Digestive Diseases and Sciences (2018) 63:1910–1919
https://doi.org/10.1007/s10620-018-4946-2

ORIGINAL ARTICLE

Efficacy of *Bifidobacterium breve* Fermented Milk in Maintaining Remission of Ulcerative Colitis

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Received: 6 October 2017 / Accepted: 23 January 2018 / Published online: 15 February 2018

Abstract

Background Fermented milk products containing *Bifidobacterium breve* strain Yakult (BFM) may improve clinical status in ulcerative colitis (UC) patients.

Aims To assess efficacy of BFM in maintaining remission in Japanese patients with quiescent UC.

Methods This double-blind study (B-FLORA) enrolled 195 patients with quiescent UC, randomized to receive one pack of BFM fermented milk per day [*Bifidobacterium breve* strain Yakult (10 billion bacteria) and *Lactobacillus acidophilus* (1 billion bacteria)] ($n = 98$) or matching placebo ($n = 97$) for 48 weeks. The primary efficacy endpoint was relapse-free survival (relapse: rectal bleeding score ≥ 2 on Sutherland disease activity index scale for 3 consecutive days and/or initiation of remission induction therapy for worsening of UC).

Results An interim analysis was conducted after inclusion and follow-up of one-third of patients for the first phase of the study ($n = 195$). Relapse-free survival was not significantly different between the BFM and placebo groups ($P = 0.643$; hazard ratio 1.16; 95% CI 0.63–2.14, log-rank test), nor was the incidence of relapse. Therefore, the study was discontinued for lack of efficacy. An exploratory analysis of fecal samples from a subgroup of patients revealed no effects of either study beverage on intestinal microbiota, but there was a significant decrease in *Bifidobacterium* species before relapse, regardless of treatment group. Three mild adverse events occurred for which a causal relationship with the study beverage could not be ruled out (placebo: abdominal bloating and stress in one patient; BFM: body odor in one patient).

Conclusions BFM had no effect on time to relapse in UC patients compared with placebo.

Study Registration UMIN000007593.

Keywords Ulcerative colitis · *Bifidobacterium breve* · Probiotics · Randomized controlled trial

Introduction

Inflammatory bowel disease (IBD) refers to a collection of diseases including ulcerative colitis (UC) that are characterized by chronic inflammation in the gut causing abdominal pain and bloody diarrhea. The annual incidence of UC is 1–20 cases/100,000 individuals, and the prevalence is 8–26 cases/100,000 [1]. In Asia, the incidence of IBD is increasing and is thought to be related to a Westernized diet and the increased use of antibiotics [2].

UC is differentiated from other IBDs by the location of inflammation, which occurs only in the rectum and colon in UC, but is widespread throughout the gastrointestinal tract in Crohn's disease. Although the pathogenesis of UC is thought to involve a genetic susceptibility component and environmental factors, it is poorly understood. However, anti-inflammatory agents targeting the immune system can be used to manage the disease. In some cases, surgical resection of the colon is necessary in patients with severe disease or in patients who do not respond to treatment.

The gut contains numerous species of bacteria (microbiota) that maintain the mucosal barrier and aid digestion. The microbiota was recently shown to affect development of the immune system [3]. Because the microbiota exists in homeostasis with

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10620-018-4946-2) contains supplementary material, which is available to authorized users.

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the human host, the dysregulation of bacterial species caused by a change in diet, antibiotic use, or disease might be involved in IBD pathogenesis by breaking immunologic tolerance, leading to an abnormal inflammatory response to the presence of commensal bacteria. However, specific species involved in IBD have not been identified to date [4].

Recently, interest has increased in the use of commensal bacteria or bacteria present in fermented foods, termed probiotics, to modulate the microbiota and confer positive effects on the immune system [5, 6] with the potential to treat IBD.

A previous study demonstrated that *Clostridium butyricum* MIYAIRI prevented pouchitis and altered the microbiota in UC patients [7]. A clinical trial showed the efficacy of *Bifidobacterium longum* 536 (BB536) supplementation for inducing remission in Japanese patients with active UC [8]. Furthermore, the use of Bio-Three tablets containing *Streptococcus faecalis, Clostridium butyricum,* and *Bacillus mesentericus* maintained clinical remission in patients with quiescent UC [9]. Some studies have also shown promising results using fermented milk products containing *Bifidobacterium breve* strain Yakult. For example, live *B. breve* strain Yakult and galactooligosaccharide improved the clinical status in UC patients compared with a control group [10]. An in vitro study of peripheral blood mononuclear cells from UC patients treated with *B. breve* reported increased production of the anti-inflammatory cytokine, IL-10 [11]. The administration of *Bifidobacterium* strains to mice with dextran sulfate-induced intestinal inflammation reduced the worsening of intestinal inflammation, indicating that it inhibited the growth of *Bacteroides vulgatus*, which might promote gut inflammation [12]. However, despite these studies showing benefits of probiotics, the mechanism of action of probiotics in UC is currently poorly understood and their effects on gut microbiota are unclear.

Mil–Mil®, a fermented milk product containing *B. breve* strain Yakult, is an easily obtainable source of bifidobacteria. Its consumption is easy, and it is a cost-effective method to provide bifidobacteria to UC patients.

Some UC patients undergo quiescent periods, the maintenance of which is beneficial. We hypothesized that *B. breve* strain Yakult fermented milk is more effective than the energy beverage (placebo) for preventing the relapse of UC in patients with UC in remission. In addition, an exploratory analysis was conducted in patients with UC in remission enrolled at one study center to assess the effects of *B. breve* strain Yakult fermented milk on intestinal microbiota.

Methods

Patients

UC patients were enrolled at study sites across Japan from April 2012 to September 2013, and eligibility was confirmed. Informed written consent was obtained from all patients at the time of enrollment. A diagnosis of UC was made by clinical, endoscopic, and histological findings according to standard criteria [13]. Eligible patients were those diagnosed with UC, in remission, who gave written informed consent, were aged 20–70 years, and within 2 years before the day of enrollment had experienced worsening symptoms defined as one or more of the following criteria: (1) persistent bloody stool for ≥ 1 week; (2) initiation of 5-aminosalicylic acid (5-ASA) treatment, dose escalation, or a change in medication type for worsening symptoms; (3) initiation or dose escalation of cytapheresis or glucocorticoids; or (4) initiation or dose escalation of immunomodulators, immunosuppressants, or anti-tumor necrosis factor (TNF)-α antibody reagents (Supplementary Data S1).

Exclusion criteria included: (1) diagnosed with proctitis-type UC; (2) visible bloody stools detected < 4 weeks before enrollment; (3) dose modifications (dose reduction or escalation) of oral 5-ASA preparation, or change in medication type for worsened UC, < 4 weeks before enrollment; (4) local administration of an 5-ASA preparation or glucocorticoid < 4 weeks before enrollment; (5) administration of cytapheresis < 4 weeks before enrollment; (6) administration of immunomodulators (azathioprine, mercaptopurine), immunosuppressants (tacrolimus, cyclosporin) or anti-tumor necrosis factor-α antibody reagents (infliximab) < 12 weeks before enrollment; (7) unable to stop regular consumption of probiotic products, other than the study beverage, or food products using lactic acid bacteria during the study period; and (8) regular consumption of *B. breve* strain Yakult used in this study < 10 days before enrollment (Supplementary Data S2).

Prohibited concomitant therapy included 5-ASA treatment, glucocorticoids, immunomodulators/immunosuppressants, cytapheresis, and antibiotics and antibacterial agents (Supplementary Data S3). Restricted treatments were allowed with conditions and included standard treatments for UC if patients were taking them at the time of enrollment (Supplementary Data S3).

Disease severity was determined based on "remission" in examinations performed less than 4 weeks before the day of enrollment.

Remission was defined using the Sutherland disease activity index (DAI) scale with a rectal bleeding score of 0 and an endoscopic score of 0 or 1 [14].

Study Design and Treatments

This was a multicenter, randomized, placebo-controlled, double-blind parallel-group study, conducted at the following study sites in Japan: Keio University School of Medicine; Tokyo Yamate Medical Center, Center for Inflammatory Bowel Disease; Toho University Medical Center

Sakura Hospital; Kitasato University Hospital; Yokohama City University Medical Center, Inflammatory Bowel Disease Center; Kannai-Suzuki Clinic; and Matsushima Clinic.

Patients received either one pack of *B. breve* strain Yakult fermented milk (Mil–Mil) per day for 48 weeks (BFM group) or placebo (one pack of energy beverage) per day for 48 weeks.

BFM and placebo consisted of 100 mL of an opaque white liquid that were identical except that BFM contained *B. breve* strain Yakult (10 billion bacteria) and *Lactobacillus acidophilus* (1 billion bacteria) as contained in a commercially available pack of Mil–Mil.

Patient data were sent to the registration center via facsimile, where randomization was implemented by the central registration method. A statistician determined the algorithm of allocation.

Patients were randomly allocated to the BFM group or placebo group at a ratio of 1:1 by dynamic allocation with the following randomization factors: age (≥ 40 years/< 40 years), sex (male/female), duration of remission maintenance during the period from the day of latest remission induction therapy to the day of enrollment (≤ 12 weeks/> 12 weeks), study site (each study site), and compliance with 5-ASA (≥ 80%/< 80%/not prescribed).

The study beverage and placebo beverage were packaged to be indistinguishable in appearance from each other and were delivered from the distribution center based on the allocation results at the time of enrollment. The distribution center, which was not informed of the groups to which patients were allocated, delivered the study beverage according to the provided numbers; this maintained blindness of the study.

Primary Endpoint

The primary endpoint was relapse-free survival of UC during the study period. Relapse was defined as persistence of a rectal bleeding score of ≥ 2 on the Sutherland DAI scale for 3 consecutive days and/or initiation of remission induction therapy for worsening of UC (as judged by the investigator).

Secondary Endpoints

The secondary study endpoints were: (1) time to "worsening" of UC during the study period; (2) time to relapse of UC for 24 weeks after enrollment; (3) change in abdominal symptom score from baseline; (4) change in each Sutherland DAI subscore; and (5) change in abdominal symptom scores (passage of flatus and bloating).

The effect of the study beverage on intestinal microbiota in 43 UC patients enrolled at Keio University Hospital was investigated as an exploratory endpoint. Examination of intestinal microbiota was performed at weeks 0, 4, 12, 24, 36, and 48 (or at study discontinuation).

Worsening of UC was defined as a condition that was consistent with the definition of relapse or if prohibited/restricted concomitant therapy was required.

"Completion" was defined as ingestion of the allocated study beverage throughout the 48-week study, and "discontinuation" was defined as incomplete ingestion of the allocated study beverage.

Clinical Procedures

The items for investigation included patient characteristics, eligibility, Sutherland DAI scores, other medical information, and evaluation of adverse events (Supplementary Table S1). Patient information was collected at enrollment (week 0), and then fecal examination was performed at weeks 4, 12, 24, and 36, and at study discontinuation (week 48) (Supplementary Table S1). Sutherland DAI scores, symptoms, use of study beverage, and other factors were assessed at weeks 12, 24, and 36, and at study discontinuation (week 48).

Analysis of the Intestinal Microbiota

The Yakult Intestinal Flora SCAN (YIF-SCAN®) system was used for the exploratory analysis of intestinal microbiota in patients enrolled at Keio University Hospital. This system determines the distribution of common intestinal bacteria by 16*S* or 23*S* rRNA-targeted RT-quantitative PCR [15, 16].

Safety

The safety endpoints were the number and proportion of patients developing adverse events for which a causal relationship with the study beverage could not be ruled out during the study period.

Adverse events were defined as any unfavorable symptoms or signs (including abnormal laboratory values) occurring in patients.

Events reported were limited to those for which a causal relationship with the study beverage could not be ruled out.

Subgroup Analysis

The post hoc subgroup analysis was based on the judgment of the executive committee examining the primary analysis results, who decided to assess the presence or absence of relapse and relationship with stress for each dose of 5-ASA.

Ethical Statement

This study was conducted in compliance with the Declaration of Helsinki and the Ethical Guidelines for Clinical Studies of the Ministry of Health, Labour and Welfare, Japan. The study was initiated after review and approval by the ethics review board of each site. This study was registered in the UMIN clinical trials registry (Trial ID: UMIN000007593).

Statistics

The number of subjects required to achieve a significance level of 0.05 for a two-sided test and a statistical power of at least 80% by log-rank analysis was calculated to be 290 per group, for a total of 580, assuming a relapse rate of 20% for the placebo group and 30% for the control group.

To allow for the number of patients discontinuing during the study, the target number of subjects was determined to be 300 per group, for a total of 600. However, it was considered unfeasible to enroll 600 patients because the study beverage has a short expiration date and it needed to be delivered to the patients' homes every week. Therefore, the study was divided into two periods (Periods I and II). It was decided to enroll one-third of the target number of patients in Period I and then to judge whether the entire study should be implemented based on the results of an interim analysis to be conducted after completion of Period I. The number of patients per group for Period I was set as 100 ($n = 200$ in total), and the target sample size for the entire study was 300 subjects per group ($n = 600$ subjects in total), which was expected to provide adequate information for group comparisons.

Kaplan–Meier analyses were used to determine relapse-free survival and worsening-free survival. Between-group comparisons were done using the log-rank test. A P value of < 0.05 was considered statistically significant. Regarding the decision to continue/discontinue the study, the Bayesian predictive power was calculated and compared with a reference range. The data analysis was performed by an independent data monitoring committee. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

Study Flow

Overall, 195 patients with quiescent UC were enrolled and randomized into either the BFM ($n = 98$) or placebo group ($n = 97$) (Fig. 1). After enrollment, no patients in the BFM group and one patient in the placebo group were withdrawn because of a change in the 5-ASA dose up to 4 weeks before enrollment (Fig. 1). One patient from the BFM group and one patient from the placebo group were excluded for not adhering to the study beverage. The compliance of the study beverage was similar between the groups (Supplementary Table S2).

The full analysis set and per-protocol analysis set were 97 and 43, respectively, in the BFM group and 95 and 39 in the placebo group (Fig. 1).

Patients discontinued the study because of discontinuation of protocol-specified treatments, relapse of UC, start of prohibited concomitant treatment, adverse events, discontinuation of study beverage, and other factors judged by the study investigator (Fig. 1). Of note, no patients in the BFM group discontinued because of adverse events compared with two patients in the placebo group. One patient in the placebo group was discontinued from the study by decision of the study investigator because the patient could not be contacted for an extended period of time.

Patient Demographics

Patient demographics are shown in Table 1. The BFM and placebo groups contained similar numbers of males and females of a similar age (mean age: BFM = 41.3 years vs. placebo = 41.8 years). In addition, there were no differences in remission maintenance therapy, location of lesions, numbers receiving 5-ASA therapy, or the Sutherland DAI scores (Table 1).

Primary Endpoint

The primary endpoint, relapse-free survival of UC during the study, was not significantly different between the BFM and placebo groups ($P = 0.643$; hazard ratio [HR] 1.16: 95% CI 0.63–2.14, log-rank test) (Fig. 2). Furthermore, the incidence of relapse was not significantly different ($P = 0.651$) between the BFM (22.7%) and placebo groups (20.0%).

These results indicate that BFM was not superior to placebo for the primary endpoint. No remission-maintaining effect of BFM was demonstrated ($P = 0.643$) (Fig. 2).

No differences were observed between the BFM and placebo groups when patients were stratified by sex, age, remission maintenance, lesion location, remission induction therapy, or Sutherland DAI scale scores (Table 2).

Secondary Endpoints

The secondary endpoint, time to worsening of UC during the study period (48 weeks after enrollment), was not significantly different between the two groups (BFM, 25.8% vs. placebo, 24.2%; $P = 0.803$).

In addition, there were no statistically significant differences in clinical deterioration ($P = 0.803$) or worsening-free survival ($P = 0.778$; HR 1.08: 95% CI 0.62–1.91, log-rank

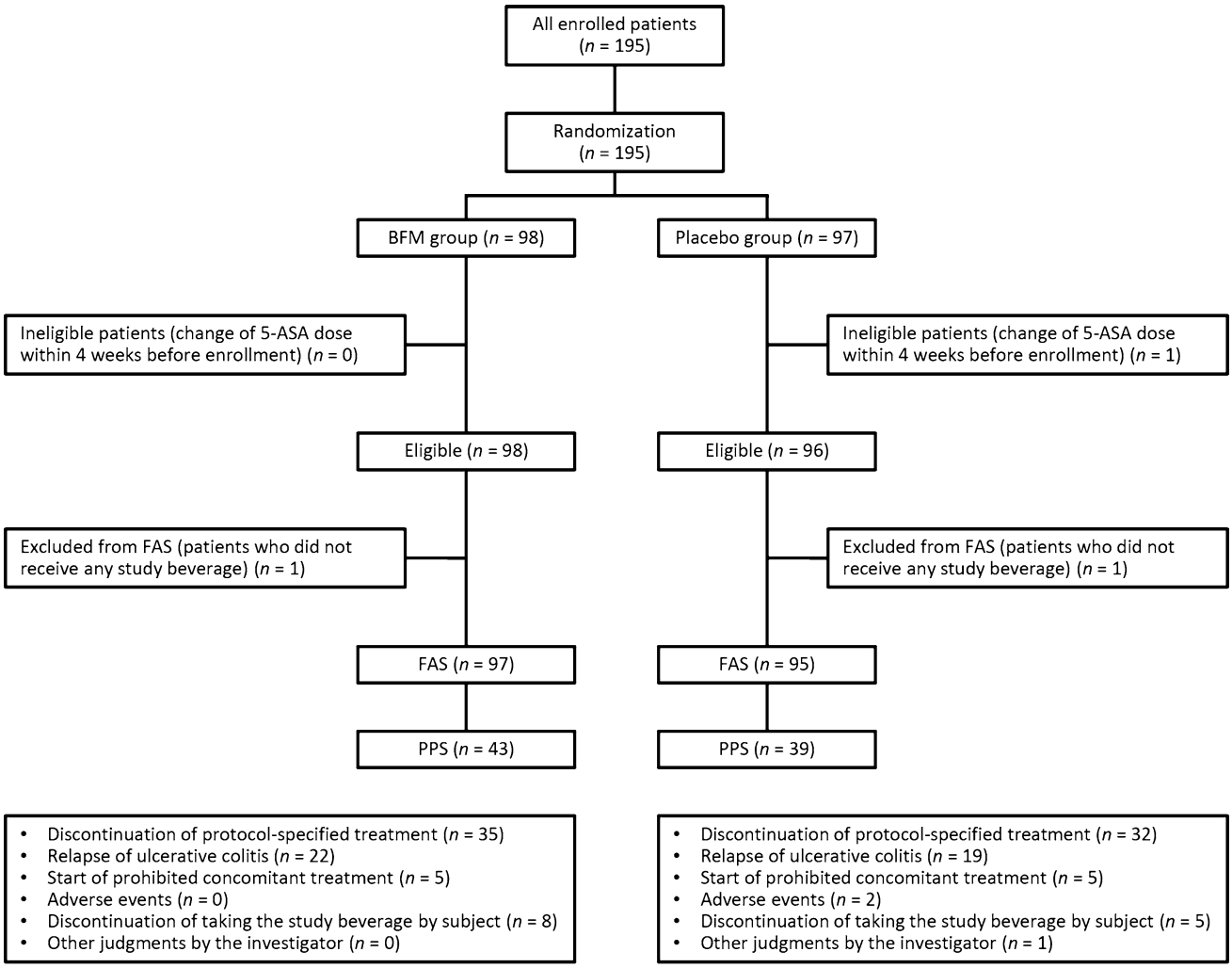

Fig. 1 Study flowchart. Some patients discontinued for more than one reason. *5-ASA* 5-aminosalicylic acid, *BFM Bifidobacterium breve* strain Yakult fermented milk, *FAS* full analysis set, *PPS* per-protocol set

Table 1 Patient characteristics

Variables	BFM group $n = 97$	Placebo group $n = 95$
Males/females (%)	51.6/48.5	52.6/47.4
Age, years, mean (range)	41.3 (20–70)	41.8 (20–66)
Remission maintenance therapy from day of latest remission induction therapy to day of enrollment, days, mean (range)	362.7 (54–750)	378.6 (41–846)
Location of lesion (%)		
Pancolitis	49.5	54.7
Left-sided colitis	50.5	45.3
Use of 5-ASA, *n* (%)	96 (99.0)	94 (98.9)
Sutherland DAI score, mean ± SD	1.08 ± 0.16	1.07 ± 0.12

Student's *t* test was used for continuous data, and Chi-squared test was used for categorical variables

BFM Bifidobacterium breve strain Yakult fermented milk, *5-ASA* 5-aminosalicylic acid, *DAI* disease activity index, *SD* standard deviation

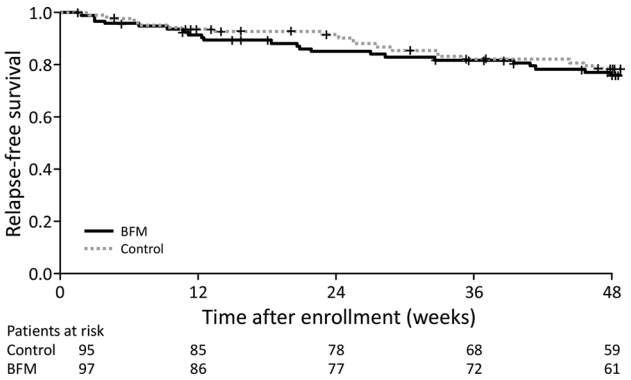

Fig. 2 Relapse-free survival rate during the study period (48 weeks after enrollment). *BFM Bifidobacterium breve* strain Yakult fermented milk

test) between the BFM and placebo groups (Supplementary Fig. S1). The median times to worsening/relapse in the BFM and control groups were 48.6 weeks and 48.9 weeks, respectively, though these times were calculated including all patients (even those who did not experience relapse, in which the relapse date was defined as the date of the final follow-up visit). However, if the median times are calculated including only patients who experienced a relapse during the study period, the median times to worsening/relapse were 19.2 weeks in the BFM group and 24.8 weeks in the control group.

Time to relapse of UC during 24 weeks after enrollment was not significantly different between the two groups (BFM, 14.4% vs. placebo, 8.4%; $P = 0.191$) or relapse-free survival ($P = 0.199$; HR 1.75: 95% CI 0.74–4.18, log-rank test) (Supplementary Fig. S2).

There were no statistically significant differences in the mean DAI scores, abdominal symptom scores, stool frequency scores, rectal bleeding scores, or physician's rating of disease activity between the BFM and placebo groups (Supplementary Table S3).

Study Discontinuation After Interim Analysis

The interim analysis results showed that the Bayesian predictive power was 3.7%, which was markedly lower than the reference range for study continuation (20–25%). Based on this, the Independent Data Monitoring Committee recommended discontinuation of the study, so the study was discontinued.

Safety

There were three serious adverse events: avascular necrosis of bilateral femoral head (in one patient in the placebo group), surgical removal of granuloma in the throat (in one patient in the BFM group), and pulmonary thromboembolism (in one patient in the placebo group). However, a causal relationship with the study beverage was ruled out for all these events.

Throughout the study period, there were three adverse events, occurring in two patients, for which a causal relationship with the study beverage could not be ruled out: abdominal bloating and stress in one patient in the placebo group, and body odor in one patient in the BFM group. All of these events were mild and resolved.

Analysis of Intestinal Microbiota

Analysis of intestinal microbiota in the cohort of 43 patients from Keio University Hospital showed no significant differences between the two treatment groups over the course of the study, except for *Clostridium leptum*, though the clinical significance of this is not clear (Supplementary Figs. S3 and S4). During the study period, patients visited the clinic as soon as possible upon relapse, which was designated as Visit 6. A comparison of intestinal bacteria between the relapsed group and the maintained remission group, regardless of treatment group, revealed a significant decrease in *Bifidobacterium* species before relapse (Fig. 3 and Supplementary Fig. S5).

Discussion

The objective of this study was to investigate the potential effect of *B. breve* strain Yakult on maintaining the beneficial state of relapse in UC patients. Unfortunately, this study demonstrated a lack of effect of *B. breve* strain Yakult on maintaining relapse-free survival or on Sutherland DAI scale scores including stool frequency, rectal bleeding, and physician's rating of disease activity when compared to placebo. Based on this information, the Independent Data Monitoring Committee recommended the discontinuation of the study and it was discontinued.

Previous studies have yielded conflicting results regarding the use of probiotics in UC, either for inducing or maintaining remission. Some have shown that probiotics might modulate gut microbiota and have positive effects on the immune system, and may be used to treat gut diseases such as UC. A randomized study by Ishikawa et al. that treated UC patients with *B. breve* strain Yakult powder and galactooligosaccharide (synbiotics group) for 1 year reported that UC clinical status was improved, as assessed by colonoscopy [10]. However, although that study showed a significant decrease in the mucosal appearance score before and after treatment in the synbiotics group, the effect was not compared between the synbiotic and control groups.

Table 2 Subgroup analysis

Group	*n*	*n* with events	HR	95% CI
Allocation factor				
Sex				
Male				
BFM	50	9	1.17	(0.45–3.04)
Placebo	50	8	Reference	
Female				
BFM	47	13	1.17	(0.53–2.62)
Placebo	45	11	Reference	
Age				
≥ 40 years				
BFM	54	9	0.76	(0.31–1.83)
Placebo	53	11	Reference	
< 40 years				
BFM	43	13	1.80	(0.74–4.34)
Placebo	42	8	Reference	
Remission maintenance therapy from day of latest remission induction therapy to day of enrollment				
≤ 12 weeks				
BFM	9	3	1.17	(0.24–5.83)
Placebo	10	3	Reference	
> 12 weeks				
BFM	88	19	1.16	(0.60–2.26)
Placebo	85	16	Reference	
Lesion				
Location of lesion				
Pancolitis				
BFM	48	7	0.65	(0.25–1.68)
Placebo	52	11	Reference	
Left-sided colitis				
BFM	49	15	1.82	(0.77–4.29)
Placebo	43	8	Reference	
Latest remission induction therapy				
Initial-onset				
BFM	4	0	–	
Placebo	3	0		
Relapse				
BFM	93	22	1.17	(0.64–2.17)
Placebo	2	19	Reference	
Sutherland DAI (Week 0)				
Stool frequency				
Normal				
BFM	83	19	1.04	(0.55–1.98)
Placebo	83	18	Reference	
1–2 stools/day more than normal				
BFM	13	3	–	
Placebo	11	1		
3–4 stools/day more than normal				
BFM	1	0	–	
Placebo	1	0		
> 4 stools/day more than normal				
BFM	0	–	–	
Placebo	0	–		
Rectal bleeding				
None				
BFM	97	22	1.16	(0.63–2.14)
Placebo	95	19	Reference	
Streaks of blood				
BFM	0	–	–	
Placebo	0	–		
Obvious blood				
BFM	0	–	–	
Placebo	0	–		
Mostly blood				
BFM	0	–	–	
Placebo	0	–		
Mucosal appearance endoscopic subscore				
0				
BFM	88	21	1.18	(0.63–2.22)
Placebo	88	18	Reference	
1				
BFM	9	1	–	
Placebo	7	1		
2				
BFM	0	–	–	
Placebo	0	–		
3				
BFM	0	–	–	
Placebo	0	–		
Physician's rating of disease activity				
Normal				
BFM	89	21	1.21	(0.64–2.26)
Placebo	89	18	Reference	
Mild				
BFM	8	1	–	
Placebo	6	1		
Moderate				
BFM	0	–	–	
Placebo	0	–		
Severe				
BFM	0	–	–	

Analysis by Cox regression model

HR hazard ratio, *CI* confidence interval, *BFM Bifidobacterium breve* strain Yakult fermented milk, *DAI* disease activity index

Yoshimatsu et al. [9] investigated the effects of Bio-Three tablets, which contain 2 mg of lactomin (*Streptococcus faecalis* T-110), 10 mg of *Clostridium butyricum* TO-A, and 10 mg of *Bacillus mesentericus* TO-A, in Japanese

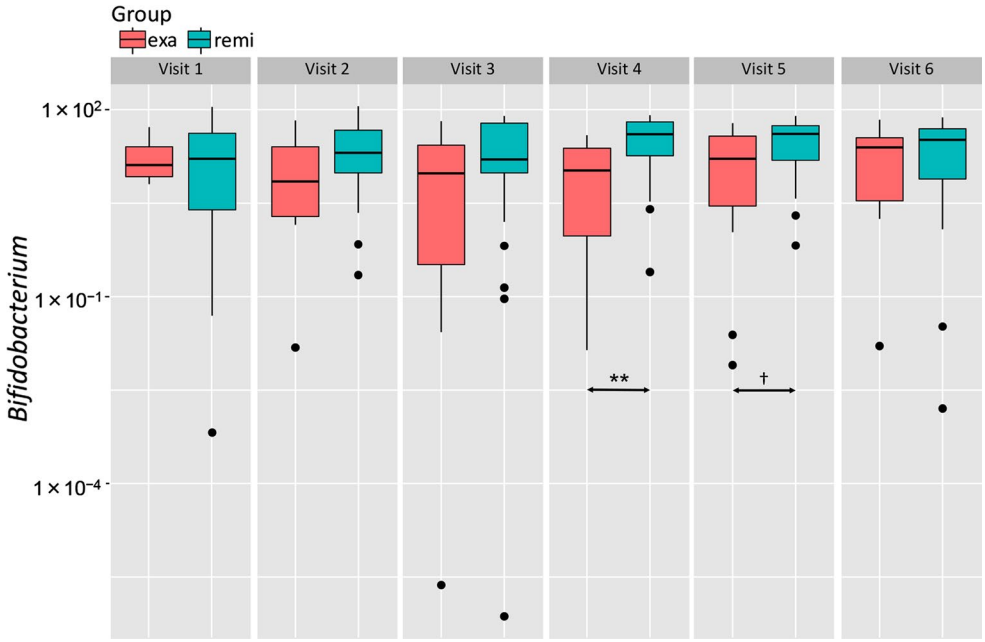

Fig. 3 *Bifidobacterium* levels in the relapse (exa) versus remission (remi) groups. $^{\dagger}P < 0.063$, $^{**}P = 0.006$ by Mann–Whitney *U* test

outpatients with UC in remission. They found that the relapse rate at 3, 6, and 9 months tended to be lower in the Bio-Three group than in the placebo group (3 months: 0.0 vs. 17.4%, $P = 0.036$; 6 months: 8.7 vs. 26.1%, $P = 0.119$; 9 months: 21.7 vs. 34.8%, $P = 0.326$). The remission rate at 12 months was 69.5% in the Bio-Three group versus 56.6% in the placebo group ($P = 0.248$). Therefore, the authors concluded that probiotic therapy may help to maintain clinical remission in patients with quiescent UC.

Tamaki et al. [8] determined the efficacy of *Bifidobacterium longum* 536 (BB536) in terms of the induction of remission in Japanese patients with mild-to-moderately active UC. In that study, the remission rates at week 8 were 63 and 52% ($P = 0.395$) in the BB536 and placebo groups, respectively. BB536, but not placebo, was associated with significant reductions in the UCDAI score, Rachmilewitz endoscopic index, and the Mayo subscore at week 8. However, that study was of very short duration (8 weeks), and longer studies are needed to determine whether these short-term benefits of BB536 are maintained for longer periods.

Sood et al. found that the probiotic VSL#3 was safe and effective in achieving clinical responses and remissions in patients with mild-to-moderately active UC [17].

Other studies have yielded less positive results regarding the efficacy of probiotics in UC patients. A Cochrane review published in 2011 found no statistically significant difference between probiotics and 5-ASA (three studies) or between probiotics and placebo (one study) in maintaining remission [18], while a more recent study from 2014 showed no benefit of *Escherichia coli* Nissle 1917 as an add-on treatment to conventional therapies for active UC [19].

Despite the apparent lack of efficacy of *B. breve* strain Yakult in our study, we found low levels of bifidobacteria levels in both groups prior to relapse. These results suggest that the maintenance of bifidobacteria levels is very important for the maintenance of remission. In addition, the results suggested that bifidobacteria levels can be used as a predictor of relapse. The reason for the absence of a significant treatment effect in the present study may be that the amount of bifidobacteria administered was too small to contribute to the maintenance of bifidobacteria throughout the intestine. Alternatively, it is also interesting to note that the relapse-free survival rates were quite high (approximately 80%) in both groups at the end of the study. It could be that the patients enrolled in this study (who tended to have relatively long periods between the latest remission induction therapy and enrollment in the study) were expected to gain long-term remission, which may have masked any potential effects of the study treatment.

Thus, a potential limitation of this study is an insufficient number of bacteria and species (*B. breve* strain Yakult: 10 billion; *L. acidophilus*: 1 billion) contained in the study beverage. Therefore, a future study using different doses of *B. breve* strain Yakult is warranted. Another potential reason for lack of efficacy may be the delivery mode. Ishikawa et al. found that *B. breve* strain Yakult powder improved UC [10]. However, their results may have been affected by the concomitant administration of galacto-oligosaccharide. Furthermore, because we did not perform endoscopic analysis we could not determine any effect of *B. breve* strain Yakult on patient mucosal status.

In summary, *B. breve* strain Yakult had no effect on the time to relapse in UC patients. However, this might reflect

the method of delivery or dosage of *Bifidobacterium* species rather than the efficacy of the bacterial culture itself, as previous studies demonstrated beneficial effects of bifidobacteria. To effectively gauge the efficacy of probiotics in UC, future studies should compare the effects of different probiotics, combinations, dosing regimens, and delivery modes and take into account specific patient populations and disease severity.

Acknowledgments The authors would like to thank J. Ludovic Croxford, PhD, Nicholas Smith, PhD, and Marion Barnett of Edanz Medical Writing for providing medical writing services. The authors would also like to thank Shigeru Kageyama, MD, PhD, of The Jikei University Hospital; Kojiro Shimozuma, MD, PhD, of Ritsumeikan University; and Kenichi Yoshimura, PhD, of Kanazawa University for participating in the Independent Data Monitoring Committee. Finally, the authors thank Kojiro Shimozuma and Tomoaki Nakamura, PhD, of the Public Health Research Foundation (PHRF) for assisting with the exploratory analysis of intestinal microbiota. The research funding was provided to the Comprehensive Support Project for Clinical Research of Lifestyle-Related Disease (CSP-LD) of the PHRF by Yakult Honsha Co., Ltd.

Funding This work was supported by Yakult Honsha Co., Ltd.

Compliance with ethical standards

Conflict of interest Takanori Kanai received a financial donation from Yakult. Yasuo Suzuki, Toshifumi Hibi, Yukari Uemura, Kaoru Yokoyama, Naoki Yoshimura, Reiko Kunisaki, and Katsuyoshi Matsuoka have no conflicts of interest to declare.

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