Short Communication

Pathological Features of *Helicobacter pylori*-Induced Gastritis in Mongolian Gerbils

Shiro Takagi¹, Fumihiro Hirayama², Shin Kohge¹, Hiroyuki Utsumi¹, Yasuhiro Kamimura¹, Masaki Takeuchi¹, Yoshifumi Ikeda³, and Tsutomu Katsuyama⁴

¹Yoshitomi Safety Evaluation Laboratory, Yoshitomi Pharmaceutical Industries Ltd., ²Kyushu Laboratories, Yoshitomi Pharmaceutical Industries Ltd., ³Seac Yoshitomi Ltd., ⁴Department of Laboratory Medicine, Shinshu University School of Medicine

Abstract: Seven-week-old male specific pathogen-free Mongolian gerbils (MGS/Sea) were orally inoculated with 2×10^8 colony-forming units per animal of a broth culture of *Helicobacter pylori* (*H. pylori*) ATCC 43504. Histopathological examination of their stomachs after 6 weeks of post inoculation revealed gastritis with hemorrhage, inflammatory cell infiltration, superficial erosion, mucosal thickening, and lymph follicles, in the pyloric mucosa and the fundic mucosa near the transitional zone. In addition, loss of fundic glands and appearance of hyperplastic epithelium with pseudopyloric glands containing paradoxical concanavalin A reactive mucins were evident in the fundic mucosa near the transitional zone. *H. pylori* bacteria were detected in the surface mucous gel layer lining inflamed mucosa or in the foveolar pits of the same regions. Galactose oxidase-cold thionine Schiff reactive mucins in the surface mucous cells were decreased. BrdU-labeled mucosal epithelial cells were markedly increased, and the generative cell zone was expanded. These features resemble human gastritis associated with *H. pylori*. (J Toxicol Pathol 1999; 12: 41~45)

Key words: Gastritis, *Helicobacter pylori*, Mongolian gerbil

Helicobacter pylori (*H. pylori*), since its first isolation from a patient with gastritis by Warren and Marshall in 1983¹, is considered to be associated with human gastritis, peptic ulcer, and gastric cancer²⁻⁴. Several animal models infected with *H. pylori* have already been developed to understand its pathogenesis⁵⁻¹¹. However, none of these models mimic the features of human *H. pylori* infection. We previously reported that *H. pylori* can colonize the gastric mucosa and induce chronic active gastritis in Mongolian gerbils^{12,13}. This appears 2 weeks after inoculation with the bacteria, and after six months, ulceration and intestinal metaplasia become prominent¹³. In the present study, we examined the pathological characteristics of *H. pylori*-induced gastritis in Mongolian gerbils at 6 weeks after inoculation, with emphasis on mucin histochemistry and proliferative activity of gastric epithelial cells.

Male specific pathogen-free Mongolian gerbils (MGS/Sea), 7 weeks old, were obtained from Seac Yoshitomi Ltd. (Fukuoka, Japan). The animals were housed in plastic cages with wood chips as bedding, and kept in an air-conditioned room. Room temperature and relative humidity were maintained at $23 \pm 2^\circ\text{C}$ and $55 \pm 5\%$, respectively. Pellet diet (CE-2, Clea Japan, Tokyo, Japan) and tap water were given *ad libitum*. Five animals were subjected to *H. pylori* inoculation and 3 served as controls.

Helicobacter pylori ATCC 43504 was obtained from the

American Type Culture Collection (Rockville, MD, USA). Broth culture was prepared according to the method of Hirayama *et al.*^{12,13}, and 0.5 ml aliquots (2×10^8 colony-forming units per animal) were orally inoculated into the stomachs of 5 gerbils by gavage. There was no bacterial contamination in this broth culture. The remaining 3 gerbils served as an untreated control.

Animals were killed under ether anesthesia at 6 weeks after the *H. pylori* inoculation. The stomachs were removed, cut along the greater curvature, and fixed in Carnoy's solution for 2 hours at 4°C , according to the method of Ota *et al.*¹⁴. Fixed samples were embedded in paraffin, sectioned at $6 \mu\text{m}$, and stained with hematoxylin and eosin (HE). To clarify the distribution of mucins, sections were stained with galactose oxidase-cold thionine Schiff (GOCTS) and then, paradoxical concanavalin A staining (PCS)¹⁵. In addition, immunohistochemical staining of *H. pylori* was performed using rabbit anti-*H. pylori* antibody (DAKO A/S, Glostrup, Denmark) and a streptavidin/biotin kit (Lipshaw, Pittsburgh, USA). Proliferative activity of gastric epithelial cells was examined using 5-bromodeoxyuridine (BrdU, Wako Pure Chemical Industries Ltd., Osaka, Japan), 100 mg/kg of BrdU being intraperitoneally injected into each animal 1 hour before the sacrifice. BrdU-labeled cells were demonstrated using anti-BrdU antibody (DAKO A/S) and a streptavidin/biotin kit (Lipshaw).

In all animals inoculated with *H. pylori*, the pyloric mucosa and the fundic mucosa near the transitional zone were thickened with hemorrhagic spots (Fig. 1). No grossly visible changes were evident in the control animals. Histopathologic examination revealed all animals inoculated with *H. pylori* exhibited severe gastritis in the pyloric mucosa

Received: 29 September 1998, Accepted: 19 January 1999

Address corresponding to: Shiro Takagi, Yoshitomi Safety Evaluation Laboratory, Yoshitomi Pharmaceutical Industries Ltd., 955 Koiwai, Yoshitomi-cho, Chikugo-gun, Fukuoka 871-8550, Japan
Tel: 0979-23-8952, Fax: 0979-24-8524

Fig. 1. Stomachs of Mongolian gerbils. A: Control gerbil. B: *H. pylori* inoculated gerbil. Note thickening of the gastric mucosa with hemorrhagic spots in the pyloric mucosa and the fundic mucosa near the transitional zone. Bar = 1 cm.

and the fundic mucosa near the transitional zone. Histological features included infiltration of polymorphonuclear cells (mainly neutrophils) and mononuclear cells (lymphocytes, monocytes, and plasma cells) in the lamina propria, invasion of neutrophils into the epithelial layer, formation of intrafoveolar microabscesses, increased mitoses in the lining epithelium, and formation of lymph follicles in

the lamina propria or submucosa. The inflamed mucosa was thickened, and occasionally showed erosive lesions with hemorrhage. In the fundic mucosa near the transitional zone, the fundic glands (chief cells and parietal cells) had disappeared and become replaced by hyperplastic epithelium with pseudopyloric glands (Figs. 2, 3). *H. pylori* bacteria were mostly detected in the surface mucous gel layer lining

Fig. 2. Gastric mucosa of Mongolian gerbils. H.E. A: Pyloric mucosa of a control gerbil. $\times 120$. B: Pyloric mucosa of an *H. pylori* inoculated gerbil. Note the inflammatory cell infiltration with lymph follicles in the lamina propria, and mucosal thickening. $\times 120$. C: Fundic mucosa of a control gerbil. $\times 75$. D: Fundic mucosa near the transitional zone of an *H. pylori* inoculated gerbil. Note the inflammatory cell infiltration in the lamina propria, superficial erosion, hemorrhage, loss of fundic glands, and appearance of hyperplastic epithelium with pseudopyloric glands. $\times 75$.

Fig. 3. High magnification of fundic mucosa detailing the findings illustrated in Fig. 2D. Note the infiltration of neutrophils and mononuclear cells in the lamina propria, superficial erosion, and invasion of neutrophils to the lining epithelium. Numerous exfoliated cell clusters are evident in the lumen. H.E., $\times 300$.

the inflamed mucosa or in the foveolar pits of the same regions (Fig. 4). No microscopic changes were observed in the control animals. In mucin histochemistry, the surface mucous cells of the controls were strongly GOCTS reactive, whereas the mucous neck cells and the pyloric gland cells exhibited PCS reactivity. In the animals inoculated with *H. pylori*, GOCTS reactive mucins in the surface mucous cells lining pyloric and fundic mucosa were markedly decreased. Pyloric gland cells demonstrated well preserved PCS reactive mucins, and pseudopyloric glands were similarly stained (Fig. 5). In proliferative activity of gastric epithelial cells, a few

BrdU-labeled mucosal cells were observed at the neck of the pyloric and fundic mucosa in controls. In the inoculated animals, in contrast, BrdU-labeled mucosal epithelial cells were abundant, and the generative cell zone was expanded to occupy almost the entire layer of the inflamed mucosa (Fig. 6).

The present study revealed the following characteristics of *H. pylori*-induced gastritis in Mongolian gerbils. The lesions occur mainly in the pyloric mucosa, showed a neutrophil response and also a chronic lymphocytic response with follicle formation. These pathological changes mimic those

Fig. 4. Gastric mucosa of an *H. pylori* inoculated gerbil. Note the *H. pylori* bacteria (arrow heads) in the foveolar pits. A: H.E., $\times 1,800$. B: Immunostaining for *H. pylori*, $\times 1,800$.

Fig. 5. Mucin histochemistry of gastric mucosa with GOCTS-PCS staining. A: Pyloric mucosa of a control gerbil. Note GOCTS reactive (blue) surface mucous cells and PCS reactive (brown) pyloric gland cells. $\times 150$. B: Pyloric mucosa of an *H. pylori* inoculated gerbil. Note decreased GOCTS reactivity and loss of mucins in the surface mucous cells. $\times 150$. C: Fundic mucosa of a control gerbil. Note GOCTS reactive surface mucous cells and PCS reactive mucous neck cells. $\times 75$. D: Fundic mucosa near the transitional zone of an *H. pylori* inoculated gerbil. Note decreased GOCTS reactivity and mucin contents of the surface mucous cells, and PCS reactive mucins with pseudopyloric glands in the basal area of the mucosa. $\times 75$.

Fig. 6. BrdU immunohistochemistry of pyloric mucosa. A: Control gerbil. Note the few BrdU-labeled mucosal epithelial cells in the neck. $\times 75$. B: *H. pylori* inoculated gerbil. Note many BrdU-labeled mucosal epithelial cells and expansion of the generative cell zone. $\times 75$.

of chronic active gastritis associated *H. pylori* infection in humans^{16–20}. In the fundic mucosa near the transitional zone, fundic glands were replaced by regenerative hyperplastic epithelium with pseudopyloric glands. These indicate the area occupied by pyloric-type mucosa is expanded. Similar changes were reported by Kimura in patients with chronic gastritis²¹. Mucin histochemistry revealed a decrease of mucins in the surface mucous cells, the major source of the surface mucous, possibly causing diminution of protective effects of the mucins. This alteration might be one of the causes of progressing mucosal damage. The obvious increase in BrdU-labeled mucosal epithelial cells indicates

that the cell cycle is activated, and this might contribute to the mucosal thickening together with the inflammatory cell infiltration in the lamina propria. An increase of proliferative activity in gastric mucosa has also been reported in *H. pylori*-related human gastritis as an increase of Ki-67 positive mucosal epithelial cells²².

Our results indicate that the histological and histochemical features of *H. pylori*-induced gastritis in Mongolian gerbils closely resemble those of *H. pylori*-related gastritis in humans. Therefore, the Mongolian gerbil is a useful experimental animal for understanding the pathogenesis of *H. pylori*-related human gastritis.

References

- Warren JR and Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; **1**: 1273-1275.
- Wyatt JJ. Histopathology of gastroduodenal inflammation: the impact of *Helicobacter pylori*. *Histopathology* 1995; **26**: 1-15.
- Leung KM, Hui PK, Chan WY, and Thomas TM. *Helicobacter pylori*-related gastritis and gastric ulcer. A continuum of progressive epithelial degeneration. *Am J Clin Pathol* 1992; **98**: 569-574.
- Correa P, Fox J, Fontham E, Ruiz B, Lin YP, Zevala D, Taylor N, Mackinley D, de Lima E, and Portilla H. *Helicobacter pylori* and gastric carcinoma. Serum antibody prevalence in populations with contrasting cancer risks. *Cancer* 1990; **66**: 2569-2574.
- Radin MJ, Eaton KA, Krakowka S, Morgan DR, Lee A, Otto G, and Fox J. *Helicobacter pylori* gastric infection in gnotobiotic beagle dogs. *Infect Immun* 1990; **58**: 2606-2612.
- Krakowka S, Morgan DR, Kraft WG, and Leunk RD. Establishment of gastric *Campylobacter pylori* infection in the neonatal gnotobiotic piglet. *Infect Immun* 1987; **55**: 2789-2796.
- Eular AR, Zurenko GE, Moe JB, Ulrich RG, and Yagi Y. Evaluation of two monkey species (*Macaca mulatta* and *macaca fascicularis*) as possible models for human *Helicobacter pylori* disease. *J Clin Microbiol* 1990; **28**: 2285-2290.
- Fukuda K, Tamura K, Yamamoto I, Kawaura A, Tonokatsu Y, Tsuyuguchi T, Ohno T, Satomi M, and Shimoyama T. Inoculation of rhesus monkeys with human *Helicobacter pylori*: Long-term investigation of gastric mucosa by endoscopy. *Dig Endosc* 1992; **4**: 19-30.
- Shuto R, Fujioka T, Kubota T, and Nasu M. Experimental gastritis induced by *Helicobacter pylori* in Japanese monkeys. *Infect Immun* 1993; **61**: 933-939.
- Karita M, Kouchiyama T, Okita K, and Nakazawa T. New small animal model for human gastric *Helicobacter pylori* infection: Success in both nude and euthymic mice. *Am J Gastroenterol* 1991; **86**: 1596-1603.
- Marchetti M, Arico B, Burrone D, Figura N, Rappuoli R, and Ghiara P. Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. *Science* 1995; **267**: 1655-1658.
- Hirayama F, Takagi S, Yokoyama Y, Iwao E, and Ikeda Y. Establishment of gastric *Helicobacter pylori* infection in Mongolian gerbils. *J Gastroenterol* 1996; **31**(Supple IX): 24-28.
- Hirayama F, Takagi S, Kusuha H, Iwao E, Yokoyama Y, and Ikeda Y. Induction of gastric ulcer and intestinal metaplasia in Mongolian gerbils infected with *Helicobacter pylori*. *J Gastroenterol* 1996; **31**: 755-757.
- Ota H and Katsuyama T. Alternating laminated array of two types of mucin in the human gastric surface mucous layer. *Histochem J* 1992; **24**: 68-92.
- Ota H, Katsuyama T, Ishii K, Nakayama J, Shiozawa T, and Tsukahara Y. A dual staining method for identifying mucins of different gastric epithelial mucous cells. *Histochem J* 1991; **23**: 22-28.
- Rauws EA, Langenberg W, Houthoff HJ, Zanen HC, and Tytgat GN. *Campylobacter pyloridis*-associated chronic active antral gastritis. *Gastroenterology* 1988; **94**: 33-40.
- Sobala GM, Crabtree JE, Dixon MF, Schorah CJ, Taylor JD, Rathbone BJ, Heatley RV, and Axon AT. Acute *Helicobacter pylori* infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. *Gut* 1991; **32**: 1415-1418.
- Satoh K, Kimura K, Yoshida Y, Kasano T, Kihira K, and Taniguchi Y. A topographical relationship between *Helicobacter pylori* and gastritis: Quantitative assessment of *Helicobacter pylori* in gastric mucosa. *Am J Gastroenterol* 1991; **86**: 285-291.
- Bayerdörffler E, Lehn N, Hatz R, Mannes GA, Oertel H, Sauerbruch T, and Stolte M. Difference in expression of *Helicobacter pylori* gastritis in antrum and body. *Gastroenterology* 1992; **102**: 1575-1585.
- Genta RM, Hammer HW, and Graham PY. Gastric lymphoid follicles in *Helicobacter pylori* infection. *Hum Pathol* 1993; **24**: 577-583.
- Kimura K. Chronological transition of the fundic-pyloric border determined by stepwise biopsy of the lesser and greater curvatures of the stomach. *Gastroenterology* 1972; **63**: 584-592.
- Fan XG, Kelleher D, Fan XJ, Xia HX, and Keeling PW. *Helicobacter pylori* increases proliferation of gastric epithelial cells. *Gut* 1996; **38**(1): 19-22.