.

changes were observed in the Bio-Three group, as compared with the placebo group, indicating that there is no problem with the safety of Bio-Three.

Bacteriological analysis

In our previous study evaluating the effectiveness of Bio-Three for inducing remission in patients with active UC[8], a cluster analysis was performed to evaluate fluctuations in fecal flora, using the T-RF data derived by digestion of the PCR products with *BslⅠ* restriction enzyme, and several findings were obtained. In the present study of the effectiveness of Bio-Three for remission maintenance, the PCR products were similarly digested with *BslⅠ* restriction enzyme, and cluster analysis of the T-RF data showed that the fecal flora can be divided into 3 clusters, consistent with the results of our previous study. To confirm whether the 3 clusters in the present study correspond to the 3 clusters in our previous study, the fecal sample data used in our previous study of remission induction were linked to the fecal sample data in the present study, and another cluster analysis was performed. The results showed that each of the fecal samples from our previous study belonged to the same clusters as those in our previous analysis. Therefore, for convenience the same names of the clusters in our previous study were used in the present study, *i.e.*, the cluster in the upper part of the figure was named cluster Ⅱ, the cluster in the middle part was named cluster Ⅲ, and the other cluster was named cluster Ⅰ (Figure 3, Table 2).

A total of 138 fecal samples belonged to cluster Ⅱ. The clinical outcomes of the 32 subjects who belonged to cluster Ⅱ at the start of treatment were remission maintained in 11 of the 16 patients in the Bio-Three group and 10 of the 16 patients in the placebo group. This difference was not significant. As a characteristic of T-RF in cluster Ⅱ, OTU124 accounted for a significantly higher proportion of total T-RF peak area in cluster Ⅱ than in the other clusters.

A total of 28 fecal samples belonged to cluster Ⅲ. The clinical outcomes of the 7 subjects who belonged to cluster Ⅲ at the start of treatment were remission maintained in 2 of the 4 patients in the Bio-Three group and 2 of the 3 patients in the placebo group. The difference between the groups was not significant.

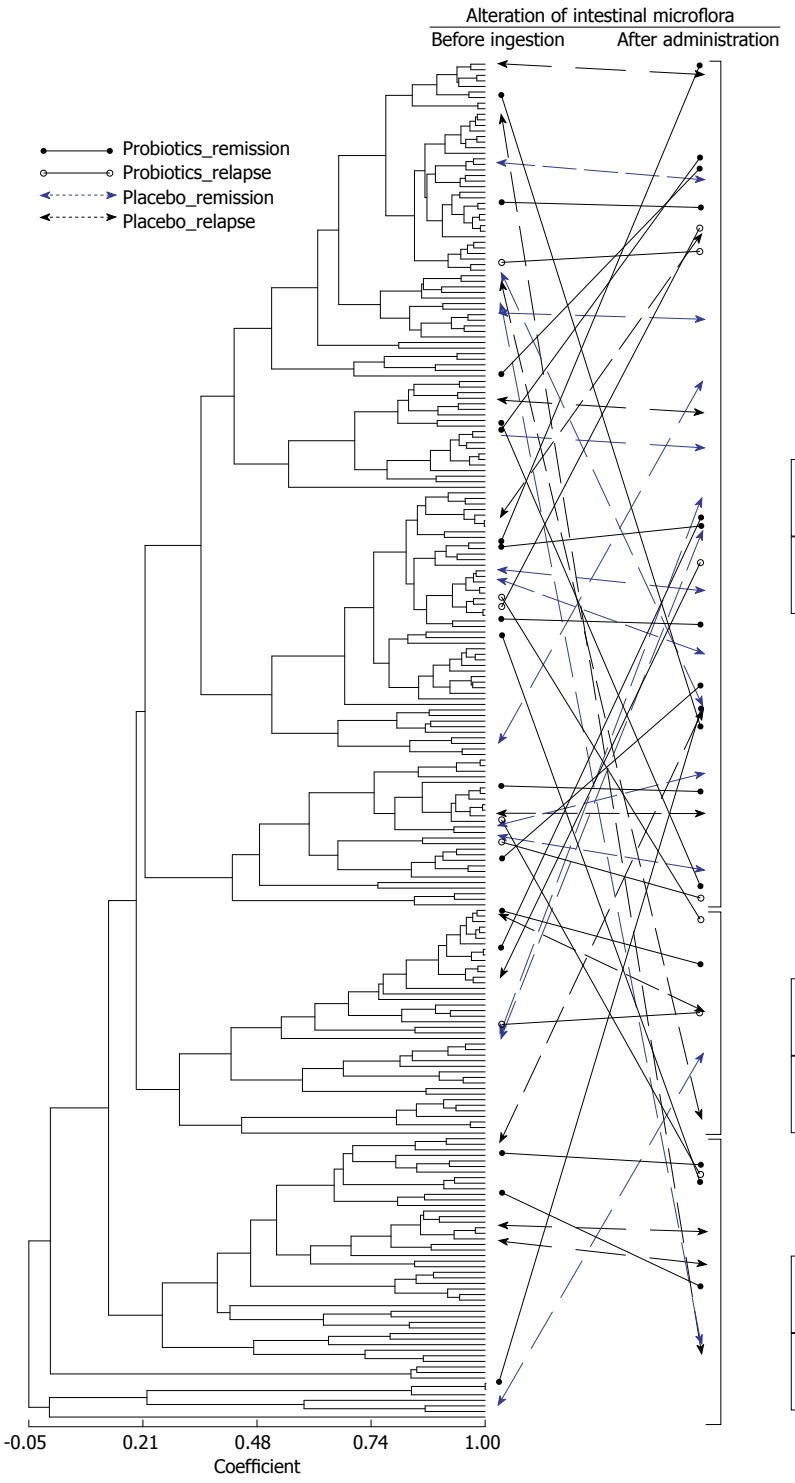
A total of 39 fecal samples belonged to cluster Ⅰ. The clinical outcomes in the 7 subjects who belonged to cluster Ⅰ at the start of treatment were remission maintained in all 3 patients in the Bio-Three group, as compared with only 1 of the 4 patients in the placebo group. Among the 39 fecal samples that belonged to cluster Ⅰ, 19 fecal samples were derived from patients in the Bio-Three group, and 17 of these samples were from patients who had a final evaluation of remission maintained. The other 20 fecal samples were from patients in the placebo group, and 8 of these samples were from patients who had a final evaluation of remission maintained. When these data were compared with the use of Pearson's χ^2 test, the *P* value was 0.0013, indicating that the rate of remission maintenance was significantly higher in the Bio-Three group than in the placebo group, and the relapse rate was significantly lower in the Bio-Three group than in the placebo group.

HPLC analysis of fecal organic acids

The fecal concentrations of short-chain fatty acids did not differ significantly between the Bio-Three group and the placebo group at any time during treatment. On comparison of the clusters derived by T-RFLP analysis, the ratio of the concentration (mmol/L) of butyrate to that of acetate (Bu/Ac ratio) was significantly higher in cluster Ⅰ than in clusters Ⅱ and Ⅲ. When fecal organic acids were compared according to clinical outcomes (*i.e.*, between fecal samples obtained at each of the specified times from patients in whom remission was maintained for 1 year and fecal samples from patients who had relapse within 6 mo after fecal collection), the concentrations of butyric acid and other short-chain fatty acids did not differ significantly between the groups. However, the Bu/Ac ratio at each of the sampling times was significantly higher in fecal samples obtained from patients who had relapse within 6 mo after fecal collection than in those obtained from patients who remained in remission (Table 3).

DISCUSSION

T-RFLP is a molecular technique that allows the diversity and colony structure of microbial complexes to be promptly compared and the diversity of ecosystems to be evaluated[11,12]. Recently, several studies have performed T-RFLP in patients with UC.



Cluster Ⅱ	No. of patients (at the start)	Clinical effectiveness (after treatment)
Probiotics	16	Remission: 11 Relapse: 5
Placebo	16	Remission: 10 Relapse: 6

Cluster Ⅲ	No. of patients (at the start)	Clinical effectiveness (after treatment)
Probiotics	4	Remission: 2 Relapse: 2
Placebo	3	Remission: 2 Relapse: 1

Cluster Ⅰ	No. of patients (at the start)	Clinical effectiveness (after treatment)
Probiotics	3	Remission: 3 Relapse: 0
Placebo	4	Remission: 1 Relapse: 3

Figure 3 Alteration of intestinal microflora after treatment in the Bio-Three group and placebo group. The dendrogram indicates the similarity of individual intestinal microflora T-RFLP patterns of fecal samples obtained after 0, 3, 6, 9, and 12 mo of treatment. Solid line and circles, Bio-Three group and remission; double line and open circles, Bio-Three group and relapse; solid dotted line and arrows, placebo group and remission; double dotted line and arrows, placebo group and relapse. The first and last samples from the same individuals are connected with lines. The tables indicate clinical effectiveness according to the cluster that the subjects belonged to at the start of this study.

However, few studies have examined time-series samples obtained from the same subjects. Most previous investigations have evaluated fecal samples obtained at a specific time[13-17].

We previously studied the effectiveness of probiotic therapy for inducing remission in patients with flare-ups of UC. On T-RFLP cluster analysis, fecal flora could be divided into 3 clusters, designated as clusters Ⅰ, Ⅱ, and Ⅲ. The flora of patients whose UC disease activity index (UCDAI) was improved by probiotic therapy was centered around cluster Ⅱ. Cluster Ⅱ flora was characterized by a high ratio of OTU124[8].

Table 2 Comparison of characteristics of fecal samples (according to cluster)

Group (sample number)	OTU124/all T-RFs (%)	Butyrate/acetate ratio (mol)
Cluster Ⅰ	2.8 ± 3.6	0.254 ± 0.172
Cluster Ⅱ (n = 138)	27.6 ± 12.0	0.184 ± 0.147
Cluster Ⅲ (n = 28)	18.0 ± 10.8	0.075 ± 0.126

Data are expressed as mean ± SD. Because equal variances were rejected for each variable, multiple comparisons were performed with Dunnett's T3 test. Different letters indicate the presence of a significant difference (P < 0.05). T-RF: Terminal restriction fragment.

Table 3 Comparison of characteristics of fecal samples (according to clinical outcomes)

Group (sample number)	OTU124/all T-RFs (%)	Butyrate/acetate ratio (mol)
Remission (before treatment) (n = 29)	22.7 ± 13.8	0.165 ± 0.143
Remission (months 3 and 6) (n = 58)	22.0 ± 14.6	0.145 ± 0.136
Remission (months 9 and 12) (n = 56)	21.7 ± 14.1	0.160 ± 0.146
Relapse (within 6 mo) (n = 28)	20.7 ± 14.7	0.260 ± 0.185

Data are expressed as mean ± SD. Because each variable had equal variances, multiple comparisons were performed with Tukey's honestly significant difference test. Different letters indicate the presence of a significant difference (P < 0.05). "Relapse (within 6 mo)" means that fecal samples were obtained within 6 mo before relapse (*i.e.*, relapse occurred within 6 mo after collection of fecal samples).

Nagashima *et al*[9], who proposed the T-RFLP technique used in our study, reported that OTU124 is derived from *Bifidobacteria*. Many studies have examined the relation between *Bifidobacteria* and UC, and most have reported that *Bifidobacteria* mitigates inflammation associated with UC[18-21]. On the basis of these findings, we previously concluded that cluster Ⅱ flora is "healthy intestinal flora (appropriate intestinal flora)" in patients with UC. Probiotic therapy may therefore be less effective for improving flora in patients who initially have cluster Ⅱ flora, leading only to marginal improvement clinically[8].

The results of the present study similarly suggest that cluster Ⅱ represents "healthy intestinal flora". Consequently, probiotic therapy is expected to be of the greatest potential benefit for intestinal flora that is most dissimilar to cluster Ⅱ. This speculation is supported by the following two points. First, in our previous study of patients with "flare-ups" of UC, only 6 (30%) of the 20 subjects had intestinal flora belonging to cluster Ⅱ before treatment[8]. In the present study of patients with UC "after induction of remission", 32 (70%) of the 46 subjects had intestinal flora belonging to cluster Ⅱ. Given that the proportion of patients with "appropriate intestinal flora" is expected to be higher among patients with remission induction and remission maintenance than among those with relapse, the fact that the majority of patients in the present study, who were already in remission, belonged to cluster Ⅱ supports our speculation that probiotic therapy is potentially most beneficial for patients with intestinal flora most dissimilar to cluster Ⅱ. Second, among the 32 subjects who belonged to cluster Ⅱ at the start of treatment in the present study, remission was maintained in 11 of the 16 patients in the Bio-Three group and 10 of the 16 patients in the placebo group. There was no difference in the remission maintenance rate between these groups. In contrast, among the 7 subjects who belonged to cluster Ⅰ (*i.e.*, the cluster farthest from cluster Ⅱ) before treatment, remission was maintained in all 3 patients in the Bio-Three group, whereas relapse occurred in 3 of the 4 patients in the placebo group. These findings indicate that probiotic therapy was less effective in patients who initially belonged to cluster Ⅱ ("appropriate intestinal flora"), with no difference from placebo. In contrast, probiotic therapy was potentially most beneficial for patients with intestinal flora belonging to cluster Ⅰ flora before treatment, the cluster that is farthest from cluster Ⅱ. Consequently, probiotic therapy was more effective than placebo for maintaining remission in this subgroup of patients. These results were very interesting and are consistent with the findings of our previous study[8].

The fecal Bu/Ac ratio differed between patients with relapse and those in whom remission was maintained for 12 mo and was significantly higher within 6 mo before relapse than at other times (Table 3). Interestingly, the Bu/Ac ratio tended to be higher in feces belonging to cluster Ⅰ than in the other clusters (Table 2). These findings may be attributed to the following mechanism. Butyrate serves as an energy source for intestinal epithelial cells and is known to induce apoptosis of colorectal cancer cells and the differentiation of intestinal epithelial cells. In addition, butyrate has been shown to inhibit the activation of nuclear factor kappa B (NF-κB) and to have anti-inflammatory properties[11,22,23]. On the other hand, the utilization efficiency of butyrate has been reported to be low in the colonic mucosa of patients with refractory UC. The anti-inflammatory activity of butyrate has prompted several studies of its effectiveness and mechanism of action in patients with UC[24-28].

In our previous study evaluating the effectiveness of Bio-Three for inducing remission in patients with UC, the decrease in the UCDAI after treatment (*i.e.*, the improvement in symptoms of UC) correlated with the decrease in the fecal butyrate concentration[8]. On the basis of this finding, we performed breath tests after administration of [1-^{13}C]-butyrate enemas in 10 patients with active UC and 12 with quiescent UC and confirmed that the utilization efficiency of butyrate was decreased in patients with high inflammatory activity[29-33]. These findings suggest that an increased Bu/Ac ratio resulting from decreased absorption of butyrate, an indicator of anti-inflammatory activity,

and an increase in fecal butyrate concentrations is associated with a higher risk of relapse in patients with UC. The mean rate of remission maintenance at 12 mo in patients who receive mesalazine preparations alone is estimated to be about 61% (range: 45%-71%) on the basis of the results of previous randomized controlled trials[34-41]. This rate is similar to the relapse rate among patients who received mesalazine preparations alone (56.6%) in the placebo group of our study.

We used the Kaplan-Meier method to compare relapse rates between the treatment groups. The relapse rate after 12 mo did not differ significantly between the Bio-Three group and the placebo group on either the log-rank test or generalized Wilcoxon test. However, detailed analysis showed that clinical effectiveness differed between the Bio-Three group and placebo group among patients who belonged to cluster Ⅰ. As mentioned above, probiotic therapy is most likely to be effective in patients with intestinal flora belonging to cluster Ⅰ, which is characterized by both a low ratio of OTU124 (which tended to be high in patients belonging to cluster Ⅱ, classified as "healthy intestinal flora") and a high fecal Bu/AC ratio (which was significantly higher in patients within 6 mo before relapse than in patients without relapse).

Combining probiotics or synbiotics with conventional drugs has been recommended as a safe and effective treatment for patients with active UC. For more than 10 years considerable attention has focused on the effectiveness of probiotic therapy for UC[42]. Nearly all studies have reported that probiotics such as VSL#3[43-45], BIFICO[46], and *E. coli* Nissle 1917[47] and prebiotics such as GBF[48-50] and BGS[51] are useful for maintaining remission maintenance and preventing relapse in patients with UC. In comprehensive Cochrane reviews of clinical studies evaluating the effectiveness of probiotics for UC, Mallon *et al*[52] and Naidoo *et al*[53] concluded that although probiotics are ineffective for the induction of remission, probiotics combined with conventional therapy are expected to be effective for maintenance of remission. A meta-analysis conducted by Sang *et al*[54]. reported that probiotics are slightly but not significantly effective for remission induction, but significantly contribute to remission maintenance.

The results of our study suggest that cluster analysis of patients' intestinal flora before treatment can contribute to the effective use of probiotic therapy. To our knowledge, our study represents an unprecedented attempt to define factors related to the effectiveness of Bio-Three for the prevention of relapse in patients with inactive UC. Not only the fecal flora, but also the fecal concentration of short-chain fatty acids differed between patients who had relapse within 1 year and those in whom remission was maintained for 1 year. This finding suggests that patient profiling on the basis of factors such as the results of cluster analysis of fecal flora and the fecal short-chain fatty acid concentration might facilitate prediction of the response to treatment and future clinical status in patients with UC.

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COMMENTS

Background

Ulcerative colitis (UC) is a chronic, idiopathic, refractory, inflammatory bowel disease (IBD) characterized by inflammatory mucosal injury of the colon, with repeated periods of remission and relapse. Recently, probiotic therapy has been acknowledged to be potentially effective and safe in patients with UC. Probiotics are defined as a live microbial feed nutritional supplement that beneficially affects the host by improving the balance of the intestinal flora.

Research frontiers

Studies of animal models of colitis have suggested that the intestinal flora has an important role in the pathogenesis of colitis. In IBD-sensitive knockout or transgenic mice, colitis develops in the presence of a normal intestinal flora, but not in mice raised in a germ-free environment, strongly suggesting that the intestinal flora participates in the development of colitis. Therefore, probiotic therapy designed to correct the intestinal flora is expected to be useful for preventing colitis.

Innovations and breakthroughs

The results confirmed that probiotic therapy can improve clinical symptoms and endoscopic findings and provided evidence that remission induction is promoted by a certain improvement in the intestinal flora. The authors also reported that probiotic therapy might be effective for maintenance of remission.

Applications

The authors conducted a single-center, randomized, double-blind, placebo-controlled study to examine whether 12 mo of probiotic therapy is useful for preventing relapse of UC in patients who were already in remission.

Peer-review

In this study, the authors designed the randomized double-blind controlled study to evaluate the effectiveness of probiotic therapy for suppressing relapse in patients with UC. They concluded that probiotics may be effective for maintaining clinical remission in patients with quiescent UC, especially those who belong to cluster Ⅰ on fecal bacterial analysis. This paper is well written and the results of the study are clinically interesting.

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