Development of Probiotics for *Helicobacter pylori* Infection

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To develop probiotics for *Helicobacter pylori* infection, we screened 203 *Lactobacillus* strains. *Lactobacillus gasseri* OLL2716 (LG21) was selected as the most effective strain to inhibit the growth of *H. pylori* by *in vitro* tests and animal study. We examined the effects of yogurt containing LG21 on *H. pylori* infection in humans. The intake of yogurt containing LG21 both decreases the number of *H. pylori* and also reduces mucosal inflammation. LG21 was shown to be effective as a probiotic for *H. pylori* infection.

Key words: *Lactobacillus gasseri*; *Helicobacter pylori*; probiotics; LG21; mucosal inflammation

INTRODUCTION

The evidence of a relationship between *Helicobacter pylori* and gastric diseases has gradually become clear since Warren and Marshall succeeded in cultivating this bacterium in 1982. Since the infection rate of *H. pylori* is very high, about 50% in Japanese, it is difficult to eradicate *H. pylori* in all people infected. The eradication therapy using antibiotics is often accompanied by side effects and not always successful because of antibiotic-resistant strains.

We found that orally administered lactobacilli could eradicate *H. pylori* in a gnotobiotic murine model (1, 6). So we screened 203 *Lactobacillus* strains to select a strain that exhibited excellent anti-*H. pylori* activity *in vitro* and in animal studies, and selected *Lactobacillus gasseri* OLL2716 (LG21) as the most suitable strain for use as a probiotic for *H. pylori* infection.

We also examined the efficacy of LG21 against *H. pylori* infection in humans.

HELICOBACTER PYLORI

H. pylori is a spiral Gram-negative rod with unipolar flagella. This bacterium inhabits the human stomach and can cause chronic inflammation and ulcers of the stomach and duodenum. *H. pylori* has also been identified as a risk factor for stomach cancer. *H. pylori* has strong urease and can catalyze urea in the stomach to ammonia and carbon dioxide. *H. pylori* can survive

under strong acidic conditions because of this ammonia production.

Selection of anti-*H. pylori* lactic acid bacteria *in vitro*

To select an anti-*H. pylori* lactic acid bacteria, anti-*H. pylori* abilities (resistance to artificial gastric juice, proliferation under acidic conditions, adherence to cultured gastric epithelial cells, suppression of *H. pylori* during co-fermentation and survival in yogurt) of 203 *Lactobacillus* strains were examined *in vitro*. *Lactobacillus gasseri* OLL2716 (LG21), *Lactobacillus gasseri* No. 6 and *Lactobacillus salivarius* WB1004 were selected as lactic acid bacteria with excellent anti-*H. pylori* properties (data not shown).

ANIMAL STUDY

Following *in vitro* experiments, the effects of the administration of *Lactobacillus* strains (LG21, *L. gasseri* No. 6, *L. salivarius* WB1004) on *H. pylori*-infected mice were examined. For *H. pylori* infection, 10⁹ cfu of *H. pylori* were orally administered to germ-free mice once a day for 3 days. Six weeks after the administration of *H. pylori*, 10⁹ cfu of *Lactobacillus* strains were administered once a week for 8 weeks. One week after finishing the administration of *Lactobacillus* strains, the number of *H. pylori* and serum anti-*H. pylori* IgG levels were measured.

No *H. pylori* was detected in the mice administered LG21 and *L. salivarius* WB1004, and the anti-*H. pylori* IgG levels of the mice administered LG21 were the lowest (Fig. 1). So LG21 was selected as the most suitable strain for use as a probiotic for *H. pylori* infection.

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Fig. 1. Effect of LG21 on the number of *H. pylori* and anti-*H. pylori* antibodies of *H. pylori*-infected mice (8 weeks, $n=5$).

EFFECTS OF LG21 ADMINISTRATION ON *H. PYLORI* INFECTION IN HUMANS

To examine the efficacy of LG21 against *H. pylori* infection in humans, we performed human clinical studies (12). Thirty-one healthy volunteers infected with *H. pylori* ingested 90 g of yogurt twice a day for 8 weeks. From 1 week after the finish of this 8-week intake of yogurt, the volunteers ingested 90 g of yogurt containing 10^9 cfu of LG21 twice a day for 8 weeks. The urea breath test and assays of serum pepsinogen I/II ratios were performed to measure the population of *H. pylori* and to evaluate the degree of mucosal inflammation in the stomach respectively, prior to intake of yogurt, 1 week after finishing the intake of yogurt and 1 week after finishing the intake of yogurt containing LG21. Moreover gastric biopsy specimens of 6 volunteers were taken for quantitative culture both before and after the intake of yogurt containing LG21.

It is known that the urea breath test provides semi-quantitative assessments of the density of *H. pylori* colonization of gastric mucosa (4, 10, 11), and that the serum pepsinogen I/II ratio is reduced in *H. pylori*-positive gastritis and gastric ulcer patients (2, 9, 13) and is correlated with the activity of gastritis (7). A high $\Delta^{13}\text{C}$ value indicates a large population of *H. pylori* and a low pepsinogen I/II ratio indicates severe mucosal inflammation in the stomach.

The $\Delta^{13}\text{C}$ value in the urea breath test did not significantly change after the intake of yogurt, but did significantly decrease following the intake of yogurt containing LG21 (Fig. 2). The pepsinogen I/II ratio did not significantly change after the intake of yogurt, but did significantly increase following the intake of yogurt containing LG21 (Fig. 3). The number of *H. pylori* in the

Fig. 2. Effect of yogurt containing LG21 on $\Delta^{13}\text{C}$ value of subjects infected with *H. pylori*.

Fig. 3. Effect of yogurt containing LG21 on serum pepsinogen I/II ratio of subjects infected with *H. pylori*.

Fig. 4. Change in number of *H. pylori* after consumption of yogurt containing LG21.

Fig. 5. Effect of yogurt containing LG21 on $\Delta^{13}\text{C}$ value of subjects infected with *H. pylori*.

Fig. 6. Effect of yogurt containing LG21 on serum pepsinogen I/II ratio of subjects infected with *H. pylori*.

gastric biopsy specimens from all 6 volunteers decreased after the intake of yogurt containing LG21 (Fig. 4).

Next, we examined the effects of long-term administration of yogurt containing LG21 (8). Thirty-one healthy volunteers infected with *H. pylori* ingested 120 g of yogurt containing 10^9 cfu of LG21 once a day for 24 weeks. The urea breath test and assays of the serum pepsinogen I/II ratios were performed both before and 1 week after finishing 24-week period of intaking yogurt containing LG21. The $\Delta^{13}\text{C}$ value significantly decreased and the serum pepsinogen I/II ratio significantly increased following the intake of yogurt containing LG21 (Figs. 5, 6). No volunteers complained about adverse effects during the test period.

These results suggest that the intake of yogurt containing LG21 both decreases the number of *H. pylori* and also reduces the mucosal inflammation in the stomach of humans infected with *H. pylori*.

CONCLUSIONS

LG21 displays inhibitory activity against *H. pylori* both *in vitro* and in animal studies. In clinical studies, the intake of yogurt containing LG21 decreases the number of *H. pylori* and improves the degree of mucosal inflammation in the stomach of humans infected with *H. pylori*. The density of *H. pylori* colonization in the stomach is of importance in the pathogenesis of infection associated with this bacterium. It was reported that there was a correlation between *H. pylori* density and gastric inflammation and duodenal ulceration (5), and no duodenal ulceration was present in subjects with antral *H. pylori* densities of less than 10^5 cfu/g tissue protein (3). Since the intake of yogurt containing LG21 suppresses *H. pylori* in addition to improving inflammation in the stomach, LG21 may reduce the risk of *H. pylori*-induced gastrointestinal diseases.

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