Suppressive effect of rice extract on *Helicobacter pylori* infection in a Mongolian gerbil model

Maki Murakami1,5, Hiroyoshi Ota2,5, Atsushi Sugiyama1, Satoshi Ishizone1, Fukuto Maruta1, Noriyuki Akita1, Yukie Okimura4, Toshiko Kumagai1, Megumi Jo1, and Takashi Tokuyama6

1Department of Gastroenterological Surgery, Shinshu University School of Medicine, Matsumoto, Japan
2Department of Laboratory Medicine, Shinshu University School of Medicine, Matsumoto, Japan
3Department of Biomedical Laboratory Sciences, School of Health Sciences, Shinshu University School of Medicine, Matsumoto, Japan
4Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Japan
5Department of Gastroenterology, Jishyukai Ueda Kidney Clinic, 322 Sumiyoshi, Ueda 386-0002, Japan
6Yushin Co. Ltd, Kagawa, Japan

**Background.** Rice extract has been shown to protect gastric mucosa from stress-induced damage. In this study, the antibiotic effect and the anti-inflammatory effect of orally administered aqueous rice extract on *Helicobacter pylori* infection and *H. pylori*-induced gastritis, respectively, in Mongolian gerbils were investigated. **Methods.** Fifty specific-pathogen-free male Mongolian gerbils, seven weeks old, were divided into four groups: uninfected, untreated animals (group A); uninfected, rice extract-treated animals (group B); *H. pylori*-infected, untreated animals (group C); and *H. pylori*-infected, rice extract-treated animals (group D). Group C and D animals were killed 12 weeks after *H. pylori* infection (i.e., at 19 weeks of age) and group A and B animals were also killed at age 19 weeks. The stomachs were removed for histopathological examination with hematoxylin-and-cosin staining and anti-5'-bromo-2'-deoxyuridine (BrdU) immunostaining. Serum anti-*H. pylori* antibody titers were also tested. **Results.** In groups A and B, the gastric mucosa showed no inflammatory cell infiltration and a few BrdU-reactive cells. Group C animals developed marked chronic active gastritis in the gastric mucosa, and BrdU-labeled cells in the gastric mucosa markedly increased in number. In group D animals, a significant reduction occurred in the degree of neutrophil polymorphonuclear cell infiltration into the gastric mucosa, in the BrdU-labeling indices of gastric epithelial cells, and in anti-*H. pylori* antibody titers in the serum (*P* < 0.01), compared with although *H. pylori* was not completely eradicated. **Conclusions.** The rice extract was effective in suppressing inflammation and epithelial cell proliferation in the gastric mucosa in *H. pylori*-infected Mongolian gerbils. The rice extract has potential to exhibit a protective effect on *H. pylori*-related gastric mucosal diseases.

**Key words:** rice extract, *Helicobacter pylori*, Mongolian gerbils, gastritis, gastric mucosal cell proliferation

**Introduction**

*Helicobacter pylori* is the most important etiological agent of chronic gastritis and peptic ulcer disease and is also demonstrated to be both epidemiologically and experimentally related to gastric carcinoma.1–4 The Mongolian gerbil (Meriones unguiculatus; MG) is useful for producing an animal model of *H. pylori*-induced chronic active gastritis that leads itself to the investigation of morbidity-related pathological epithelial alterations in the gastric mucosa that ultimately lead to intestinal metaplasia and gastric neoplasia.3–10 We observed, in this MG model of chronic gastritis, that marked neutrophil infiltration on a background of chronic inflammation appeared in the gastric mucosa 4 weeks after *H. pylori* inoculation and persisted for up to 52 weeks in all infected MGs, and that intestinal metaplasia, multiple hyperplastic polyps, and gastric ulcers also appeared 26 weeks after inoculation in some animals.8 We also found that *H. pylori* exerted co-initiating and promoting effects on N-methyl-N-nitrosourea (MNU)-induced gastric carcinogenesis in this animal model.3 Accumulating evidence has demonstrated that eradication of *H. pylori* in the stomach by the administration of oral antimicrobial agents results in the resolution of *H. pylori*-associated gastroduodenal diseases.11 However, such bacterial eradication treatment is not always successful, and it is occasionally associated with adverse effects.

A rice extract has been shown to prevent the ulcer formation that is induced in the stomach mucosa of
mice by oral administration of ethanol and HCl, or by holding animals in cold water (15°C) (R. Yamashita, T. Tokuyama, and Z. Ogita, unpublished data). This rice extract was also reported to prevent the histopathological damage and cell proliferation in the gastric mucosa induced by NaCl in rats. In the present study, we examined the antibiotic and the anti-inflammatory effects of the rice extract on *H. pylori* infection and *H. pylori*-induced gastritis, respectively, using an MG model.

**Materials and methods**

**Chemicals**

Aqueous rice extract (Rice Power Extract No. 101) was obtained from Yushin (Kagawa, Japan). The rice extract was prepared by the concentration of the cultural filtrate produced after saccharization of rice, using *Aspergillus oryzae*, followed by fermentation using *Saccharomyces cerevisiae* and heating at 85°C. The extract has a pH of 4.50 and a specific gravity of 1.156, and contains 36% saccharides, but no protein and no lipid.

**Animals**

Fifty specific-pathogen-free, male MGs (MGS/Sea; Seac Yoshitomi, Fukuoka, Japan), 7 weeks old, were used in this study. They were housed in an air-conditioned biohazard room designed for experimentally infected animals, with a 12-h light/12-h dark cycle, and were allowed free access to food (CE-2; Clea Japan, Tokyo, Japan).

**Bacterial strains and inoculation**

*H. pylori* ATCC43504 (cagA+; vacA+; American Type Culture Collection, Manassas, VA, USA) was grown in *Brucella* broth (Becton Dickinson, Cockeysville, MD, USA), containing 10% (v/v) horse serum, at 35°C under microaerophilic conditions (15% CO₂), at high humidity for 40h, with shaking (150rpm). After a 24-h fast, gerbils were inoculated via an oral catheter with 0.8-ml aliquots of *H. pylori* culture containing 10⁹ colony-forming units/ml of organisms. Four hours later, the animals were again allowed free access to water and food.

**Experimental protocol**

The animals were divided into four experimental groups (A, B, C, and D; Fig. 1). Group A comprised five gerbils that received no treatment and were given autoclaved distilled water ad libitum (control group). Group B comprised five gerbils that were not inoculated with *H. pylori* and were given aqueous rice extract ad libitum from 9 weeks of age to the end of the experimental period (RE). Group C comprised 20 gerbils that were inoculated with *H. pylori* at 7 weeks of age and were given distilled water ad libitum (Hp). Group D comprised 20 gerbils that were inoculated with *H. pylori* at 7 weeks of age and were given aqueous rice extract ad libitum from 2 weeks after *H. pylori* infection until the end of experimental period (Hp+RE). We have demonstrated, in our previous study, that inflammation appeared 2 weeks after the inoculation of *H. pylori* and it reached the highest point around 12 weeks after the inoculation of *H. pylori*. To examine the anti-inflammatory effect of the rice extract, animals were given the rice extract from 2 weeks after *H. pylori* inoculation until 12 weeks after the *H. pylori* inoculation (i.e., for 10 weeks).

At 19 weeks of age, all animals in each group were killed after 24-h fasting. Thirty minutes before the gerbils were killed, each animal was given 200mg/kg 5'-bromo-2'-deoxyuridine (BrdU) intraperitoneally. Under deep ether anesthesia, they were laparotomized, with excision of their stomachs. The stomachs were opened along the greater curvature, starting at the esophagogastric junction and ending at the proximal portion of the duodenum, and observed macroscopically.

**Bacterial cultures**

To assess the bacterial burden in the gastric mucosa, the mucosa from ten animals in each of groups C and D were subjected to a culture study. Whole stomachs were homogenized with 5ml of *Brucella* broth and the resulting samples were diluted serially, from 1:10 to