ELSEVIER

• *Helicobacter pylori* •

Anti-*Helicobacter pylori* therapy significantly reduces *Helicobacter pylori*-induced gastric mucosal damage in Mongolian gerbils

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Abstract

AIM: To investigate the effectiveness of 4 d' anti-*Helicobacter pylori* therapy on the *H pylori*-infected Mongolian gerbils based on physiological and pathological changes.

METHODS: We used 6-wk-old male gerbils orally inoculated with *H pylori* (ATCC43504, 2×10^8 CFU/mL). Seven weeks after *H pylori* inoculation, the animals of study group received 4 d' anti-*H pylori* triple therapy (*H pylori*-eradicated group). Seven days later, all animals of the *H pylori*-eradicated and control groups (*H pylori*-infected & *H pylori*-uninfected groups) were sacrificed. We examined gastric mucosal lesions macroscopically, studied gastritis microscopically and determined the stomach weight ratio, myeloperoxidase (MPO) activity and prostaglandin (PG) E_2 level.

RESULTS: The results showed that both macroscopic and histological gastric damages were significantly less in *H pylori*-eradicated group than *H pylori*-infected group. Stomach weight ratio, MPO activity and PGE$_2$ levels were significantly higher in *H pylori*-infected group than those in the other two groups.

CONCLUSION: Four days' anti-*H pylori* therapy was effective in the improvement of *H pylori*-induced gastric lesions in Mongolian gerbils.

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INTRODUCTION

Helicobacter pylori (*H pylori*) is considered a major cause of acute and chronic gastritis as well as peptic ulcer disease, and is also highly associated with the development of gastric mucosa-associated lymphoid tissue lymphoma and gastric cancer[1-3]. Both National Institute of Health (NIH) of USA and International Agency for Research on Cancer (IARC) have issued statements on the importance of *H pylori* eradication and carcinogenic risks in patients with *H pylori* infection[4,5]. At present, *H pylori* eradication therapy is performed in patients with peptic ulcer diseases. In 1996, Hirayama *et al*[6] reported that *H pylori* infection in Mongolian gerbils can induce gastric ulcer and intestinal metaplasia. This animal model is considered to be very similar to human *H pylori* infection. However, little progress has been made in the *H pylori*-eradication with a simple regimen and a short-term course. Therefore, we conducted this study to compare histological and physiological changes in the stomach of Mongolian gerbils including those receiving 4 d' anti-*H pylori* therapy after a successful inoculation of *H pylori*, with those infected with *H pylori* but not receiving anti-*H pylori* therapy, and those not infected with *H pylori*.

MATERIALS AND METHODS

Animals

Six-week-old specific pathogen-free male Mongolian gerbils (MGS/Sea) weighing 40-50 g, were used. The animals were kept in an isolated clean room with regulated temperature (approximately 20-22 ℃) and humidity (approximately 55%) with a 12/12-h light-dark cycle. The animals were allowed free access to water and sterile solid food (CE-2). There were 3 groups of experimental animals prepared for this study: group A, not inoculated with *H pylori* (*H pylori*-uninfected group, *n* = 7); group B, inoculated with *H pylori* but not received anti-*H pylori* therapy (*H pylori*-infected group, *n* = 7); and group C, inoculated with *H pylori*, and treated with anti-*H pylori* triple therapy for 4 d (*H pylori*-eradicated group, *n* = 7).

Bacteria

ATCC43504, a standard *H pylori* strain (CagA and VacA-positive), was used. The bacteria were incubated overnight in a brain-heart infusion broth (Difco Laboratories, Detroit,

Michigan) containing 10% fetal bovine serum (Gibco BRL, Grand Island, New York) at 37 ℃ under a microaerophilic atmosphere (N_2 85%, CO_2 10%, O_2 5%), and allowed to grow to a concentration of approximately $2.0×10^8$ colony-forming units (CFU)/mL.

H pylori inoculation

The animals were deprived of food for 24 h before and 4 h after *H pylori* inoculation, but were otherwise afforded free access to food and tap water. One mL of *H pylori* ($2.0×10^8$ CFU/mL) was orally inoculated into animals of groups B and C.

Regimen for eradication of H pylori

The regimen of anti-*H pylori* triple therapy is same as the therapy used in human beings. The drugs including lansoprazole, amoxicillin and clarithromycin were suspended in 0.5% w/w carboxymethyl cellulose (CMC) sodium salt solution and administrated orally twice a day for four days at a dose of 10, 3, and 30 mg/kg body weight respectively.

The animals of group C received 4 d' anti-*H pylori* therapy at 7 wk after *H pylori* inoculation, while the animals of other 2 groups (groups B and C) received vehicle (CMC) for 4 d.

All animals were sacrificed 7 d after cessation of medication.

Diagnosis of H pylori infection or eradication

We performed *H pylori* culture and rapid urease test to confirm the existence of *H pylori*.

The gerbils were sacrificed with ether anesthesia, and then their half-stomachs (right side) were excised. After approximate 50-100 g of the stomach was punched out for MPO activity examination, the remaining part of the stomach was homogenized in 10 mL phosphate buffered saline with a Polytron, followed by dilution with the same buffer. Aliquots (100 μL) of the dilutions were applied to Brucella agar plates containing 10% horse blood (Nippon Bio-Test Laboratories, Tokyo, Japan), 2.5 μg/mL amphotericin B, 9 μg/mL vancomycin, 0.32 μg/mL polymyxin B, 5 μg/mL trimethoprim and 50 μg/mL 2,3,5-triphenyltetrazolium chloride. The plates were incubated at 37 ℃ under microaerophilic atmosphere (N_2 850 mL/L, CO_2 100 mL/L, O_2 5%) for seven days. *H pylori* was identified as gold colonies in spiral shape under a microscope and positive for rapid urease test.

Determination of gastric inflammatory activity

Gross observation The stomachs were opened along the greater curvature and washed with phosphate-buffered saline, then were spread gently and fixed with pins on a cork board. All the stomachs were observed by a researcher who was unaware of the treatment of the animals. Under dissecting microscope, variable size and type of gastric lesions were checked and recorded with a square grid.

Microscopic observation Half of each stomach (left side) was fixed in 10% formalin and embedded with paraffin. Four μm thick sections were prepared and stained with hematoxylin and eosin. The diagnosis of gastritis was made according to the modified criteria, which was slightly modified from Rauws *et al*[7]. The parameters of chronic active gastritis were as follows: lymphocyte infiltration (0:

none, 1: mild infiltration to lamina propria, 2: moderate infiltration to lamina propria, 3: severe infiltration and lymphoid follicle formation), polymorphonuclear leukocyte infiltration (0: none, 1: the number of cells in lamina propria <30 in the field of magnification ×400, 2: 30-100/field, 3: >100/field), superficial erosions (0: none, 1: deletion of surface epithelial cells). The total score of these variables varied from 0 to 7, and was used as a measure of the activity of gastritis. Microscopic gastritis was classified as non-gastritis (score: 0), mild gastritis (score: 1-3), moderate gastritis (score: 4-5) and severe gastritis (score: 6-7). An experienced pathologist determined the gastric pathology without himself being aware of the prior treatment.

Determination of PGE₂ synthesis and MPO activity

PGE_2 production in the gastric mucosa of the gerbils was determined according to the method of Lee and Feldman[8]. We punched out about 50-100 mg gastric specimen from the border of gastric antrum and corpus (right half side) in each gerbil. The specimen was placed in 50 mmol/L Tris HCl (pH 8.4) buffer and then minced with a pair of scissors. After the tissue samples were washed and resuspended in 2 mL of buffer, each sample was subjected to vortex mixing at room temperature for 1 min to stimulate PGE_2 production, followed by centrifugation at 10 000 *g* for 15 s. The PGE_2 levels in the resulting supernatants were determined by means of enzyme immunoassay (PGE_2 EIA kit; Cayman Chemicals, Ann Arbor, Michigan, USA). PGE_2 production was expressed as picograms of PGE_2 per minute per milligram of tissue.

After removal of supernatants for PGE_2 analysis, the MPO activity in the remaining tissue was determined with the method described by Takahashi and Keto[9]. Each sample (approximately 50-100 mg) was first homogenized with a Polytron in 1.0 mL of 50 mmol/L phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (Sigma), and then subjected to three sessions of freezing-thawing. Subsequently, the homogenates were centrifuged at 1 600 *g* for 10 min at 4 ℃. After 5 μL aliquot of each supernatant was mixed with 145 μL of phosphate buffer (pH 6.0) containing 0.167 mg/mL *o*-dianisidine dihydrochloride (Sigama) and 0.0005% H_2O_2, the change in the rate of absorbance at 450 nm was measured with microplate reader (Thermo Max; Molecular Devices, Sunnyvale, CA). The MPO activity was expressed as the degradation of H_2O_2 μmoL/min/g tissue.

Statistical analysis

The data was presented as mean±SE and were analyzed with Student's *t*-test. *P* value less than 0.05 was regarded as statistically significant.

RESULTS

Among the *H pylori*-eradicated animals, 6 out of 7 gerbils were free of *H pylori* infection. The eradication rate was around 85.7%. The mean body weights of animals in groups A, B and C were 76.3±3.4, 73.0±1.6 and 76.1±2.5 g respectively. The difference of mean body weight in the 3 groups was not statistically significant (*P*>0.05). There was

no gastritis in group A (Figure 1A). Mild to moderate gastritis including erosions and hyperplasia of mucosa were found in group C (Figure 1B). However, remarkable erosions and hyperplasia of mucosa were found in group B (Figure 1C). The mean area of erosions (mm^2) of gastric mucosa in groups A, B and C were 0±0, 2.2±0.6, 0.4±0.3 respectively. The differences in area of gastric erosions between group A and group B and between group B and group C were statistically significant ($P<0.05$) (Table 1). The mean area (mm^2) of hyperplasia of gastric mucosa in groups A, B and C were 0±0, 62.8±6.3, 0.9±0.6 respectively. The differences in area of hyperplasia of gastric mucosa between group A and group B and between group B and group C were statistically significant ($P<0.05$) (Table 1). The mean scores of gastritis by histological study in groups A, B and C were 0±0, 6.9±0.1, 2.0±0.5 respectively. The difference was statistically significant between two groups (group A *vs* group B, $P<0.001$; group B *vs* group C, $P<0.001$) (Table 1).

In addition, the weight of stomach increased remarkably after *H pylori* infection. The mean wet weights (gram) of the stomach in groups A, B and C were 8.9±0.3, 14.1±0.3, 9.8±0.2 respectively. The differences between groups A and B ($P<0.001$) and groups B and C ($P<0.001$) were of statistical significance (Table 1).

The values of MPO activity (μmol H_2O_2/min/g tissue) in groups A, B and C were 4.6±2.3, 100.4±5.9, 5.7±1.3 respectively. The differences between groups A and B ($P<0.001$) and between groups B and C ($P<0.001$) were statistically significant (Table 1).

The values of PGE_2 synthesis (pg/mg wet weight/min) in groups A, B and C were 26.9±14.4, 77.0±15.3, 30.6±11.9 respectively. The differences between groups A and B ($P<0.001$) and between groups B and C ($P<0.001$) were statistically significant (Table 1).

Although we did not measure the thickness of the stomach, it became more thicker in *H pylori*-infected gerbils than *H pylori*-uninfected or *H pylori*-eradicated gerbils (Figure 2A-C).

DISCUSSION

Hirayama *et al*[6,10] has demonstrated gastritis, gastric erosions, gastric ulcer and intestinal metaplasia from the stomach of Mongolian gerbils infected with *H pylori* in different periods. Sawada *et al*[11] found acute inflammation, immature epithelium and erosion can be observed 2 wk after *H pylori* infection, while chronic inflammation can be noted 4 wk after *H pylori* infection. In addition, intestinal metaplasia and gastric ulcers develop 12 and 24 wk after *H pylori* infection respectively. Takahashi *et al*[9] study found
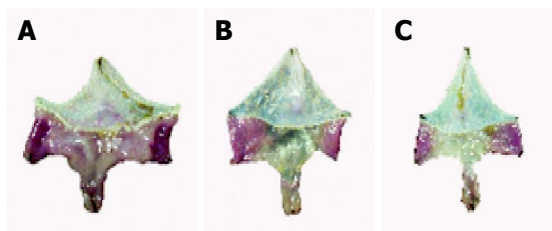

Figure 1 Gross appearance of the stomach in Mongolian gerbils. A: Non-gastritis of group A Mongolian gerbils; B: Mild to moderate gastritis of group C Mongolian gerbils; C: Severe gastritis of group B Mongolian gerbils.
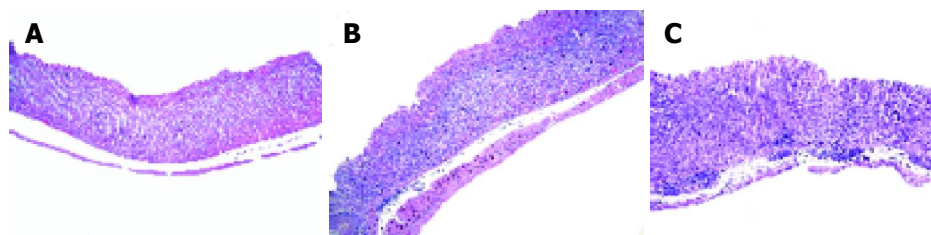

Figure 2 Histological study of gastritis activity in Mongolian gerbils (H E stain, original magnification ×50) A: Non-gastritis in group A Mongolian gerbils; B: Moderate gastritis in group C Mongolian gerbils; C: Severe gastritis in group B Mongolian gerbils.

Table 1 Comparison of gastric inflammation among *H pylori*-infected, *H pylori*-eradicated and *H pylori*-uninfected gerbils (mean±SE)

	Hp-infected Group A (n: 7)	*Hp*-eradicated Group B (n: 6)	*Hp*-uninfected Group C (n: 7)	*P* value
Stomach weight ratio (×1000)	14.1±0.3	9.8±0.2	8.9±0.3	<0.05[a] <0.05[c]
Hyperplasia area (mm^2)	62.8±6.3	0.9±0.6	0	<0.05[a] <0.05[c]
Erosion and ulcer area (mm^2)	2.2±0.6	0.4±0.3	0	<0.05[a] <0.05[c]
Score of gastritis	6.9±0.1	2.0±0.5	0	<0.05[a] <0.05[c]
MPO activity (μmol H_2O_2/min/g tissue)	100.4±5.9	5.7±1.3	4.6±2.3	<0.05[a] <0.05[c]
PGE_2 synthesis (pg/mg wet weight/min)	77.0±15.3	30.6±11.9	26.9±14.4	<0.05[a] <0.05[c]

Stomach weight ratio: Weight of stomach over weight of body. [a]$P<0.05$: *H pylori*-infected *vs H pylori*-eradicated; [c]$P<0.05$: *H pylori*-infected *vs H pylori*-uninfected
P value less than 0.05 was considered statistically siE 8.

edematous and/or congestive mucosa and white substance in the stomachs from Mongolian gerbils 1-4 wk after infection with *H pylori*. However, there is no report about the efficient and rapid eradication of *H pylori* in Mongolian gerbils. Keto *et al*[12] concluded that combined treatment with omeprazole and clarithromycin for 4 wk can enhance healing of *H pylori*-induced gastric ulcers in Mongolian gerbils, but it took a long period of time to eradicate *H pylori* in their experiment. In addition, Kusuhara *et al*[13] eradicated *H pylori* in Mongolian gerbils 5 d after dual therapy (amoxicillin plus omeprazole or clarithromycin plus omeprazole). However, dual therapy for *H pylori* eradication in human beings is used less frequently. In our study, we used anti-*H pylori* triple therapy, which is commonly used in the treatment for humans infected with *H pylori*. The drugs we used were amoxicillin, clarithromycin and lansoprazole. The eradication rate was 85.7% in our study.

From gross observation, there were less superficial lesions (erosions and hyperplasia) in *H pylori*-eradicated gerbils than in *H pylori*-infected gerbils. It revealed that effective eradication of *H pylori* can reduce the gastric inflammation in Mongolian gerbils. Probably, the interval between stopping use of antibiotics and sacrifice of animals was too short, so that gastric inflammation in *H pylori*-eradicated animals did not heal completely. The scores of gastritis in *H pylori*-uninfected and *H pylori*-eradicated animals were less than that in *H pylori*-infected animals.

From our study, we observed that *H pylori* eradication can effectively reduce gastric inflammation including erosion and hyperplasia of gastric mucosa in Mongolian gerbils. Anti-*H pylori* triple therapy can be applied in the study of Mongolian gerbils infected with *H pylori* for one to two years. Probably, it is helpful in the investigation - can the eradication of *H pylori* decrease adenomas and/or hyperplastic polyps in Mongolian gerbils?

There are many risk factors involved for development of gastritis including *H pylori*, non-steroidal anti-inflammatory drugs (NSAIDs), cigarette smoking, ethanol, food, *etc*. Recently, Suzuki *et al*[14] reported that ethanol intake preceding *H pylori* inoculation promotes gastric mucosal inflammation in Mongolian gerbils. Takahashi *et al*[15] reported that NSAIDs induce acute gastric injury in Mongolian gerbils. Tanigawa *et al*[16] suggested that fish meal contains factors, which greatly enhance *H pylori*-induced gastritis in Mongolian gerbils. Perhaps, we can investigate the effects of eradicating *H pylori* prior to administration of ethanol, NSAIDs and fish meal in Mongolian gerbils. It is very important to elucidate the necessity of eradicating *H pylori* in patients who use NSAIDs and those who are heavy drinkers.

In conclusion, 4 d' anti-*H pylori* therapy is a rapid and effective modality in eradicating *H pylori* in Mongolian gerbils and it can be used in the study of gastric mucosal changes in gerbils infected with *H pylori*.

ACKNOWLEDGEMENTS

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