Effect of *Bifidobacterium bifidum* Fermented Milk on *Helicobacter pylori* and Serum Pepsinogen Levels in Humans

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ABSTRACT

Helicobacter pylori infection is an important risk factor for gastric diseases. Some probiotics are useful for suppressing *H. pylori* infection. *Bifidobacterium bifidum* YIT 4007 can improve the experimental gastric injury in rats and the disease stages on the gastric mucosa in peptic ulcer patients. We evaluated the fermented milk using a clone (BF-1) having the stronger ability to survive in the product than this parent strain to clarify the in vitro suppressive effect of BF-1 on *H. pylori* and the in vivo efficacy of BF-1 fermented milk on *H. pylori* and gastric health. In the mixed culture assay of BF-1 and *H. pylori*, the number of pathogens was decreased such that it was not detected after 48 h in the *Brucella* broth with a decrease in pH values. In the cell culture experiment with human gastric cells, the *H. pylori* infection-induced IL-8 secretion was suppressed by the preincubation of BF-1. In a human study of 12-wk ingestion (BF-1 group, n = 40; placebo group, n = 39) with a randomized double-blind placebo-control design, the *H. pylori* urease activity and gastric situation were evaluated using a urea breath test (UBT) and the serum pepsinogen (PG) levels as biomarkers for inflammation or atrophy, respectively. In the *H. pylori*-positive subjects, the difference (Δ UBT) of the UBT value from the baseline value in the BF-1 group (n = 34) was lower than that in the placebo group (n = 35) at 8 wk. The baseline UBT values showed a negative correlation with Δ UBT values at 8 and 12 wk in the BF-1 group but not in the placebo. In the PG-positive subjects classified by the PG test method, the BF-1 group was lower in Δ UBT values than the placebo group at 8 and 12 wk. In the active gastritis class by PG levels, the BF-1 group was lower in their Δ UBT values than the placebo at 8 and 12 wk. The PG I levels in the BF-1 group were lower than the placebo at 12

wk. The PG II levels in the BF-1 group did not change during the ingestion period, but the placebo was increased. The PG I/II ratios slightly decreased from baseline at 12 and 20 wk in the BF-1 and placebo groups. These patterns were also observed in the *H. pylori*-positive subjects. The improving rates of upper gastrointestinal symptomatic subjects and total symptom numbers in the BF-1 group were higher than those in the placebo. These results indicate that BF-1 fermented milk may affect *H. pylori* infection or its activity, gastric mucosal situation, and the emergence of upper gastrointestinal symptoms.

Key words: *Bifidobacterium bifidum*, *Helicobacter pylori*, pepsinogen, probiotics

INTRODUCTION

Helicobacter pylori causes inflammation of the gastric mucosa and the promotion of gastric acid secretion, and then persistent infection leads to chronic gastritis, atrophic gastritis, and peptic ulcer (Morris and Nicholson, 1987; Kawaguchi et al., 1996). Atrophic gastritis is closely related to gastric cancers (Uemura et al., 1997; Yoshihara et al., 1998). The *H. pylori*-infected subjects with atrophic gastritis are high-risk populations (Uemura et al., 2001). The grade of atrophy in the gastric mucosa, which is judged by the serum pepsinogen (PG) levels (Samloff et al., 1982; Miki et al., 1987, 1993, 2003), is related with the detection rate of gastric cancers by using endoscopy (Miki, 2001). These findings indicate that it is important to maintain the mucosa function and prevent gastric diseases to suppress the *H. pylori* infection and improve the gastric mucosa.

Antibiotics, such as amoxicillin, clarithromycin, or nitroimidazoles, are effective drugs against *H. pylori* infection, and triple or quadruple combination therapy using these antibiotics and acid-suppression drugs (proton pump inhibitors or H₂-receptor antagonists) are performed for the eradication of *H. pylori* (Malfertheiner et al., 2002; Oderda, 2003). However, there are some failures in the eradication therapy by antibiotics (Lind

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et al., 1999; Mascitelli and Pezzetta, 2003), which have been indicated to be the adverse potential for the emergence of drug-resistant *H. pylori* strains (Matsumoto et al., 1997) and the occurrence of side effects, such as reflex esophagitis due to an increase in acid secretion (Hamada et al., 2000).

Probiotics are microorganisms exerting beneficial effects on their host's health by improving the intestinal flora balance (Fuller, 1991). The genera *Lactobacillus* and *Bifidobacterium* as candidates of probiotics, often used for fermented dairy products or food supplements, are expected to suppress deleterious bacteria in the gastrointestinal tract by changing the microbiological environment. The ingestion of probiotics contributes to the improvement of bacteria and viral infection, diarrhea due to antibiotics, inflammatory bowel disease, and irritable bowel syndrome (de Vrese and Schrezenmeier, 2002). Several reports describe that probiotics have the potential to inhibit *H. pylori* growth in vitro (Midolo et al., 1995; Coconnier et al., 1998), to improve mucosal inflammation in *H. pylori*-infected animals (Sgouras et al., 2004, 2005), and to suppress the secretion of IL-8, a potent proinflammatory cytokine, in the *H. pylori*-infected gastric cells (Sgouras et al., 2005; Tamura et al., 2006). Furthermore, some strains can decrease biomarker values for *H. pylori* in humans (Sakamoto et al., 2001; Cruchet et al., 2003; Pantoflickova et al., 2003; Linsalata et al., 2004). These findings indicate that selected probiotics may act on *H. pylori* in vivo.

Bifidobacteria preferably inhabits the colon and produces acetic acid as well as lactic acid under anaerobic conditions, contributing to the health of the lower gastrointestinal tract. Therefore, the *Bifidobacterium*, unlike the *Lactobacillus*, has not been an intriguing probiotic against *H. pylori* in the stomach and the upper gastrointestinal tract. However, some reports showed the potential of *Bifidobacterium* to suppress *H. pylori* infection and to improve the gastric mucosa pathology. Nagaoka et al. (1994) reported that *Bifidobacterium bifidum* YIT 4007 improved the ethanol- and the acetic acid-induced acute gastric injuries in rats. The administration of *B. bifidum* YIT 4007 shifted the stages of peptic ulcer to the mild state and restricted the *H. pylori* infection on the gastric mucosa in patients (Yamamoto et al., 1994). Wang et al. (2004) demonstrated that *B. animalis* Bb12 could inhibit in vitro growth of *H. pylori*, and ingestion of this strain-containing yogurt decreased the urease activity of *H. pylori*. Furthermore, *Bifidobacterium*-fermented milk containing *B. bifidum* YIT 4007 decreased the number of Enterobacteriaceae in the human flora (Asahara et al., 1999), of which *Escherichia coli* can produce lots of molecular hydrogen (McKay et al., 1982). This reminds us that probiotic *Bifidobacterium* might

change the generation of molecular hydrogen in the stomach, and subsequently affect the severity of *H. pylori* infection, because *H. pylori* uses molecular hydrogen as energy for the infection (Olson and Maier, 2002), which is frequently detected in the intragastric gas (Urita et al., 2006).

Taken these facts into consideration, we have attempted to develop a dairy food beneficial to gastric mucosal health, and then spontaneously obtained a microbiologically identical clone (**BF-1**) of *B. bifidum* YIT 4007, which has the stronger ability to survive in dairy products than this parent strain. Here, for the purpose of knowing the evaluation of the fermented milk using BF-1, we studied the effect of the ingestion of BF-1 fermented milk on *H. pylori* and gastric health in 2 in vitro assays (*H. pylori* proliferation and IL-8 secretion) and in a human study with a randomized double-blind placebo-control design.

MATERIALS AND METHODS

Bacteria

All strains were obtained from the Culture Collection Research Laboratory of Yakult Central Institute for Microbiological Research (Tokyo, Japan). The BF-1 was kept as *B. bifidum* YIT 10347 in the laboratory.

Sample Beverages

The BF-1 fermented milk was prepared by anaerobic culture of these strains in sterilized milk at 37°C for 24 h. The placebo was prepared from untreated milk by adjusting the organic acids (1.3 mg of lactic and 1.3 mg of acetic acids per mL) to the same level as in the fermented milk. These samples were supplied as indistinguishable packaged beverages (100 mL/1 bottle) to subjects at intervals of 1 wk; BF-1 beverage contained more than 1×10^7 cfu/mL of BF-1 (mostly approximately 5×10^8 at d 1 to approximately 1×10^8 cfu/mL at d 7 of a supplying interval) and approximately 1×10^7 cfu/mL of *Streptococcus thermophilus* YIT 2021 (mostly constant during the interval), which was used for the adjustment of taste and flavor.

Mixed Culture Assay

Helicobacter pylori YIT 10238 was cultured in *Bruccella* broth (Becton Dickinson Co., Sparks, MD) with 10% heat-inactivated horse serum under microaerobic (5% O₂, 10% CO₂, and 85% N₂) and mild shaking conditions at 37°C for 48 h. The BF-1 was cultured in m-ILS medium (Shimakawa et al., 2003) under anaerobic conditions at 37°C for 20 h. Portions of culture broths of *H. pylori* (1×10^5 cfu/mL) and BF-1 (1×10^7 cfu/mL)

were mixed in the serum-added *Brucella* broth with 1% glucose and cultured under the above conditions for *H. pylori* for 48 h. The counts of *H. pylori* and BF-1 in the mixed culture were measured on a modified Helicobacter agar plate (Nissui Pharmaceutical, Tokyo, Japan) under microaerobic conditions and TOS-propionate agar plate (Yakult Pharmaceutical, Tokyo, Japan) under anaerobic conditions, respectively.

Cell Culture Assay

The human gastric epithelial-like stomach cancer cell line GCIY RCB0555 (Nozoe et al., 1991) was obtained from the Riken Cell Bank (Tsukuba, Japan). The cells were cultured in Eagle's MEM (Nissui Pharmaceutical) supplemented with 15% fetal bovine serum (Sigma) at 37°C in humidified air containing 5% CO₂. Cell viability was assayed with the method of Trypan Blue exclusion (Freshney, 1987).

IL-8 Assay

The GCIY cells were seeded in the above medium (100 µL/well) onto collagen-coated 96-well plates (Sumiron, Sumitomo Bakelite, Tokyo, Japan) at a density of 4 to 5 × 10⁵ cells/mL. The confluent cell sheet was carefully rinsed with the fresh medium once and preincubated with BF-1 or *S. thermophilus* YIT 2021 suspension of the medium for 3 h. Then, the sheet was rinsed with the fresh medium 3 times and incubated with *H. pylori* (1 × 10⁷ cfu/mL) for 24 h. Cell culture medium was used for pH measurement and IL-8 assay. The IL-8 levels were quantified with the human IL-8 immunoassay kit (Biosource International Inc., Camarillo, CA), according to the manufacturer's instructions.

Subjects

Healthy adult volunteers were recruited by Allegro Inc. (Tokyo, Japan). The content and methods of the study were fully explained to all participants, and their written informed consent was obtained in writing prior to enrollment. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Clinical Examination Ethics Committee of the Arima Memorial Medical Fund (Tokyo, Japan). This human study was the pilot trial to investigate the potentials of the BF-1 fermented milk on gastric events. Eighty candidates were selected from applicants satisfying the following eligibility criteria: men and women concerned about their own stomach health, those with a urea breath test (UBT) value at 5% and over, or those judged as positive in the PG test. The sample size and UBT value were decided in consideration of the previous

Figure 1. Growth and survival curves of *Helicobacter pylori* in mixed culture of BF-1. A) *H. pylori* alone was cultured in the *Brucella* broth. B) *H. pylori* and BF-1 were mixed and cultured in the broth.

reports on other probiotics (Felley et al., 2001; Pantoflickova et al., 2003). The exclusion criteria included subjects with a history of milk allergies and lactose intolerance; those with diseases, such as hepatic, biliary, circulatory, respiratory, gastrointestinal, renal, urinary, neural, mental and blood diseases; those taking antibiotics and stomach medicines such as proton-pump inhibitors, bismuth salt, H₂ blockers, and so on.

Protocol

A randomized double-blind placebo-controlled comparative study was performed to evaluate the effect of the BF-1 beverage. Following the entry period, the study was carried out for a period of 22 wk, which included a 2-wk preingestion period, a 12-wk beverage-ingestion period, and an 8-wk postingestion period. In

Figure 2. Effect of BF-1 on IL-8 secretion by a human gastric cell line GCIY infected with *Helicobacter pylori*. Values are expressed as means $\pm$ SD ($n = 3$). P -values are obtained from comparison with the control (*H. pylori* alone) using Dunnett's test.

the entry period and preingestion period, a preclinical examination and a dietary survey were carried out to screen the qualified subjects, as mentioned above. After the clinical examination during the preingestion period, the 80 subjects were randomly assigned to the BF-1 beverage-ingestion group (BF-1 group) or the placebo beverage-ingestion group (placebo group) with stratification according to age, height, weight, serum PG levels (PG I, PG II, and PG I/II ratio), and UBT values and were subjected to beverage-ingestion examination. The subjects in each group were instructed to drink one bottle of the beverage daily. After the beverage-ingestion period, the postingestion period immediately followed.

The subjects were advised not to change their current dietary habits or lifestyle and to not take foods and supplements that contained any probiotics, and which might affect *H. pylori* infection or gastric symptoms during the study period. The subjects were instructed to keep a lifestyle diary about items as follows: 1) time of ingesting the beverages; 2) description of physical conditions (disease, injury, upper and lower intestinal symptoms, bowel movement, mental activity, and complaints); and 3) description of drugs, supplements, alcohol, and smoking. Furthermore, the subjects were instructed to record the contents of all foods consumed for 3 consecutive days nutrients (protein, lipid, and carbohydrate) and the energy taken from foods in every preingestion period, beverage-ingestion period (4, 8, and 12 wk), and postingestion period and to record the distance walking on a pedometer for the total consumed

Figure 3. Urea breath test (UBT) values and the difference (Δ UBT) between the baseline (BL) and each time in the ingestion of BF-1 fermented milk ($n = 34$, closed diamond) and of the placebo milk ($n = 35$, open square) among those of *Helicobacter pylori*-positive subjects. A) UBT value at each time. B) Δ UBT values.

energy during the same period as the intake of food was recorded on the stylized dietary and physical diaries, respectively.

Clinical examination (blood test for sialic acid, aspartate transaminase, alanine transferase, γ -glutamyl-transferase, alkaline phosphatase, lactate dehydrogenase, total protein, albumin, albumin:globulin ratio, total bilirubin, urea nitrogen, creatinin, uric acid, protein fractions (albumin, α_2 , β , γ , albumin:globulin ratio), total cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, glucose, Na, K, Ca, Cl, Mg, red blood cells, white blood cells, platelets, hematocrit, hemoglobin, mean cell hemoglobin, mean cell volume, basophil, eosinophil, lymphocyte, monocyte, neutrophil; urine test for pH, gravity, glucose, protein, urobilinogen, ketone, hemocult), vital sign check (systolic pressure, diastolic pressure, pulse, and body temperature), and doctor's interview for upper gastrointestinal symptoms (gastric pain, disgust, nausea, pyrosis, belch, stomach complaint) and for general health conditions were performed once (baseline) in the preingestion period, 3 times (4, 8, and 12 wk) in the beverage-ingestion period and once (20 wk) in the postingestion period. Expiratory air for UBT analysis, blood, and urine for

Figure 4. Correlation between urea breath test (UBT) values at baseline (BL) and the difference (Δ UBT) between the baseline and 8 wk (A and B) or 12 wk (C and D), in the ingestion of BF-1 fermented milk ($n = 34$, A and C) and of the placebo milk ($n = 35$, B and D). ρ is Spearman's rank correlation coefficient.

clinical analysis were collected the morning after the subjects had fasted for at least 10 h. All clinical specimens were analyzed in the General Institute of BML Inc. (Kawagoe, Japan). Two weeks after the start of the beverage-ingestion period, a subject in the placebo group retired for a personal reason independent of the study. Finally, the 79 subjects completed the study according to the protocol, in which the BF-1 group had 40 persons (sex ratio, men:women = 22:18; average age = 43.5 ± 8.8 yr; age range = 24 to 65 yr), and the placebo group had 39 persons (sex ratio, men:women = 22:17; average age, 44.4 ± 8.8 yr; age range, 28 to 58 yr).

Statistical Analysis

Data were expressed as means and SD. Data of cell culture experiment were analyzed using the Dunnett's comparison test compared with the control group. Analysis was performed using measured values of UBT and a PG test at each examination time and differences of UBT and PG values (Δ UBT, Δ PGI, Δ PGII, and Δ PG I/II) between each time point and the baseline within

the group, for the comparison of both beverage groups using the Mann-Whitney test and for the effect of ingestion by the Wilcoxon test using SAS version 8.2 for Windows (SAS Institute Japan, Tokyo, Japan). The correlation between the UBT value at baseline and the Δ UBT at each time was analyzed with the nonparametric Spearman rank correlation using Kyplot software for Windows (Kyence Inc., Tokyo, Japan). The χ^2 test was used for the analysis of rate of stomach symptoms between the BF-1 and placebo groups. Probability values < 0.05 were considered to be significant. We furthermore performed stratified analysis using UBT values and serum PG levels to know what class was affected by BF-1 fermented milk. *Helicobacter pylori* infection was judged by the UBT value [*H. pylori*-positivity (5% of UBT value and over: $n = 34$ and 35 in the BF-1 and placebo groups, respectively) and *H. pylori*-negativity (below 5%: $n = 6$ and 4 in the BF-1 and placebo groups, respectively) subjects]. The serum PG test method, often used as a primary screening test for detecting gastric cancer (Miki et al., 1987, 1993, 2003), was used for the classification of the *H. pylori*-positive subjects in

this study. In the *H. pylori*-positive subjects, the PG positive (n = 13 and 10 in the BF-1 and placebo groups, respectively) with levels of PG I <70 ng/mL and PG I/II ratio <3.0 were assumed to be high-risk individuals with atrophic gastritis, and the remaining subjects (the PG negative, n = 21 and 25, respectively) were assumed to be low-risk. Furthermore, we used another classification criterion for the diagnosis of histological gastritis using the serum PG levels (Kiyohira et al., 2003). According to this criterion, the *H. pylori*-positive subjects were classified into normal stomach (n = 0 and 0, in the BF-1 and placebo groups, respectively) with PG I <85 ng/mL, PG II <15.0 ng/mL, and PG I/II $\geq$ 6.5; borderline (n = 11 and 11, respectively) with PG I <85 ng/mL, PG II <15.0 ng/mL, and PG I/II 3.0 to <6.5; atrophic (n = 4 and 3, respectively) with PG I <85 ng/mL, PG II <15.0 ng/mL, and PG I/II <3.0; and active gastritis (n = 19 and 21, respectively) with PG I $\geq$ 85 ng/mL and PG II $\geq$ 15.0 ng/mL.

RESULTS

Mixed Culture Experiment

Helicobacter pylori proliferated well in the *Brucella* broth, reaching more than 10^8 cfu/mL without BF-1 (Figure 1). In the mixed culture with BF-1, *H. pylori* was decreased such that it could not be detected after 48 h, but BF-1 grew slowly, accompanying the decrease in pH values (pH 5.51 at 0 h, pH 5.40 at 5 h, pH 5.02 at 24 h, and pH 4.52 at 48 h).

Cell Culture Experiment

The IL-8 secretion from GCIY cells after *H. pylori* infection was suppressed by the pretreatment with BF-1, but slightly inhibited with high dose of *S. thermophilus* YIT 2021 (Figure 2). There was no change in pH under this culture condition. The preincubation of BF-1 or *S. thermophilus* YIT 2021 did not affect the viability of *H. pylori*-infected cells (data not shown).

Urea Breath Test

Of all subjects, 69 persons had more than the cutoff level of UBT at the preingestion period to be judged as positive for *H. pylori* infection. The BF-1 group had low UBT values in the beverage-ingestion period compared with baseline; the UBT value at 8 wk was lower ($P = 0.029$) than that at baseline (Figure 3). However, the placebo group had high UBT values. The Δ UBT value of the BF-1 group was lower than that of the placebo group at 8 wk ($P = 0.027$). Furthermore, the UBT values of the preingestion period showed a negative correlation with Δ UBT values at 8 wk ($\rho = -0.533$, $P = 0.002$) and

12 wk ($\rho = -0.360$, $P = 0.039$) in the BF-1 group but not in the placebo group (Figure 4). Next, the 2 beverage groups were compared with 2 criteria for their serum PG levels. First, the Δ UBT values of the BF-1 group were lower than those of the placebo group at 8 wk ($P = 0.010$) and 12 wk ($P = 0.088$) in the PG-positive class (Figure 5), but not different in the PG-negative class. Second, the Δ UBT values of the BF-1 group were lower than those of the placebo group at 8 wk ($P = 0.011$) and 12 wk ($P = 0.028$) in the active gastritis class (Figure 6), but were not different in the other classes (data not shown).

Serum Pepsinogen Concentration

The PG I levels of both groups during the ingestion period were not different from baseline, but those of the placebo group rose along after completion of the ingestion ($P = 0.020$ at 20 wk; Figure 7A and 7B). The BF-1 group was lower than in the placebo group in PG I level at 12 wk ($P = 0.038$) and Δ PG I level at 12 wk ($P = 0.087$). In the BF-1 group the PG II levels did not change during the beverage-ingestion period but rose after the cessation of ingestion ($P = 0.001$ at 20 wk; Figure 7C and 7D). The PG II levels of the placebo group were increased even during the ingestion period ($P = 0.025$ at 12 wk) and at the postingestion period ($P < 0.001$ at 20 wk). However, no group difference in the PG II and Δ PG II levels was observed. The PG I/II ratios decreased at 12 wk ($P < 0.001$ and $P = 0.039$) and 20 wk ($P < 0.001$ and $P = 0.003$) compared with the baseline in the BF-1 and placebo groups, respectively, but no group differences were observed (Figure 7E and 7F). Furthermore, in the *H. pylori*-positive subjects, the group difference of the Δ PG I levels could not be shown ($P = 0.138$ at 12 wk), whereas the Δ PG II levels (BF-1, $P < 0.001$ at 20 wk; placebo, $P = 0.022$ at 12 wk and $P < 0.001$ at 20 wk) and the Δ PG I/II ratios (BF-1, $P = 0.002$ at 12 wk and $P < 0.001$ at 20 wk; placebo, $P = 0.017$ at 12 wk and $P = 0.001$ at 20 wk) showed similar patterns as observed in Figure 7D and 7F.

Upper Gastrointestinal Symptoms

Twenty-eight subjects (n = 14, men:women = 5:9 in the BF-1 group; n = 14, men:women = 5:9 in the placebo group) had one or more upper gastrointestinal symptoms according to doctors' interviews at the preingestion period. As for the rate of the number of symptom-relieved subjects, the BF-1 group (4 wk, 79%, n = 11; 8 wk, 79%, n = 11; 12 wk, 93%, n = 13; 20 wk, 93%, n = 13) was higher (4 wk, $P = 0.022$; 8 wk, $P = 0.115$; 12 wk, $P = 0.065$; 20 wk, $P = 0.001$) than the placebo group

Figure 5. Urea breath test (UBT) values and the difference (Δ UBT) between baseline (BL) and each time in the ingestion of BF-1 fermented milk ($n = 13$, closed diamond) and of the placebo milk ($n = 10$, open square) in the pepsinogen (PG) test method positive subjects (A and B), and in the ingestion of BF-1 fermented milk ($n = 21$, closed diamond) and of the placebo milk ($n = 25$, open square) in the PG-negative subjects (C and D). A and C, UBT values at each time. B and D, Δ UBT values. Identification of PG-positive and PG-negative subjects was as described in Materials and Methods.

Figure 6. Urea breath test (UBT) values, and the difference (Δ UBT) between baseline (BL) and each time in the ingestion of BF-1 fermented milk ($n = 19$, closed diamond) and of the placebo milk ($n = 21$, open square) in the active gastritis subjects classified by serum pepsinogen levels. A) UBT values at each time. B) Δ UBT values. Identification of active gastritis subjects was as described in Materials and Methods.

(4 wk, 36%, $n = 5$; 8 wk, 50%, $n = 7$; 12 wk, 64%, $n = 9$; 20 wk, 36%, $n = 5$). Thirty-five symptoms (BF-1, $n = 18$; placebo, $n = 17$) were apparent in 28 symptomatic subjects. Regarding the rate of total numbers of improved symptoms, the BF-1 group (4 wk, 78%, $n = 14$; 8 wk, 78%, $n = 14$; 12 wk, 89%, $n = 16$; 20 wk, 83%, $n = 15$) was higher (4 wk, $P = 0.004$; 8 wk, $P = 0.027$; 12 wk, $P = 0.042$; 20 wk, $P = 0.004$) than the placebo group (4 wk, 29%, $n = 5$; 8 wk, 41%, $n = 7$; 12 wk, 59%, $n = 10$; 20 wk, 35%, $n = 6$).

Clinical Examination

Among the vital signs, clinical laboratory values, results of doctors' interviews, and contents of the lifestyle diary in the study period, there was no problem derived from ingestion of beverages. The nutrients, total energy obtained from foods, and total energy consumed from walking were not different between the BF-1 and placebo groups.

DISCUSSION

Some probiotics have potential activity on the inhibition of *H. pylori* in vitro and in vivo and the improve-

Figure 7. The pepsinogen (PG) I levels, PG II levels, and PG I/II ratios, and the differences (Δ PG I, Δ PG II and Δ PG I/II) between baseline (BL) and each time in the ingestion of BF-1 fermented milk (n = 40, closed diamond) and of the placebo milk (n = 39, open square). A) PG I levels at each time; B) Δ PG I levels; C) PG II levels at each time; D) Δ PG II levels; E) PG I/II ratios at each time; F) Δ PG I/II ratios.

ment of gastritis in *H. pylori* infection (Hamilton-Miller, 2003). To be previously reported, *B. bifidum* YIT 4007 alleviated drug-induced acute injury in the rat stomach (Nagaoka et al., 1994) and improved the stage of disease in peptic ulcer patients (Yamamoto et al., 1994). To understand the potential interference to *H. pylori* infection, we investigated the effect of a new clone, BF-1, on the pathogen growth in vitro and the efficacy of the fermented milk on the *H. pylori* and serum gastric markers in vivo in humans.

The BF-1 inhibited the *H. pylori* growth in mixed culture under suitable conditions of *H. pylori*. The pattern of decrease in *H. pylori* is similar to the pattern of pH decrease. Live *H. pylori* was not detected after 48 h (pH 4.5), but BF-1 was proliferated. This indicated that BF-1 could restrict the activity of *H. pylori* (growth, survival, or action) through the action of organic acids like lactic and acetic acids as metabolic products. How-

ever, some bifidobacteria could produce heat-stable antimicrobial peptides against *H. pylori* (Collado et al., 2005), and then a strain of *B. bifidum* produces a bacteriocin, bifidocin B, which has not been reported for *H. pylori* (Yildirim and Johnson, 1998). Therefore, it would be necessary to consider other factors than organic acids for the inhibition of *H. pylori*.

Helicobacter pylori-associated gastritis is histologically characterized by infiltration of neutrophils, lymphocytes, and macrophages into the mucosa (Rauws et al., 1988), accompanying expression of various cytokines (Yamaoka et al., 1997). The IL-8 is the potent chemotactic activity for neutrophils (Crabtree et al., 1994), and its level in the mucosa is closely correlated with the density of *H. pylori* and neutrophil infiltration in the antrum (Yamaoka et al., 1998). The BF-1 suppressed the *H. pylori*-induced IL-8 secretion from GCIY cells, where pH values and cell viability were constant

during the experiment. These results showed that organic acids were not major inhibitory factors for IL-8 secretion and that cell injury was not associated with the suppression. The BF-1 had the ability to adhere to GCIY cells, and the treatment of BF-1 after the infection did not suppress the IL-8 secretion due to *H. pylori* (unpublished data). These findings indicate that the interaction of BF-1 with the gastric cells before *H. pylori* infection might be essential for the suppression of IL-8 secretion.

Their ingestion of BF-1 fermented milk affected the *H. pylori* biomarker values in the *H. pylori*-positive population, but the placebo did not. Some probiotics reported to be active against *H. pylori* show the activity to decrease the UBT biomarker values in the human study (Sakamoto et al., 2001; Cruchet et al., 2003; Pantoflickova et al., 2003; Linsalata et al., 2004; Wang et al., 2004), but there have been no reports about the eradication of the pathogen, as seen in our study. The efficacy of BF-1 fermented milk was clearer in the active gastritis class with higher levels of PG I and PG II, the BF-1 group was lower in Δ UBT values than the placebo group; this class is characterized as 100% infection of *H. pylori* and higher incidences of active inflammation assessed by neutrophil infiltration and chronic inflammation by mononuclear cell infiltration (Kiyohira et al., 2003). The results indicate that BF-1 fermented milk could suppress *H. pylori* activity during ingestion, which might calm the gastric inflammation.

Furthermore, we observed that subjects with higher UBT values at baseline had more significant rates of reduction after ingestion of BF-1 fermented milk (Figure 4). Because of correlations of UBT values with the histological grade of *H. pylori* colonization (Perri et al., 1998) and with the density of *H. pylori* genomes (Kobayashi et al., 2002) in the gastric mucosa, higher UBT subjects were predicted as higher *H. pylori* carriers, which are presumably exposed to higher risk for stomach diseases and disorders. Our finding indicates that BF-1 fermented milk might effectively suppress the higher risk of the pathogens, but it remains speculative.

The BF-1 fermented milk contained *S. thermophilus* YIT 2021, in addition to BF-1. Because there were considerable numbers of live *S. thermophilus* (1/50 to 1/10 cfu of BF-1) in the BF-1 fermented milk, this bacterium had possibilities to partially participate in the effect, but we have not known yet whether it affects gastric events. It would be important and inevitable to clarify contribution rate of each strain to better understand the mechanism of the anti-*H. pylori* activity of BF-1 fermented milk.

Helicobacter pylori infection increases in serum PG I and PG II levels (Kuipers et al., 1995; Asaka et al.,

1992; Knight et al., 1995; Ohkusa et al., 2004) and is well correlated with inflammatory scores (Wagner et al., 1994; Lopes et al., 2006). Especially, the PG II level is increased compared with the PG I level, proposing the use as a marker of *H. pylori* infection or eradication and of histological features of the gastric body (Miki et al., 2003; Kiyohira et al., 2003; di Mario et al., 2004). During the ingestion of BF-1, the serum PG II levels were maintained at baseline level, but they were increased after the completion of ingestion. This pattern of the PG II levels is the case of the *H. pylori*-positive subjects. The results show that the ingestion of BF-1 fermented milk might affect the development of inflammation in the stomach. The PG I/II ratio was decreased by both samples in the whole and *H. pylori*-positive subjects. The successful eradication of *H. pylori* decreases the PG I and PG II and increases the PG I/II ratio (Hunter et al., 1993; Haruma et al., 1999). The BF-1 fermented milk did not eradicate *H. pylori*; therefore, the decrease in the PG I/II ratio might be not observed despite the suppression of the PG I and PG II levels. Although these observations could help to presume the effect of the BF-1 fermented milk on the gastric mucosa infected by *H. pylori*, because we have not observed any group differences in pepsinogen levels in the *H. pylori*-positive subjects in this study, further investigations using histological approaches would be required to clarify the direct effects of BF-1 on the mucosa.

The BF-1 fermented milk increased the number of subjects with relief from gastrointestinal symptoms, improved gastrointestinal symptoms during the ingestion period, and increased the feeling of relief following complete ingestion. The symptoms or complaints are not always linked with clear pathophysiological abnormalities (Colin-Jones and a working party, 1988), in which *H. pylori* infection is a risk factor for dyspepsia along with complaints (Suzuki et al., 2005; Wildner-Christensen et al., 2006). To know whether the improving effect of BF-1 fermented milk is related with *H. pylori* infection, therefore, a larger scale intervention study targeting *H. pylori*-negative as well as *H. pylori*-positive subjects would be necessary.

Helicobacter pylori infection is a serious risk for the incidence of upper gastrointestinal diseases, such as peptic ulcer, atrophic gastritis, and stomach cancers. In many cases, the carriers remained asymptomatic, not compelled to cure the pathogen, despite their own risky situation. Therefore, it is important to remove or suppress *H. pylori* activity and toxicity using various anti-*H. pylori* techniques other than drugs, such as probiotics or functional foods against *H. pylori*. Some probiotics are useful for suppressing *H. pylori* infection, relieving stomach symptoms, and improving gastric in-

flammation. We reported here that the ingestion of BF-1 fermented milk affected 1) the UBT values of *H. pylori*-positive subjects, 2) the serum PG I and PG II levels, and 3) the upper gastrointestinal symptoms, indicating that the fermented milk with probiotics might be useful for the maintenance of stomach health.

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