This study examined the effects of indomethacin and rofecoxib on normal and *Helicobacter pylori* (*H. pylori*)-infected gastric mucosa of Mongolian (M.) gerbils. M. gerbils (6-wk-old) were orally administered *H. pylori* (ATCC43504, $2 \times 10^8$ CFU/ml) after fasting for 24 hours. Beginning 3 mo after inoculation, indomethacin (2 mg/kg, s.c) or rofecoxib (10 mg/kg, p.o.) was administered once daily for 2 wk to the gerbils. At autopsy, gastric mucosal ulcer area, myeloperoxidase (MPO) activity, prostaglandin (PG) E$_2$ synthesis, and *H. pylori* viability were determined. Histamine-stimulated gastric acid secretion was measured with the acute gastric fistula method. Histological study was performed with H&E staining. *H. pylori* infection caused severe mucosal damage and production of lymphoid follicles in the gastric submucosa. In *H. pylori*-infected gerbils, indomethacin aggravated the gastric mucosal damage induced by *H. pylori* infection. Furthermore, indomethacin by itself induced gastric ulcers at an incidence of 6/10. In contrast, rofecoxib did not aggravate the *H. pylori*-induced mucosal damage. Indomethacin and rofecoxib significantly reduced *H. pylori* viability. MPO activity was significantly increased in *H. pylori*-infected gerbils compared with *H. pylori*-uninfected gerbils. Indomethacin and rofecoxib reduced MPO activity in *H. pylori*-infected gerbils. PGE$_2$ synthesis was markedly increased in *H. pylori*-infected gerbils (approximately 3-times) compared with the normal gerbils. Indomethacin significantly inhibited PGE$_2$ synthesis in the gastric mucosa, both in normal and *H. pylori*-infected gerbils. Rofecoxib did not reduce PGE$_2$ synthesis in normal gerbils, however, PGE$_2$ synthesis was reduced to normal levels in *H. pylori*-infected gerbils. In *H. pylori*-infected gerbils, histamine-stimulated gastric acid secretion was reduced compared with normal gerbils. Indomethacin significantly increased histamine-stimulated gastric acid secretion and rofecoxib tended to increase secretion in *H. pylori*-infected gerbils. It was concluded that indomethacin enhances development of gastric mucosal damage in normal gerbils and aggravates *H. pylori*-induced gastric damage, resulting in gastric ulcers. Rofecoxib did not induce gastric damage in normal gerbils.
and did not aggravate damage in *H. pylori*-infected gerbils, suggesting that rofecoxib is less damaging to the stomach than indomethacin.

**Key words:** *H. pylori*, *M. gerbil*, COX-1, COX-2.

**INTRODUCTION**

Our group has already reported that a single inoculation of *Helicobacter pylori* (*H. pylori*) could induce atrophic gastritis, as well as ulcer and cancer-like mucosal changes in the stomachs of Mongolian gerbils (*M. gerbils*) (1, 2). In addition, we have reported that various non-steroidal anti-inflammatory drugs (NSAIDs) invariably induce acute gastric lesions and delay ulcer healing in animals. It had been generally believed that the mechanism of action for NSAIDs is inhibition of both cyclooxygenase-1 (COX-1) and COX-2 isoenzymes in the arachidonic acid cascade. Both COX enzymes produce prostaglandin (PG), but COX-1 is considered to be responsible for maintenance of gastric mucosal integrity. In contrast, COX-2 is induced by such processes as inflammation. It is well known that PGs regulate gastric acid secretion, gastric mucus and bicarbonate secretion, gastric mucosal blood flow, and gastric motility (3 - 6). Accordingly, NSAIDs cause gastric mucosal damage by inhibiting both COX-1 and COX-2, resulting in reduced PG synthesis. Consequently, NSAID-induced gastric mucosal damages are invariably inhibited by PG supplementation (7). In order to reduce such gastric damage, COX-2 selective inhibitors, such as celecoxib and rofecoxib were developed and clinically used (8, 9). Indeed, the incidence of gastric ulcers in patients taking COX-2-selective NSAIDs is significantly reduced compared with the incidence for those taking non-selective NSAIDs (10, 11). Such data indicates that PG production induced by COX-2 plays an important role in gastric mucosal repair. Consequently, *H. pylori* infection and NSAIDs are considered to represent the most important risk factors for gastric mucosal damage.

Data concerning the effect of NSAIDs on *H. pylori*-infected patients remain controversial, i.e., NSAIDs were found to aggravate gastric mucosal damage induced by *H. pylori* infection (12), as well as exert no effect (13). In addition, it was reported that *H. pylori* eradication achieved prior to administration of NSAIDs could reduce the incidence of gastric ulcers (14). Another study concluded that NSAIDs had no effect on the gastric ulcer recurrence and delay of gastric ulcer healing (15). Accordingly, the interplay between *H. pylori* infection, NSAIDs, and the timing of *H. pylori* eradication remains uncertain.

The present study examined the effects of rofecoxib and indomethacin on the gastric mucosa of normal and *H. pylori*-infected *M. gerbils*. 
MATERIALS AND METHODS

Animals

Male Mongolian gerbils (6-wk-old, 40-50 g) were purchased from Seac Yositomi (Fukuoka, Japan). The gerbils were kept in an isolated clean room with regulated temperature (approximately 20-22°C), humidity (approximately 55%), and light/dark cycle (12/12 h). The gerbils 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. A total of 5 to 10 animals were used for each study. The maintenance of the animals and the experimental procedures were carried out in accordance with the guidelines of the Ethics Committee of Kyoto Pharmaceutical University.

H. pylori preparation and inoculation

Cag A- and Vac A-positive standard strains of H. pylori (ATCC43504; American Type Culture Collection, Rockville, MD) were used for this study. The bacteria were incubated overnight in a brain-heart infusion broth (Difco Laboratories, Detroit, MI) containing 10% fetal bovine serum (Gibco BRL, Grand Island, NY) at 37°C under a microaerophilic atmosphere; the bacteria were allowed to grow to a concentration of approximately $2.0 \times 10^8$ colony-forming units (CFU)/mL. H. pylori ($2.0 \times 10^8$ CFU/mL) were orally inoculated into each animal at a dose of 1.0 mL/animal.

Drugs

In H. pylori-infected animals, drug treatment was started 3 mo after inoculation. Indomethacin (Sigma), suspended in a trace of Tween 80 and saline, was subcutaneously administered at a dose of 2 mg/kg once daily for 2 wk to normal and H. pylori-infected gerbils beginning 3 mo post-infection. Rofecoxib (Nippon Chemiphar Co., Tokyo) was suspended in 0.5% hydroxypropylcellulose and orally administered at a dose of 10 mg/kg daily for 2 wk to normal and H. pylori-infected gerbils beginning 3 mo post-infection. Control animals received vehicle alone. To measure histamine-stimulated gastric acid secretion, histamine (Sigma) was suspended in saline and subcutaneously administered at a dose of 10 mg/kg to normal and H. pylori-infected gerbils beginning 3 mo post-infection. Indomethacin and rofecoxib were also subcutaneously administered to normal and H. pylori-infected gerbils 1 h before stimulation with histamine.

Macroscopical Studies

The animals were killed with an overdose of ether. The stomach of each animal was removed, opened along the greater curvature, and then spread upon a corkboard. The lesion area (mm$^2$) was promptly measured under a dissecting microscope ($\times 10$; Olympus, Tokyo, Japan). The author (S.O.) who determined the ulcer size was blinded with regards to the treatment that any given animal received.

Quantification of viable H. pylori

After sacrificing the animals with ether anesthesia, the stomach of each animal was homogenized in 10 ml of phosphate-buffered saline with a Polytron (Kinetica, Steinhofhalde, Sweden) and then diluted with the same buffer. Aliquots (0.1 ml) of the dilutions were applied to Brucella agar plates (Gibco BRL) containing 10% horse blood (Nippon Bio-Test Laboratories, Tokyo), 2.5 µg/ml amphotericin B (Sigma, St. Louis, MO), 9 µg/ml vancomycin (Sigma), 0.32
µg/ml polymyxin B (Sigma), 5 µg/ml trimethoprim (Sigma), and 50 µg/ml 2, 3, 5-triphenyltetrazolium chloride (Wako Pure Chemicals, Osaka). The plates were incubated at 37°C under a microaerophilic atmosphere for 7 days. All colonies were either black or gold and were identified as *H. pylori* by appreciating the characteristic spiral shape under a microscope (×2,000; Olympus). The number of colonies was determined and the viable *H. pylori* count was expressed as the ratio CFU/stomach.

**Measurement of MPO activity**

We previously reported that MPO activity was negligible in normal gerbils. To elucidate changes in MPO activity induced by the test drugs, activity was measured by the method of Krawisz et al. (16). Gastric tissue (approximately 50 mg) was extracted from each stomach, homogenized with a Polytron in 1.0 mL of 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl-ammonium bromide (Sigma), and then subjected to freeze-thawing three times. The homogenates were centrifuged at 1,600 g for 10 min at 4 °C. After an aliquot (5 µL) of each supernatant had been mixed with 145 µL of phosphate buffer (pH 6.0), containing 0.167 mg/mL *o*-dianisidine dihydrochloride (Sigma) and 0.0005% H$_2$O$_2$, the change in the rate of absorbance at 450 nm was measured with a microplate reader (Thermo Max; Molecular Devices, Sunnyvale, CA). MPO activity was expressed as the degradation of H$_2$O$_2$ µmol/min/g tissue.

**Determination of PGE$_2$ synthesis**

PGE$_2$ production in the gastric mucosa of the gerbils was determined according to the method of Lee and Feldman (17, 18). Gastric specimens were extracted from the fundic-antral area. Each gastric specimen was placed in 50 mM Tris-HCl (pH 8.4) buffer and then minced with scissors. After the tissue samples had been washed and re-suspended in 1 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 sec. The PGE$_2$ levels in the resulting supernatants were determined by means of an enzyme immunoassay (PGE$_2$ EIA kit; Cayman Chemicals, Ann Arbor, MI). PGE$_2$ production was expressed as pg of PGE$_2$/min/mg tissue.

**Measurement of histamine-stimulated gastric acid secretion**

The effect of indomethacin and rofecoxib on histamine-stimulated gastric acid secretion was determined in normal and *H. pylori*-infected gerbils with the acute gastric fistula method. The gerbils were fasted for 23 h, then anesthetized with an intraperitoneal injection of urethane at a dose of 1.25 g/kg. After tracheotomy, a polyethylene tube was inserted into the trachea to ensure a patent airway, the abdomen was incised, and the stomach and duodenum were exposed. An acute fistula was achieved by insertion of a polyethylene tube towards the direction of the antrum through an incision in the duodenum. A total of 1 milliliter of saline was injected and collected every 15 minutes; acid output (µmol H$^+$/15 min) was determined by titration with 10 mmol/L NaOH. Animals were subcutaneously injected with 2 mg/kg of indomethacin or 10 mg/kg of rofecoxib 1 h before stimulation with histamine (10 mg/kg subcutaneous dose).

**Histological observation**

After gross observation, several specimens were extracted from each stomach, fixed with buffered 10% formalin, and embedded in paraffin. Subsequently, 4 µm paraffin samples were prepared and stained with hematoxylin and eosin.
Statistical Analysis

Data are presented as means ± SEM. Statistical differences were evaluated using the Student's t-test or Dunnett's multiple comparison test, with a P value of < 0.05 regarded as significant.

RESULTS

Effects of indomethacin and rofecoxib on the gastric mucosa of normal and H. pylori-infected M. gerbils

In untreated normal gerbils, no gastric damage was observed. Indomethacin induced a few erosions in the region of the corpus, while rofecoxib caused no gastric erosion in the gastric mucosa. The erosion area in indomethacin-treated animals was 1.8 ± 0.3 mm². Three mo after H. pylori infection, some superficial damage and erosions were observed, but no ulcers were noted. The areas for superficial damage and erosions were 33.3 ± 24.4 mm² and 2.8 ± 0.6 mm², respectively. Indomethacin aggravated gastric damage induced by H. pylori and resulted in gastric ulcers in the fundic-antral area at an incidence of 6/10. The areas for superficial damage, erosions, and ulcers were 12.2 ± 2.6 mm², 6.1 ± 2.0 mm², and 7.1 ± 3.4 mm², respectively. Moreover, in indomethacin-treated infected gerbils, largest ulcer area was 31 mm². Rofecoxib did not affect H. pylori-induced gastric mucosal damages and did not precipitate development of gastric ulcers. The areas for superficial damage and erosions were similar to the H. pylori-infected control group, i.e., 33.6 ± 14.2 mm² and 2.2 ± 1.2 mm² (Figs 1-2).

Fig.1. Effects of indomethacin and rofecoxib on the gastric mucosa of normal and H. pylori-infected M. gerbils. Indomethacin (2 mg/kg, s.c.) and rofecoxib (10 mg/kg, p.o.) were administered once daily for 2 wk to M. gerbils with and without H. pylori infection. Drug treatment was started 3 mo after inoculation. Note that indomethacin clearly induced gastric ulcers in H. pylori-infected animals, while rofecoxib did not. Data are presented as means±SEM for 5-10 animals.
Effects of indomethacin and rofecoxib on histological changes in normal and \textit{H. pylori}-infected \textit{M. gerbils}

In normal gerbils, no gastric damage was observed, hence the mucosal structure was normal. In the indomethacin-treated animals, the mucosa epithelial cells were observed to peel away from the underlying cells. In contrast, rofecoxib-treated animals exhibited normal mucosa. In \textit{H. pylori}-infected mucosa, superficial damage and erosions were observed to a small extent. Moreover, lymphoid follicles, neutrophil infiltration, gastric hyperplasia were

\textit{Fig. 2.} Gross appearances of the gastric mucosa in normal (A) and \textit{H. pylori}-infected \textit{M. gerbils} (B), with and without indomethacin (2 mg/kg, s.c.) or robecoxib (10 mg/kg, p.o.). Note that gastric ulcers were observed only after indomethacin treatment was administered to \textit{H. pylori}-infected gerbils.
also observed. Indomethacin treatment aggravated such mucosal damage observed in *H. pylori*-infected mucosa and *H. pylori*-induced gastric ulcers, which were found to penetrate the muscularis and serosa and adhere to the liver. In rofecoxib-treated animals, mucosa superficial damage and erosions were observed to a small extent, however, gastric ulcers were not induced as in the indomethacin-treated gerbil group (Fig. 3).

**Effects of indomethacin and rofecoxib on *H. pylori* viability in *H. pylori*-infected *M. gerbils***

*H. pylori* was detected in all the gerbil stomachs that were examined immediately after treatment with vehicle, indomethacin, or rofecoxib. *H. pylori* viability was $3.1 \times 10^5$ CFU/stomach in indomethacin control animals. Indomethacin significantly decreased *H. pylori* viability to $1.8 \times 10^5$ CFU/stomach. In rofecoxib control animals, *H. pylori* viability was $3.6 \times 10^5$ CFU/stomach.

![Fig. 3. Histological appearances of the gastric mucosa of normal (A) and *H. pylori*-infected *M. gerbils* (B) with or without indomethacin (2 mg/kg, s.c.) or rofecoxib (10 mg/kg, p.o.). (a) Control, (b) Indomethacin (2 mg/kg, s.c.), (c) Rofecoxib (10 mg/kg, p.o.). (H&E staining; x50).](image)
which was also significantly reduced to 2.0x10^5 CFU/stomach with rofecoxib treatment (Fig. 4).

Effects of indomethacin and rofecoxib on the MPO activity of normal and H. pylori-infected M. gerbils

In the indomethacin and rofecoxib normal control groups, MPO activity in the gastric mucosa was negligible (7.2±3.0 µmol H_2O_2/min/g tissue and 6.4±1.8µmol H_2O_2/min/g tissue, respectively). In indomethacin-treated gerbils, MPO activity in the gastric mucosa was significantly increased, however, rofecoxib did not affect MPO activity (26.7±6.3 µmol H_2O_2/min/g tissue and 10.0±3.4 µmol H_2O_2/min/g tissue). MPO activity in H. pylori-infected gastric mucosa was markedly increased compared with normal gerbils. In indomethacin-treated H. pylori-infected gerbils, MPO activity in the gastric mucosa was significantly higher than that of normal gerbils, however, the level was reduced compared with the H. pylori-infected control group (907.2±218.5 µmol H_2O_2/min/g tissue vs. 1690.7±128.1 µmol H_2O_2/min/g tissue). In the rofecoxib-treated H. pylori-infected gerbils, MPO activity in the gastric mucosa was also significantly higher than that of normal gerbils, but less than the H. pylori-infected control group (1255.1±236.0 µmol H_2O_2/min/g tissue vs. 1906.4±47.2 µmol H_2O_2/min/g tissue) (Fig. 5).
**Effects of indomethacin and rofecoxib on PGE\textsubscript{2} synthesis in normal and H. pylori-infected M. gerbils**

PGE\textsubscript{2} synthesis in the indomethacin-treated gastric mucosa was significantly reduced compared with normal gerbils (8.0 ± 2.4 pg/min/mg tissue vs. 49.8 ± 25.6 pg/min/mg tissue). PGE\textsubscript{2} synthesis in the rofecoxib-treated mucosa was similar to the normal control mucosa (37.8 ± 7.0 pg/min/mg tissue vs. 42.0 ± 14.5 pg/min/mg tissue). In *H. pylori*-infected gastric mucosa, PGE\textsubscript{2} synthesis was markedly increased to approximately 3 times that of the control gastric mucosa. Indomethacin significantly inhibited PGE\textsubscript{2} synthesis less than the normal control group. In rofecoxib-treated gastric mucosa, PGE\textsubscript{2} synthesis was significantly inhibited, resulting in a value similar to the control gastric mucosal PGE\textsubscript{2} synthesis (Fig. 6).

**Effects of indomethacin and rofecoxib on histamine-stimulated gastric acid secretion in normal and H. pylori-infected M. gerbils**

In normal gerbils, histamine was found to significantly increase gastric acid secretion. The maximum level of gastric acid secretion was 75 min after stimulation with histamine. After indomethacin treatment 1 h before histamine stimulation, the maximum gastric acid secretion tended to increase compared with that of vehicle group (42.5±31.0 µEq/15 min vs. 17.6±4.9 µEq/15 min) 90 min after stimulation with histamine. In rofecoxib-pretreated group, the

![Fig. 5. Effects of indomethacin and rofecoxib on myeloperoxidase (MPO) activity in the gastric mucosa of normal and *H. pylori*-infected M. gerbils. Indomethacin (2 mg/kg, s.c.) or rofecoxib (10 mg/kg, p.o.) was administered once daily for 2 wk to M. gerbils with or without *H. pylori* infection. Drug treatment was started 3 mo after inoculation. Data are presented as means ± SEM for 4-5 animals. *, # Significantly different from the normal and *H. pylori*-infected groups respectively, P<0.05.](image)
maximum gastric acid secretion was the same as that of the vehicle group (17.8±3.6 µEq/15 min vs. 17.6±4.9 µEq/15 min) 75 min after stimulation with histamine. In H. pylori-infected gerbils, the maximum gastric acid secretion was reduced (5.5±3.0 µEq/15 min vs. 17.6±4.9 µEq/15 min) 60 min after stimulation with histamine. Moreover, total gastric acid secretion 2 h after stimulation with histamine was also significantly reduced compared with that of normal gerbils (31.3±0.6 µEq/2 hr vs. 97.6±2.1 µEq/2 hr). In contrast, in the indomethacin pretreatment group, maximum gastric acid secretion was enhanced 75 min after stimulation with histamine (11.1±4.1 µEq/15 min vs. 5.5±3.0 µEq/15 min). Furthermore, total gastric acid secretion 2 h after stimulation with histamine was significantly increased compared with that of H. pylori-infected gerbils (62.8±1.3 µEq/2 hr vs. 31.3±0.6 µEq/2 hr). In the rofecoxib pretreatment group, the maximum gastric acid secretion was enhanced 75 min after stimulation with histamine (7.4±2.8 µEq/15 min vs. 5.5±3.0 µEq/15 min) (Fig. 7).

**DISCUSSION**

This study demonstrated that indomethacin aggravated gastric mucosal damage induced by H. pylori, resulting in gastric ulcers. Moreover, the study also confirmed that rofecoxib, a COX-2 selective inhibitor, had an effect on not only normal mucosa, but also H. pylori-induced mucosal damage. In general, the mechanisms of NSAID-induced gastric damage and H. pylori-induced mucosal
damage are thought to be independent, however, factors such as neutrophil activation and gastric acid secretion are thought to be involved in the pathogenesis of gastric damage.

Gastric acid is recognized as a caustic agent for the stomach. In the present study, although gastric acid secretion was increased by stimulation with histamine in *H. pylori*-infected gerbils, the increase was low compared with normal gerbils. Nonetheless, pretreatment with indomethacin increased histamine-stimulated gastric acid secretion more than the vehicle in *H. pylori*-infected M. gerbils. It is known that gastric acid secretion is decreased by *H. pylori* infection and that such a phenomenon might be caused by a component of *H. pylori*, inflammatory cytokines induced by *H. pylori* infection, or a decrease in the number of parietal cells caused by atrophic gastritis (19 - 21). It has been reported that expression of several inflammatory cytokines, such as Interleukin (IL)-1ß and tumor necrosis factor (TNF)-α resulting from gastric ulcers, inhibits gastric acid secretion (22) and elevates expression of COX-2 mRNA in gastric mucosa (23, 24). It has also been reported that PG inhibits gastric acid secretion by binding to EP3 receptors (25). In our study, PGE2 synthesis in *H. pylori*-infected gerbils was increased 3

Fig. 7. Effects of indomethacin and rofecoxib on histamine-stimulated gastric acid secretion in normal and *H. pylori*-infected M. gerbils. Indomethacin (2 mg/kg, s.c.) or rofecoxib (10 mg/kg, p.o.) was administered 60 min before histamine (10 mg/kg, s.c.) treatment. It was of note that *H. pylori* infection significantly reduced histamine-stimulated gastric acid secretion. Indomethacin, but not rofecoxib, significantly suppressed the reduced acid secretion seen in infected animals. Data are presented as means ± SEM for 6-7 animals. *, # Significantly different from the normal and *H. pylori*-infected groups respectively, P<0.05.
times greater than that of normal gerbils. In *H. pylori*-infected gerbils, treatment with indomethacin significantly decreased PGE₂ synthesis in the gastric mucosa; the level was lower than that of normal gerbils. Such findings indicate that PG induced by COX-2 in the inflammatory process is associated with a decrease in gastric acid secretion seen in *H. pylori* infection. Indomethacin increased gastric acid secretion as a result of inhibition of PG synthesis. The present study demonstrated that in normal gerbils, rofecoxib decreased PGE₂ synthesis to the same level as that of normal gerbils; pretreatment with rofecoxib tended to increase gastric acid. Xiao (26) has also reported that etodolac, a COX-2 selective inhibitor, increased gastric acid secretion in *H. pylori*-infected mice. Accordingly, PG production induced by COX-2 is involved in the inhibition of gastric acid secretion observed in *H. pylori* infection.

In *H. pylori* infection, IL-1, as well as TNF-α produced by monocytes and macrophages, induced IL-8 production (27). *H. pylori* stimulate IL-8 production in gastric epithelial cells (28). It has also been reported that IL-8-enhanced neutrotaxis, as well as expression of CD11b/CD18 and free radicals, resulted in continuous infiltration of inflammatory cells, in addition to cell injury (29). On the other hand, NSAIDs decrease PG synthesis through COX inhibition. PGI₂ produced in endothelial cells and PGE₂ produced in gastric mucosal and endothelial cells inhibit neutrophil activation, production of cytokines, and expression of adhesion molecules (30). Moreover, PGE₂ also inhibits neutrophils from producing elastase and free radicals (31). The decrease of PG synthesis resulting from NSAIDs caused neutrophil activation, leading to gastric mucosal injury. In this study, gastric mucosal MPO activity was very low level in normal gerbils treated with vehicle or rofecoxib. In contrast, MPO activity was increased in normal gerbils treated with indomethacin. *H. pylori*-infected gerbils had significantly higher MPO activity and exhibited severe neutrophil infiltration. As was noted in a previous study, consumption of NSAIDs during *H. pylori* infection resulted in more severe injury due to combination of neutrophil activation induced by NSAIDs with neutrophil infiltration induced by *H. pylori* infection. Nonetheless, indomethacin and rofecoxib decreased MPO activity in gerbil gastric mucosa. Two possible mechanisms are involved. One possibility is that indomethacin induces lower IL-8 levels than *H. pylori* infection alone. Moreover, activated neutrophils caused endothelial cell injury in only blood vessels, not in the gastric mucosa. Another possibility is that rofecoxib inhibits only COX-2, therefore, PGE₂ and PGI₂ produced by COX-1 inhibits neutrophil activation, resulting in decreased gastric mucosal MPO activity.

Gastric mucous is one gastric mucosal protective factor. The mechanism of action involves binding to EP₄ receptors (3). As seen in macroscopic views of stomachs, gastric mucous is significantly increased by *H. pylori* infection. Nonetheless, indomethacin and rofecoxib decreased the volume of gastric mucous in *H. pylori* infection. Moreover indomethacin and rofecoxib significantly decreased *H. pylori* viability. Accordingly, the decrease in *H. pylori*
viability might be caused by the decrease in gastric mucous, an element essential for \textit{H. pylori} survival, as a result of decreasing PG synthesis.

The other gastric protective actions of PG include PGE$_2$ binding to EP1, resulting in inhibition of gastric motility, and to EP2/EP3/EP4, resulting in an increase of gastric blood flow (5, 30). It has been reported that the gastric mucosal injury induced by non-selective inhibitors, such as indomethacin, results in acceleration of gastric motility and a decrease in gastric mucosal blood flow (32). On the other hand, it is known that rofecoxib has no effect on gastric motility (33) and celecoxib, a COX-2 selective inhibitor, does not decrease gastric mucosal blood flow (34). Consequently, COX selective inhibition allows reduction of the incidence of gastric mucosal injury.

In a clinical study, \textit{H. pylori} and non-selective NSAIDs were found to result in severe gastric damage compared with COX-2 selective NSAIDs, due to inhibition of both COX-1 and COX-2. Nonetheless, it is not clear that NSAIDs aggravate gastric mucosal damage induced by \textit{H. pylori}. If such a possibility holds true, then the timing of NSAID ingestion and \textit{H. pylori} infection, as well as basal gastric acid secretion and the degree of gastric mucosal injury, become important. In particular, the timing of \textit{H. pylori} infection is thought to be very important for induction of a gastric mucosal injury. We previously performed such a study 4 wk after induction of \textit{H. pylori} infection, determining erosion area, MPO activity, PGE$_2$ synthesis, and \textit{H. pylori} viability in the gastric mucosa of gerbils. The results were as follows. At baseline, 4 wk after \textit{H. pylori} infection induction, some mucosal erosions were observed. Indomethacin aggravated the gastric mucosal erosion induced by \textit{H. pylori} infection, resulting in gastric ulcer production (1/6) 4 wk after inoculation (35). In this study, treatment with indomethacin beginning 3 mo after \textit{H. pylori} infection inoculation resulted in gastric ulcer production at an incidence of 6/10. Accordingly, the longer the period of \textit{H. pylori} infection, the more severe the gastric mucosal injury is due to persistent inflammatory reaction.

It was expected that COX-2 selective inhibitors would exhibit a strong anti-inflammatory effect with less gastric mucosal injury than non-selective NSAIDs. Nonetheless, COX-2 selective NSAIDs still possess problems. One study reported that COX-2 selective NSAIDs induced gastric mucosal injury without inhibition of PG in normal gastric mucosa (36). Another study reported that COX-2 selective NSAIDs delay the healing of gastric ulcers. Such reports indicate that COX-2 might be needed to maintain normal conditions in the gastric mucosa. Consequently, prescribing COX-2 selective NSAIDs to \textit{H. pylori} positive patients warrants prudence.

The present study found that indomethacin aggravated gastric mucosal injury induced by \textit{H. pylori} infection through inhibition of PG synthesis, resulting in a decrease of gastric mucosal protective factors and an increase in gastric acid secretion. Moreover, rofecoxib had no effect on gastric mucosa of normal and \textit{H. pylori}-infected gerbils. Such a result would be expected with preservation of
normal PG synthesis levels and inhibition of neutrophil activation. Therefore, these results indicate that non-selective NSAIDs enhance gastric mucosal injury induced by \textit{H. pylori} infection to a greater extent than COX-2 selective NSAIDs. Accordingly, our study further elucidates the effects of \textit{H. pylori} infection and COX-2 selective NSAIDs on gastric mucosal injury.

In conclusion, it was found that indomethacin, a non-selective NSAID, aggravated gastric mucosal damage induced by \textit{H. pylori} infection, leading to development of gastric ulcers. In contrast, rofecoxib, a COX-2 selective inhibitor, had no effect on normal and \textit{H. pylori}-infected gastric mucosa. This difference may result from different inhibition levels of PG synthesis that is the difference between inhibition of both COX-1 and COX-2, versus only COX-2. Rofecoxib thus appears to be safer to use than indomethacin in regards to the stomach.

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