Effects of ingesting Lactobacillus- and Bifidobacterium-containing yogurt in subjects with colonized Helicobacter pylori\textsuperscript{1–3}

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ABSTRACT

Background: Evidence suggests that ingesting lactic acid bacteria exerts a suppressive effect on Helicobacter pylori infection in both animals and humans. Supplementing with Lactobacillus- and Bifidobacterium-containing yogurt (AB-yogurt) was shown to improve the rates of eradication of H. pylori in humans.

Objective: We administered AB-yogurt to subjects with asymptomatic H. pylori to test whether the yogurt could inhibit H. pylori growth.

Design: The in vitro inhibition of H. pylori growth was determined by inoculating Lactobacillus acidophilus La5 or Bifidobacterium lactis Bb12 on plates that were inoculated with H. pylori. Assessment of the viability of H. pylori was performed by the mixed culture method with La5 or Bb12. In an intervention study, 59 adult volunteers infected with H. pylori were given AB-yogurt (10\textsuperscript{7} colony-forming units of both La5 and Bb12/mL) twice daily after a meal for 6 wk. Eleven subjects positive for H. pylori infection were treated with milk placebo as control subjects. H. pylori bacterial loads were determined with use of the \textsuperscript{13}C-urea breath test, which was performed before and 4 and 8 wk after the start of AB-yogurt supplementation.

Results: Bb12 exerted an in vitro inhibitory effect against H. pylori, whereas La5 did not show an effect. Administration of AB-yogurt decreased the urease activity of H. pylori after 6 wk of therapy (P < 0.0001).


KEY WORDS Probiotics, Bifidobacterium lactis Bb12, Lactobacillus acidophilus La5, urea breath test, yogurt, Helicobacter pylori

INTRODUCTION

Helicobacter pylori is a spiral-shaped, gram-negative rod that can colonize epithelial cells lining the antrum of the stomach and survive in the acidic environment. H. pylori causes chronic gastritis, plays an etiologic role in the development of peptic ulcer disease, and is considered a risk factor in the development of gastric malignancies such as gastric mucosa-associated lymphoid tissue lymphomas and gastric adenocarcinoma (1–3). Despite the effective antibiotic-based therapies, we were concerned about their possible induction of resistance to antibacterial drugs. Furthermore, the side effects of these kinds of therapies are a common cause of treatment discontinuation. Probiotics are live microbial food supplements that beneficially affect the host by improving its microbial balance (4). Lactobacillus and bifidobacteria are added to several fermented dairy products and are known to have an inhibitory growth effect on a wide range of intestinal pathogens in humans and animals. Midolo et al (5) demonstrated that inhibition of H. pylori growth occurred as a result of organic acid production by Lactobacillus acidophilus strains in vitro. Cocconier et al (6) showed that L acidophilus supernatant decreased H. pylori viability in vitro and decreased urease activity and the histopathologic degree of gastric lesions in mice infected with Helicobacter felis. Conventional yogurt is fermented milk produced by adding Lactobacillus bulgaricus and Streptococcus thermophilus to milk, and conventional yogurt bacteria have a poor resistance to acid and bile (7). However, L acidophilus and bifidobacteria can tolerate pH 3 environments and 2–8% concentrations of bile acid (8). Administration of yogurt supplemented with Bifidobacterium spp. and L acidophilus was shown to enhance mucosal and systemic immunoglobulin A responses to the cholera toxin immunogen (9). Sheu et al (10) discovered that supplement with yogurt containing Lactobacillus and Bifidobacterium could improve the intention-to-treat eradication rate of H. pylori and restore the depletion of Bifidobacterium in stools after therapy. On the basis of the increasing evidence that L acidophilus and bifidobacteria have therapeutic properties, we investigated the effects of yogurt containing L acidophilus La5 and Bifidobacterium lactis Bb12 on H. pylori infection in vitro and in humans.

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SUBJECTS AND METHODS

Preparation of the yogurt

The AB-yogurt (230 mL; President Enterprise Corporation, Tainan, Taiwan) used in this research was fermented milk containing sugar, high-fructose corn syrup, pectin, galactooligosaccharide, and an approximately equal mixture of L. acidophilus La5, B. lactis Bb12, L. bulgaricus, and S. thermophilus at a concentration of at least 10⁷ bacteria/mL.

In vitro study

Disk diffusion test

H. pylori was isolated from the gastric biopsy specimens of 8 patients (2 gastric cancers, 2 gastric ulcers, 3 duodenal ulcers, and 1 case of gastritis). La5 and Bb12 were grown separately in medium consisting of 10% skim milk and 0.1% yeast extract incubated at 37 °C for 16 h. The suspension of La5 or Bb12 was inoculated into broth, and the concentration was adjusted according to the MacFarland standard [≈6 × 10⁷, 9 × 10⁷, 12 × 10⁷, 15 × 10⁷, and 21 × 10⁷ colony-forming units (CFU)/mL, respectively] to reach the concentration of 9 × 10⁶ CFU/mL. Diluted culture isolates (10, 30, and 50 µL) were aspirated and incorporated into filter paper disks. The paper disks were placed onto previously inoculated H. pylori (concentration was adjusted to 3 × 10⁸ CFU/mL) blood agar plates and then kept under microaerophilic conditions for 72 h. The inhibitory growth effect of H. pylori was interpreted by the inhibition clear halo zone, and the size of each inhibition zone was measured.

Mixed culture method

The concentration of 3 clinical isolates of H. pylori was adjusted to 3 × 10⁸ CFU/mL. Both 7.5 g Bacto agar (Difco Laboratory, Sparks, MD) and 14 g Brucella Broth (Difco Laboratory) were dissolved in 500 mL distilled water. After autoclaving at 121 °C for 20 min, 50 mL 5% fetal calf serum was added at the temperature of ≈50 °C. Subsequently, broths of La5 and Bb12 (concentration was 10⁸ CFU/mL), La5-containing yogurt, and AB-yogurt were poured into the culture dish when the temperature was ≈40 °C. When the medium became dry, the H. pylori isolates were administered for coculture. We counted the number of formed bacterial colonies under microaerophilic conditions after 72 h.

Subjects

Seventy volunteers infected with H. pylori, as identified by means of positive ¹³C-urea breath test (UBT) and positive serology, were enrolled. Control samples were taken from individuals who were asymptomatic or who were dyspeptic but willing to undergo endoscopic examination. Volunteers with upper gastrointestinal tract lesions other than minimal antral erosions were excluded and treated with appropriate medical care. Other exclusion criteria included subjects who had taken antibiotics, bismuth salt, or proton pump inhibitors in the previous month; who had gastric or duodenal bleeding; and who had a history of gastric surgery or other life-threatening conditions. Informed consent was obtained, and the ethics committee of Kaohsiung Medical University Hospital approved the study.

Study protocol

Volunteers were assigned to consume a bottle of AB-yogurt (230 mL) or to consume an unfermented milk placebo twice a day after their morning and evening meals for the first 6 wk (AB-yogurt group: average age, 39 ± 10 y, range, 22–59 y; men: women = 22:37; n = 59. Placebo group: average age, 33 ± 9 y, range, 25–53 y; men:women, 5:6; n = 11). Subjects were instructed to maintain a regular dietary pattern and to avoid dairy products, Chinese herbal medicines, honey, spicy foods, cranberries, and products containing live lactic acid bacteria during the period of treatment.

¹³C-UBT was performed on subjects who had fasted overnight on 3 occasions: weeks 0 (before the study), 4, and 8 (2 wk after end of treatment), respectively. A baseline sample of expiratory air was obtained just before the ingestion of 75 mg ¹³C urea diluted in 75 mL water (Proto Pylorikit; Altachem Pharma Ltd for Isodiagnostika, Edmonton, Canada). Excess δ¹⁵CO₂ was measured by isotope mass spectrometry. A value of > 3.5/ml was considered a positive result.

Anti-H. pylori immunoglobulin G antibodies were measured by using a specific enzyme immunoassay (Meridian Diagnostics Inc, Cincinnati, OH) according to the supplier’s instructions. The test, with use of spectrophotometric single wavelength, was considered positive when the OD₅₄₀ was more than 0.120. Specimens of gastric antrum and body (2 biopsies each with a total of 4 biopsies) were obtained at the initial time by gastroendoscopy and repeated at 4 wk after the end of treatment from 14 randomly selected subjects among the AB-yogurt group.

Histology

Biopsies were fixed in 10% formalin and stained with hematoxylin and eosin. Gastritis was graded according to the modified Sydney system (11, 12): the density of H. pylori was graded from 0 to 4, and the activity of gastritis (the density of infiltration with neutrophils) and gastric inflammation (the density of infiltration with mononuclear cells) was graded from 0 to 4.

Statistical analysis

Pretreatment and posttreatment values were compared by repeated measurements of two-factor analysis of variance. P < 0.05 was considered statistically significant. To identify the specific groups that were significantly different, we performed Scheffé multiple comparison procedures for comparisons of pairs of means. To express the variability of the results, SE was used. The correlation between pretreatment UBT data and its interval change among the AB-yogurt group was analyzed with use of Pearson correlation coefficient. The paired t test was used for comparison of pretreatment and posttreatment values of biopsies. All calculations in this study were determined by SAS version 8.0 (SAS Institute Inc, Cary, NC) and SPSS version 8.0 (SPSS Inc, Chicago) programs.

RESULTS

In vitro study

The concentration of inoculum of H. pylori was ≈3 × 10⁸ CFU/mL. Bb12 had an inhibitory effect on H. pylori in samples from 8 patients, whereas La5 did not have any obvious inhibition effect. The minimal concentration of Bb12 to be inhibitory to H.
pylori was $9 \times 10^8$ CFU/mL. No inhibitory effect of the supernatant of La5 or Bb12 was observed. In samples of H. pylori from another 3 patients, cocultures of Bb12, La5, La5-containing yogurt, and both Bb12- and La5-containing yogurt with H. pylori were performed, respectively. As the volume of the preparations of Bb12 and La5 plus Bb12 yogurt increased, colonies of H. pylori first appeared to be absent in both conditions at 0.125 mL. H. pylori became totally absent at the volume of 0.25 mL, whereas La5 and La5 yogurt did not show any suppressive effect at the different concentrations. This finding demonstrated that only Bb12 possesses a suppressive effect (Table 1).

In vivo study
Seventy adult subjects were confirmed to have H. pylori infections. Serial UBT was performed on all 70 subjects. There was a significant interaction of time and treatment; administration of AB-yogurt lowered the $^{13}$C-UBT values after 6 wk of therapy (Figure 1). Among the AB-yogurt group, there were significant differences in the results of $^{13}$C-UBT values between week 0 and week 4, and between week 0 and week 8 ($36.2 \pm 19.4$ compared with $30.1 \pm 15.8$, $P < 0.05$ by using Scheffé multiple comparison method). Also, the pretreatment amounts of excess $^{13}$CO$_2$ showed a significant negative correlation with the difference of excess $^{13}$CO$_2$ amounts between weeks 0 and 4 and between weeks 0 and 8 (Figure 2) in the subjects in AB-yogurt group. For subjects whose $^{13}$C-UBT values declined at week 8, we had a further follow-up at weeks 12 and 16. One of the patients whose excess $^{13}$CO$_2$ amount was negative at week 8 returned to a positive status at weeks 12 and 16, suggesting that H. pylori was not eradicated completely. Examination of antral biopsies showed reduced H. pylori density and gastritis activity ($P < 0.006$ and $P = 0.015$, respectively) from 14 subjects. No significant change was observed in the gastric body (Table 2).

DISCUSSION
In the present study, we demonstrated that yogurt containing B. lactis Bb12 had a suppressive effect on H. pylori in vitro. Our results indicated that a 6-wk ingestion of yogurt containing La5 and Bb12 significantly decreased the values of UBT. In addition, endoscopic biopsies of gastric mucosa taken from the antrum
were shown to survive in the intestine and could play an important role in the maintenance of gastrointestinal health. Also, ingestion of yogurt could increase the numbers of stool bifidobacteria and suppress coliform bacteria (23). *Clostridium perfringens* and *Escherichia coli* are organisms that produce copious amounts of hydrogen gas.

It could be possible that the decreases in these organisms after a short-term treatment, which increases colonic bifidobacteria, will result in decreased colonic hydrogen gas production. Therefore, we speculated that yogurt containing Bb12 might change the production of hydrogen gas and thereby lessen the severity of *H. pylori* infection.

The activity of gastritis, used as a measure of response to therapy, decreased after ingestion of AB-yogurt. This decreased activity could be related particularly to the decreased density of *H. pylori*. No significant change in gastric inflammation represented by the density of mononuclear cells in the lamina propria was observed. Because *H. pylori* nearly always has a predominantly antral distribution (24), similar changes were not observed in the body of the stomach. However, the temporary inhibitory effect after stopping ingestion of AB-yogurt could be partially because of its stimulation of immunity. Administering fermented milk with lactic acid bacteria (*L. bulgaricus* and *S. thermophilus*) apparently enhanced the immune response in animal studies by activating macrophages and lymphocytes (25). In humans fed yogurt, results showed an increase of B and natural killer cells in the lymph nodes and increased interferon-γ production (26–28). The long-term anti-inflammatory effect and its immunomodulatory properties of AB-yogurt merits further investigation.

Pedrosa et al (29) demonstrated that neither *S. thermophilus* nor *L. bulgaricus* was recovered from the stomach or small intestine of subjects fed yogurt. Bb12 was considered to be responsible for the efficacy for suppressing *H. pylori* in our study. The mechanisms could be explained through reciprocal inhibition between different bacterial species. In our in vitro study, Bb12 exerted a significant inhibitory effect on *H. pylori* growth on the plate (mixed culture method), which might be related to its competition for nutrients. Bacterial disk diffusion method showed an annular radius of inhibition on the plate by the Bb12. Moreover, we found that subjects with higher intragastric bacterial load of *H. pylori* predicted by the 13C-UBT values had more significant rates of reduction after treatment. Because the ability to adhere to epithelial cells and thereby colonize the stomach is probably of crucial importance in its interference with *H. pylori*, this phenomenon could be explained by the barrier effect as a result of the attachment of La5 or Bb12 to gastric epithelial cells.

Because there is a high prevalence of lactose intolerance in our country, it is difficult to have a larger number of control subjects. The reason we administered milk placebos in our study was because of their considerable difference from fermented yogurt with regard to bacteriologic composition and lactase activity. When the ingestion of AB-yogurt was stopped, the UBT values returned to pretreatment levels at week 16 in some subjects (data not shown). Therefore, the necessity for continuous ingestion is apparent. It was also necessary to give doses regularly instead of assuming that a few doses would allow the organism to colonize the gut permanently (30). However, we need more research to standardize the dosages and timing of taking probiotics to reduce *H. pylori* infection in humans.

**Table 2**

<table>
<thead>
<tr>
<th>Aspects and biopsy site</th>
<th>Before treatment (week 0)</th>
<th>After treatment (week 12)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helicobacter pylori</em> density</td>
<td>Antrum 6.00 ± 0.65</td>
<td>3.86 ± 0.54</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Body 3.25 ± 0.82</td>
<td>2.00 ± 0.35</td>
<td>0.119</td>
</tr>
<tr>
<td>Activity of gastritis</td>
<td>Antrum 3.43 ± 0.27</td>
<td>2.36 ± 0.17</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Body 1.83 ± 0.32</td>
<td>1.67 ± 0.26</td>
<td>0.586</td>
</tr>
<tr>
<td>Gastric inflammation</td>
<td>Antrum 4.57 ± 0.17</td>
<td>5.14 ± 0.29</td>
<td>0.071</td>
</tr>
<tr>
<td></td>
<td>Body 3.67 ± 0.38</td>
<td>3.33 ± 0.28</td>
<td>0.220</td>
</tr>
</tbody>
</table>

*All values are x ± SE, n=14 specimens. Treatment was 6 wk long and the posttreatment period was defined as 12 wk.*

*P < 0.05 was considered significant (paired t test)*

*3 Gastritis was graded according to the modified Sydney system (11–12). Biopsies were performed twice at each site. Each value is the sum of the grades of the 2 biopsies.*

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**References**

1. Kabir et al (20) found that *L. acidophilus* La1 to demonstrate a marked decrease in 13C-UBT activity of gastritis and increased incidence of peptic ulcers, we showed significantly decreased gastritis activity and *H. pylori* density. Values of UBT provided a semiquantitative assessment of the density of *H. pylori* colonization of gastric mucosa, in addition to using histologic examination (13).

Michetti et al (14) used the cultured supernatant of *L. acidophilus* La1 to demonstrate a marked decrease in 13C-UBT values in humans. Felley et al (11) demonstrated that a 3-wk intake of acidified milk containing *Lactobacillus johnsonii* La1 decreased *H. pylori* density in humans. Furthermore, a decrease in antral inflammation and antral gastritis was also observed. The design of polymicrobial preparation in this study obtained results similar to previous experiments, whereas only live *B. lactis* Bb12 had a bacteriostatic effect on *H. pylori* in vitro. Because increased density of *H. pylori* on the gastric mucosa was related to more severe gastritis and increased incidence of peptic ulcers, we inferred that a reduction of the density here might help to decrease the risk of developing pathologies (15–17).

Current well-studied probiotics are usually lactobacilli but not bifidobacteria. Lactic acid bacteria in fermented milk need to be present in high concentrations and to survive the digestive process to exert the maximal influence on gastrointestinal functions and to act as probiotics. *Lactobacilli* are the predominant bacteria found in a normal fasting stomach, although their number is small, ranging from 0 to 10^3/mL fluid (18). *L. acidophilus* is a strain of human origin that can tolerate very low acidic pH and can survive passage through the gastrointestinal tract (19). Being acid resistant, *L. acidophilus* persists in the stomach longer than other bacteria (Drasar BS, unpublished observations, 1987). Kabir et al (20) found that *L. acidophilus* can exhibit a significant inhibitory effect on the attachment of *H. pylori* to the gastric epithelial cell lines. Wendakoon et al (21) examined the efficacy of a specially designed fermented milk product containing selected lactic acid bacteria with anti-*H. pylori* properties (including *L. casei*, *L. acidophilus*, and *S. thermophilus*). After administration of the yogurt for 1 mo, only 1 subject of 27 was found to have had a complete eradication.

However, Olson and Maier (22) found that hydrogen is available in the gastric mucosa of mice and that its use greatly increased the stomach’s colonization by *H. pylori*. Bifidobacteria showed an annular radius of inhibition on the plate by the Bb12. Because there is a high prevalence of lactose intolerance in our country, it is difficult to have a larger number of control subjects. The reason we administered milk placebos in our study was because of their considerable difference from fermented yogurt with regard to bacteriologic composition and lactase activity. When the ingestion of AB-yogurt was stopped, the UBT values returned to pretreatment levels at week 16 in some subjects (data not shown). Therefore, the necessity for continuous ingestion is apparent. It was also necessary to give doses regularly instead of assuming that a few doses would allow the organism to colonize the gut permanently (30). However, we need more research to standardize the dosages and timing of taking probiotics to reduce *H. pylori* infection in humans.
PROBIOTICS SUPPRESS *HELIcobacter pylori* INFECTION

In summary, probiotic preparations of yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium* BB12 are determined to be effective in suppressing *H. pylori* infection in humans for a 6-wk treatment.

S-NL participated in the interpretation of the data and in writing the manuscript. C-SL participated in the interpretation of the data. T-NW participated in the design of the study, in the analysis, and in the interpretation of the data. WMW contributed to various stages of the study, including the design of the experiment, collection of the data, analysis of data, and writing of the manuscript. D-SP, Y-CS, D-CW, C-MJ, and C-HL participated in the data collection. No author had any financial or personal relations with the company or organization sponsoring the research. None of the authors had a conflict of interest.

REFERENCES