Efficacy of probiotic treatment with *Bifidobacterium longum* 536 for induction of remission in active ulcerative colitis: A randomized, double-blinded, placebo-controlled multicenter trial

Hiroyuki Tamaki,1 Hiroshi Nakase,2 Satoko Inoue,2 Chiharu Kawanami,5 Toshinao Itani,4 Masaya Ohańa,6 Toshihiro Kusaka,3 Suguru Uose,7 Hiroshi Hisatsune,8 Masahide Tojo,9 Teruyo Noda,1 Souichi Arasawa,1 Masako Izuta,1 Atsushi Kubo,1 Chikara Ogawa,1 Toshihiro Matsunaka1 and Mitsushige Shibatouge1

1Department of Gastroenterology, Takamatsu Red Cross Hospital, Takamatsu, 2Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University Hospital, 3Digestive Disease Center, Kyoto-Katsura Hospital, Kyoto, 4Department of Gastroenterology, Nishi Kobe Medical Center, 5Department of Gastroenterology, Kobe City Medical Center Hospital, Kobe, 6Department of Gastroenterology, Tenri Hospital, Tenri, 7Department of Gastroenterology, Kansai Electric Power Hospital, 8Department of Gastroenterology, Saiseikai Noe Hospital, Osaka, and 9Department of Gastroenterology, Otsu Red Cross Hospital, Otsu, Japan

**Background and Aim:** We conducted a randomized, double-blinded, placebo-controlled trial to investigate the efficacy of *Bifidobacterium longum* 536 (BB536) supplementation for induction of remission in Japanese patients with active ulcerative colitis (UC).

**Methods:** Fifty-six patients with mild to moderate UC were enrolled. Three patients had pancolitis, 36 had left-sided colitis, and 17 had proctitis. Patients were randomly treated with 2–3 × 10^11 freeze-dried viable BB536 (28 patients) or placebo (28 patients) for 8 weeks.

**Results:** In total, 63% of patients receiving BB536 showed clinical remission (UC disease activity index [UCDAI] ≤ 2) at week 8 compared to 52% of those receiving placebo (P = 0.395). We observed a significant decrease of UCDAI scores (3.8 ± 0.4 at baseline to 2.6 ± 0.4 at week 8) in the BB536 group (P < 0.01), whereas there was no significant decrease in the placebo group (P = 0.88). There was also a significant decrease in the Rachmilewitz endoscopic index (EI) and the Mayo subscore at week 8 in the BB536 group, whereas there was no significant decrease in the placebo group. A single patient in the BB536 group complained of a mild side-effect, but no other adverse effects were observed.

**Conclusion:** Supplementation with BB536 was well tolerated and reduced UCDAI scores, EI and Mayo subscores after 8 weeks in Japanese patients with mild to moderately active UC.

**Key words:** induction of remission, microbiota, probiotic, randomized controlled trial, ulcerative colitis

**INTRODUCTION**

Ulcerative colitis (UC) is a chronic inflammatory bowel disease of unknown etiology that is characterized by acute exacerbations followed by remissions. Although the etiology is still unclear, one of the main hypotheses is that UC is caused by an excessive immune response to endogenous bacteria in genetically predisposed individuals.1 Therefore, manipulation of the mucosal microbiota to reduce the inflammatory potential of colonizing bacteria is an attractive therapy for UC. Most conventional UC therapies, including 5-aminosalicylic acid (ASA) compounds, corticosteroids, and immunosuppressant agents, suppress intestinal inflammation associated with UC. However, a subpopulation of patients is intolerant or refractory to these therapies because of their significant adverse effects. In this regard, treatments that directly modulate gut microbiota have been studied as adjunctive therapies or as alternative options to conventional drug therapies.2–4

Probiotics contain viable organisms, sufficient amounts of which reach the intestine in an active state and thus exert positive health effects.5 They mostly include lactic acid-producing bacteria, such as bifidobacteria and lactobacilli, but other
organisms, such as Escherichia coli and the yeast Saccharomyces boulardii, have been reported to have some beneficial effects in maintaining remission in patients with inflammatory bowel disease. Although their mechanisms of action have not been established, some studies suggest that these probiotics modulate membrane permeability and the mucosal immune system.6–8

Bifidobacterium longum 536 (BB536) is a probiotic isolated in 1969 from the feces of a breast-fed infant. The efficacy of 4 weeks of symbiotic treatment (Bifidobacterium longum with Synergy 1; Orafti, Tienen, Belgium) in treating patients with active UC has been reported by Furrie et al.7 We have also reported the molecular mechanism of BB536 on cytokine production and expression of molecules related to mucosal barrier function with in vivo and in vitro experiments. Pretreatment of heat-inactivated BB536 induced up-regulation of T-bet in splenocytes of T-cell receptor alpha-knockout mice, and inoculation of BB536 to mice resulted in increased gene expression of tight junction molecules (claudin-1 and ZO-1).8 Taken together, these data suggest that treatment with BB536 could be a promising therapeutic option for patients with active UC. However, despite growing data on the efficacy of BB536, there have been few placebo-controlled trials on the management of mild to moderately active UC. Hence, the aim of the present study was to investigate the efficacy and safety of BB536 in achieving clinical response and remission at week 8 in Japanese patients with mild to moderately active UC.

METHODS

This was a multicenter, double-blinded, placebo-controlled, randomized trial. The study was conducted at nine hospitals in Japan. The protocol was approved by the Investigational Review Board of Takamatsu Red Cross Hospital, Kyoto University Hospital, Kobe City Medical Center Hospital, Nishi Kobe Medical Center, Tenri Hospital, Koto-Katsura Hospital, Kansai Electric Power Hospital, Saiseikai Noe Hospital, and Otsu Red Cross Hospital. All patients provided written informed consent for their participation.

Patients

A total of 56 consecutive adult patients (>18 years) with mild to moderate UC (UC disease activity index [UCDAI] score 3–9; Table 1) were enrolled in the present study (27 men, 29 women; mean age 44 ± 14 years). Diagnosis of UC was established using standard clinical, radiological, endoscopic, and histological criteria. Patients were excluded if they had evidence of severe disease (UCDAI >10), concurrent enteric infection, use of antibiotics within the past 2 weeks, change in dose of oral 5-ASA within the past 4 weeks, and use of rectal 5-ASA or steroids within 7 days before entry into the study. Patients requiring hospitalization and imminent need for surgery, lactating and pregnant women, and those who received any investigational medicines within 3 months were excluded. Patients with significant hepatic, renal, endocrine, respiratory, neurological, or cardiovascular diseases were also excluded.

Randomization

Each center enrolled patients according to a randomization list. Patients who fulfilled the eligibility criteria specified above were randomly assigned to receive either BB536 or a placebo in a random order, using only one randomization list. The randomization number was strictly given according to the order of the patient’s enrollment, with each patient assigned the first available number on the randomization list. The randomization number, or the reason for not enrolling the patient, was reported for each patient in the appropriate forms. Randomization was carried out in a double-blind manner using 1:1 allocation to the two groups.

Study procedures

All study procedures were conducted for each patient enrolled in the study. At the screening visit, the patient’s baseline characteristics, including demographic information and past surgical and medical therapy, were obtained. All laboratory tests were carried out at local laboratories. Individual disease activity was assessed at the baseline visit and after 4 and 8 weeks. At each visit, a detailed physical examination and history were carried out. All patients underwent colonoscopic examination at baseline and after 8 weeks. Disease activity was assessed by UCDAI score.9 At the baseline visit and after 8 weeks, the Rachmilewitz endoscopic index (EI) and the Mayo subscore were calculated to assess mucosal state.10,11

Table 1  Demographics and baseline characteristics of patients with mild to moderate ulcerative colitis

<table>
<thead>
<tr>
<th></th>
<th>BB536</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male : female)</td>
<td>11:17</td>
<td>16:12</td>
</tr>
<tr>
<td>Age in years (mean ± SD)</td>
<td>44.9 ± 14.5</td>
<td>45.5 ± 13.8</td>
</tr>
<tr>
<td>UCDAI at entry (mean ± SD)</td>
<td>5.07 ± 2.25</td>
<td>6.32 ± 3.43</td>
</tr>
<tr>
<td>Disease extent (no. patients) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proctosigmoiditis</td>
<td>10 (35.71%)</td>
<td>7 (25.0%)</td>
</tr>
<tr>
<td>Left-sided colitis</td>
<td>17 (60.71%)</td>
<td>19 (67.86%)</td>
</tr>
<tr>
<td>Pancolitis</td>
<td>1 (3.57%)</td>
<td>2 (7.14%)</td>
</tr>
<tr>
<td>No medications</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

UCDAI, ulcerative colitis disease activity index.

© 2015 Japan Gastroenterological Endoscopy Society
All adverse events were documented, classified, and graded according to the World Health Organization recommendations for the evaluation of active and subjective toxicity. Daily disease activity records were written by the study participants, who were provided with diary cards to assess and record their symptoms. Participants’ compliance in taking the study medications (BB536 and placebo) was assessed by the investigators.

**Treatment**

Eligible patients were randomly treated with \(2 - 3 \times 10^{11}\) freeze-dried viable BB536 (Morinaga Milk Industry Co., Ltd, Tokyo, Japan; 28 patients) or placebo (containing dextrin; 28 patients) three times daily for 8 weeks. The study product, BB536, was provided in sealed plastic individual dose sachets, and placebo was supplied in identical sachets.

**Concomitant treatment**

Patients taking 5-ASA, prednisolone, azathioprine, and 6-mercaptopurine continued to receive these medications at stable doses during the study period. Any change in dosing of these drugs throughout the 8-week study period was considered a protocol violation. Rectal administration of 5-ASA, steroids, antibiotics, non-steroidal anti-inflammatory drugs, and antidiarrheal drugs was not permitted during the course of the study.

**Primary end-point**

Primary end-point was the beneficial effects of BB536 in patients with mild to moderate UC, assessed by serial change of UCDAI scores between baseline and at week 8 of treatment.

**Secondary end-points**

Secondary end-points were the possible beneficial effects of BB536 on the following: (i) UCDAI scores and the proportion of patients achieving remission (defined by UCDAI scores of 0–2) at week 8; (ii) change in objective symptoms (rectal bleeding, mucosal findings, and stool frequency) and subjective symptoms (physician rating of disease activity); and (iii) improvement in endoscopic scores, assessed by the Mayo subscore at week 8.

**Sample size**

Planned sample size was 50 patients per arm, which would result in 80% power to detect 75% improvement of response probability in the BB536 group assuming response probability of 41% in the placebo group. This calculation was based on the results of our preliminary examination of additional treatment with BB536 in the patients with UC, which resulted in a 75% (6/8) response rate. For the placebo group, we referred to the reported response rate of standard treatment with 5-ASA in the patients with UC.\(^\text{12}\)

**Statistical analysis**

Baseline characteristics of patients were compared using Student’s \(t\)-test for independent samples or Pearson’s \(\chi^2\)-test as appropriate. Continuous variables are expressed as means ± standard errors, and categorical data are expressed as percentages. UCDAI scores, EI, and the Mayo subscore at each visit were compared with the baseline visit score using the Wilcoxon test. \(P\)-values < 0.05 were considered significant.

**RESULTS**

**Participant flow**

A TOTAL OF 56 patients (28 in the BB536 group and 28 in the placebo group) were enrolled between January 2007 and May 2009. Twenty-four patients in the BB536 group and 23 patients in the placebo group completed the study (86% and 82%, respectively). Nine patients withdrew from the study during treatment. Two patients in the BB536 group withdrew during the follow-up period. Among the remaining seven patients, there were protocol violations with two patients in the BB536 group and five in the placebo group (dose escalation of a concomitant drug or other additional treatments were required for exacerbation of UC; Fig. 1).

**Clinical characteristics**

Clinical characteristics of the enrolled patients in the two groups are shown in Table 1. No significant differences were identified in terms of demographic characteristics (mean age; sex; extent of disease; mean UCDAI scores; and the use of steroids, 5-ASA, or immunosuppressive drugs).

**Primary end-point**

There was a significant decrease in UCDAI scores at week 8 in the BB536 group (3.8 ± 0.4 at baseline vs 2.6 ± 0.4 at week 8; \(P < 0.01\)), whereas there was no significant decrease in these scores in the placebo group (4.5 ± 0.5 at baseline vs 3.2 ± 0.6 at week 8; \(P = 0.88\)) (Fig. 2).

**Secondary end-points**

Although the UCDAI scores at week 8 tended to be lower in the BB536 group than in the placebo group, this difference was not significant (2.75 ± 2.01 in the BB536 group vs 3.43 ± 3.03 in the placebo group; \(P = 0.50\)) (Fig. 3A). Nineteen patients (79.2%) in the BB536 group showed clinical improvement compared to 14 patients (60.9%) in the placebo group.
and 15 patients (62.5%) in the BB536 group showed clinical remission (UCDAI ≤ 2) at week 8 compared to 12 patients (52.2%) in the placebo group (PP, P = 0.39; ITT, P = 0.63) (Fig. 3B); however, these differences were not statistically significant.

To assess the factors that contributed to the improvement in UCDAI score, we evaluated the changes in each component of the UCDAI score: rectal bleeding, mucosal findings, stool frequency, and physician rating of disease activity. Patients receiving BB536 had a significant reduction in rectal bleeding (0.79 ± 0.19 at baseline vs 0.5 ± 0.17 at week 8; P = 0.038) and mucosal findings (2.23 ± 0.51 at baseline vs 1.73 ± 0.6 at week 8; P = 0.017) whereas there was no significant decrease in these scores in the placebo group (Fig. 4). In contrast, there were no significant differences in stool frequency and physician’s global assessment in either group (data not shown).

We evaluated the endoscopic improvement in each group using EI and the Mayo subscore because we found that treatment with BB536 significantly improved rectal bleeding and mucosal findings, as mentioned above. Accordingly, there was a significant decrease in EI scores at week 8 in the BB536 group (6.7 ± 0.5 at baseline vs 3.7 ± 0.6 at week 8; P < 0.01), whereas there was no significant decrease in the placebo group (6.7 ± 0.6 at baseline vs 4.5 ± 0.6 at week 8; P = 0.073) (Fig. 5A). Similarly, there was a significant decrease in Mayo subscores at week 8 in the BB536 group (2.2 ± 0.10 at baseline vs 1.5 ± 0.73 at week 8; P < 0.01), whereas there was no significant decrease in the placebo group (2.3 ± 0.10 at baseline vs 1.9 ± 0.18 at week 8; P = 0.078) (Fig. 5B). Seven patients (29.2%) in the BB536 group achieved mucosal healing (Mayo subscore = 0 or 1) at week 8 compared to four patients (17.4%) in the placebo group (Fig. 5C); however, this difference was not statistically significant (PP, P = 0.14; ITT, P = 0.41).

Safety and tolerability

No major adverse event was reported in either group. One patient in the BB536 group complained of a dry cough after 2 weeks of receiving BB536.

DISCUSSION

IN THE PRESENT study, BB536 was significantly superior to placebo in reducing UCDAI scores, and a higher proportion of patients in the BB536 group experienced clinical remission at week 8. Moreover, we observed a significant decrease in EI scores and the Mayo subscore at week 8 in the BB536 group. Furthermore, no major adverse events were reported in either group. Overall, this study demonstrated that giving BB536, in addition to standard treatment, improved clinical symptoms and endoscopic findings in Japanese patients with mild to moderately active UC.
Bifidobacteria are rod-shaped, non-gas-producing, anaerobic micro-organisms and are present in the feces of breast-fed infants. To date, 30 species in the genus *Bifidobacterium* have been identified, 10 of which are from human sources. Several reports have indicated that giving *Bifidobacterium infantis*, *Bifidobacterium breve*, *Bifidobacterium bifidum*,...
and Bifidobacterium longum had favorable effects in reducing colonic inflammation in active UC patients. In particular, two randomized placebo-controlled trials reported the positive effects of Bifidobacterium longum on patients with mildly active UC. Fujimori et al. reported the effects of Bifidobacterium longum on quality of life in patients with UC who were in remission or had only mildly active UC by assessing inflammatory bowel disease questionnaire scores. The results of this trial showed significant improvement in the synbiotic group (treated with a combination of Bifidobacterium longum and prebiotics) in comparison with Bifidobacterium longum-alone or prebiotics-alone groups. Another trial evaluated synbiotic therapy (Bifidobacterium longum and Synergy 1 which contained fructo-oligosaccharide/inulin growth substrate for the probiotic strain; Orafti, Tienen, Belgium) for induction of remission in patients with active ulcerative colitis. Although this was a pilot trial with a small study population, 4 weeks of this synbiotic treatment resulted in improvement in UC activity. These data support the favorable effect of BB536 on patients with mild to moderate UC, consistent with the results of our study.

© 2015 Japan Gastroenterological Endoscopy Society

Figure 5  (A) Serial changes in Rachmilewitz endoscopic index (EI) (Wilcoxon t-test). There was a significant decrease in EI scores at week 8 in the BB536 group (6.7 ± 0.5 at baseline vs 3.7 ± 0.6 at week 8; P < 0.01), whereas there was no significant decrease in the placebo group (6.7 ± 0.6 at baseline vs 4.5 ± 0.6 at week 8; P = 0.073). (B) Serial changes in Mayo subscore (Wilcoxon t-test). There was a significant decrease in Mayo subscores at week 8 in the BB536 group (2.2 ± 0.10 at baseline vs 1.5 ± 0.73 at week 8; P < 0.01), whereas there was no significant decrease in the placebo group (2.3 ± 0.10 at baseline vs 1.9 ± 0.18 at week 8; P = 0.078). (C) Mucosal healing rate at week 8 (Mayo subscore = 0 or 1). Although the mucosal healing rate at week 8 tended to be higher in the BB536 group than in the placebo group, there was no statistical difference (PP P = 0.14, ITT P = 0.41).
The mechanisms by which probiotics exert biological effects are still poorly understood. Oelschlaeger et al. described three possible effects of probiotics: (i) probiotics may modulate the host’s defenses, including both the innate and acquired immune system; (ii) probiotics may also have a direct effect on other micro-organisms; and (iii) probiotic effects may be based on actions affecting host products and microbial products, such as toxins.\(^{15,16}\) Furrie et al. reported that synbiotic treatment with *Bifidobacterium longum* reduced levels of mucosal inducible inflammatory markers, including human beta-defensin, tumor necrosis factor-α, and interleukin-1α.\(^7\) Our colleagues previously reported that giving BB536 up-regulated gene expression of tight junction molecules (claudin-1 and ZO-1) via Toll-like receptor 2, leading to enhancement of intestinal barrier function in colonic tissue.\(^8\) In general, the intestinal barrier maintains epithelial integrity, which protects the organism against bacterial or food antigens. Thus, BB536 may attenuate colonic inflammation in UC by enhancing the mucosal barrier.

The current study has certain limitations. Regarding the data on induction of remission, 52% of patients in the placebo group experienced remission, which, in comparison with other studies, is a high rate of remission associated with placebo. Su et al. reported that the pooled estimate of the placebo rate of remission was 13% (95% confidence interval, 9–18%; range, 0–40%; median, 12%) based on a meta-analysis of 40 placebo-controlled, randomized clinical trials evaluating UC therapy.\(^17\) This suggests that trial length, number of study visits, remission definitions, and design features affected placebo remission rates. Su et al. also suggested that the most important factor related to a high placebo response might be the standard medical treatments that were given to all enrolled patients. In other words, standard therapies alone improve the pathological condition of mild to moderately active UC during clinical trials. However, one of the factors influencing response to BB536 treatment might be diversity of intestinal microflora. Yoshimatsu et al. reported that the clinical efficacy of probiotics as maintenance therapy in patients with UC could be altered by variations in intestinal microflora, as evaluated by terminal restriction fragment length polymorphism and cluster analysis.\(^18\) Fecal analysis of microflora in each patient with UC has the potential to identify the subpopulation most likely to respond to BB536 therapy. Another factor regarding response to BB536 treatment is the dosage. Recently, several studies have reported dose-dependent effects of probiotics on the immune response *in vitro* and *in vivo*.\(^19,22\) Therefore, we speculate that treatment with an increased dose of BB536 may more clearly exhibit positive clinical effects. Consequently, our data demonstrated that addition of BB536 to standard treatment was effective in inducing remission in a subpopulation of Japanese patients with mild to moderately active UC.

In summary, we found that giving BB536, in addition to standard treatment, improved clinical symptoms and endoscopic findings in Japanese patients with mild to moderately active UC. Further studies are required to confirm the efficacy and safety of giving BB536 for active UC.

**ACKNOWLEDGMENTS**

We wish to acknowledge the help of Mr Kazuyoshi Nanba as a statistical consultant. This work was supported by the Japanese Society for the Promotion of Science ‘KAKENHI’ Grants-in-aid for Scientific Research (24509411, 25860532, 26460967, and 26893122) and Health and Labor Sciences Research Grants for Research on Rare and Intractable Disease from the Ministry of Health, Labor and Welfare, Japan.

**CONFLICT OF INTERESTS**

Authors declare no conflict of interests for this study.

**REFERENCES**

8. Takeda Y, Nakase H, Namba K et al. Upregulation of T-bet and tight junction molecules by *Bifidobacterium longum* improves

© 2015 Japan Gastroenterological Endoscopy Society


