Role of cyclooxygenase-2 in Helicobacter pylori-induced gastritis in Mongolian gerbils

SATORU TAKAHASHI,1,2 TAKUYA FUJITA,2 AND AKIRA YAMAMOTO2
Departments of 1Applied Pharmacology and 2Biopharmaceutics, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan

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Takahashi, Satoru, Takuya Fujita, and Akira Yamamoto. Role of cyclooxygenase-2 in Helicobacter pylori-induced gastritis in Mongolian gerbils. Am J Physiol Gastrointest Liver Physiol 279: G791–G798, 2000.—Cyclooxygenase (COX)-2 expression is induced in the gastric mucosa of Helicobacter pylori-infected patients, but its role remains unclear. We examined the effects of NS-398 and indomethacin on gastric pathology in H. pylori-infected Mongolian gerbils. COX-1 was detected in both normal and H. pylori-infected mucosa, whereas COX-2 was expressed only in the infected mucosa. PGE2 production was elevated by H. pylori infection, and the increased production was reduced by NS-398, which did not affect PGE2 production in normal mucosa. Indomethacin inhibited PGE2 production in both normal and infected mucosa. Hemorrhagic erosions, neutrophil infiltration, lymphoid follicles, and epithelial damage were induced by H. pylori infection. NS-398 and indomethacin aggravated these pathological changes but did not increase viable H. pylori number. H. pylori-induced production of neutrophil chemokine and interferon-γ was potentiated by NS-398 and indomethacin. Neither NS-398 nor indomethacin caused any pathological changes or cytokine production in normal animals. These results indicate that COX-2 as well as COX-1 might play anti-inflammatory roles in H. pylori-induced gastritis.

prostaglandin; nonsteroidal anti-inflammatory drug; infection

Helicobacter pylori is recognized as a major etiologic factor of chronic gastritis and gastric/duodenal ulcers. H. pylori infection causes marked infiltration of inflammatory cells into the gastric mucosa and expression of many cytokines. Consequently, it is generally suspected that cytokine-related inflammatory responses might be involved in H. pylori-induced mucosal injuries (3, 5, 10, 43). Similarly, mucosal defense mechanisms might also be activated by H. pylori infection. Prostaglandins (PGs) are well-known mucosal defense factors, protecting the gastric mucosa against injury caused by a variety of toxic stimuli (9, 28). PGE2 stimulates the secretion of gastric mucus and bicarbonate, increases mucosal blood flow, inhibits acid secretion, and reduces gastric motility (9). PGs are synthesized through cyclooxygenase (COX), which is a target of nonsteroidal anti-inflammatory drugs (NSAIDs) (38). COX exists in two isoforms, of which COX-1 is constitutively expressed in many tissues, including the stomach, and COX-2 is normally undetectable in most tissues, its expression being induced at inflammatory sites (13, 23). In the normal gastric mucosa, COX-1-derived PGs play a crucial role in maintaining mucosal integrity (14, 18). In contrast, COX-2 is not expressed in normal mucosa, but its induction results in a marked and sustained increase in PGE2 production in the damaged mucosa (24, 32, 37). In addition to COX-1, COX-2 also plays an important role in the healing of gastric ulcers (24, 32). Recent studies showed that COX-2 expression is observed in the gastric mucosa of H. pylori-positive patients (22, 31, 33). In addition, Romano et al. (29) reported that H. pylori directly induces COX-2 mRNA expression and increases PGE2 production in gastric cancer MKN28 cells. However, the role of COX-2 in H. pylori-induced gastritis in vivo remains unclear.

Mongolian gerbil models of H. pylori infection have been established and widely used (15, 16, 20, 21, 34). We have shown that chronic gastritis and ulcers are generated in all H. pylori-infected animals (20, 34). In addition, Watanabe et al. (41) recently reported that gastric cancers are also developed by H. pylori infection in gerbils. Thus the gerbil model is quite suitable for in vivo study of the pathogenesis of H. pylori-induced gastric diseases, since this model exhibits pathological features that mimic those of human patients with H. pylori.

To investigate the role of COX-2 in H. pylori-induced gastritis, we examined the effect of NS-398, a COX-2-selective inhibitor, on H. pylori-induced pathological changes in Mongolian gerbils, compared with indomethacin, a nonselective COX inhibitor.

METHODS

Animals. Male Mongolian gerbils (6 wk old, 40–50 g) were kindly supplied from Nihon SLC (Hamamatsu, Japan). The animals were kept in an isolated clean room with regulated temperature (20–22°C) and humidity (~55%) with a 12:12-h light/dark cycle. The animals were fasted for 24 h before H. pylori infection.
H. pylori inoculation, and drinking water was also withheld after the inoculation. From 4 h after the inoculation, both food and water were freely available to the animals.

H. pylori preparation and inoculation of Mongolian gerbils.

The preparation and inoculation of H. pylori were performed as we previously described (34). A cagA- and vacA-positive standard strain of H. pylori (NCTC 11637; American Type Culture Collection, Rockville, MD) was used. The bacteria were incubated in a brain-heart infusion broth (Difco Laboratories, Detroit, MI) containing 10% fetal bovine serum (GIBCO BRL, Gaithersburg, MD) at 37°C overnight under a microaerophilic atmosphere and allowed to grow to a density of $-2.0 \times 10^8$ colony-forming units (CFU)/mL. H. pylori (2.0 $\times 10^8$ CFU; 1.0 ml) was orally administered to each animal. Normal animals received 1.0 ml of the medium alone.

Western blot analysis of COX-1 and COX-2 proteins.

Expression of COX-1 and COX-2 proteins in the gastric mucosa was examined by Western blotting. Gastric specimens (the fundus near the antrum) were taken from normal and H. pylori-infected animals. COX proteins were partially purified according to the method of Gierse et al. (12). The specimens were homogenized in 25 mM Tris-$\cdot$HCl (pH 8.0) buffer containing 250 mM sucrose, followed by centrifugation at 10,000 $g$ for 20 min. The pellet was resuspended in 25 mM Tris-$\cdot$HCl (pH 8.0) buffer containing 1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, and the mixture was gently stirred for 2 h at 4°C. The supernatant was recovered after centrifugation at 30,000 $g$ for 30 min and applied onto a DEAE-Sepharose CL-4B (Amersham Pharmacia Biotech, Little Chalfont, UK) column that had been equilibrated with 25 mM Tris-$\cdot$HCl (pH 8.0) buffer containing 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 1 mM phenylmethylsulfonyl fluoride, and 0.2 mM EDTA. After the column was washed with the same buffer supplemented with 50 mM NaCl, elution was performed with 200 mM NaCl. After aliquots (20 $\mu$g) of the eluted proteins and purified COX proteins (50 ng; Cayman, Ann Arbor, MI) had been subjected to SDS-polyacrylamide gel electrophoresis (10%), the separated proteins were electrophoretically transferred onto Hybond-P membranes (Amersham Pharmacia Biotech). The membranes were incubated with the antibody (Cayman) against COX-1 or COX-2 protein after nonspecific binding sites had been blocked with bovine serum albumin. COX proteins were detected on X-ray films (Fuji Film, Tokyo, Japan) with an enhanced chemiluminescence kit (Immuno Star; Wako Pure Chemicals, Osaka, Japan).

Determination of PGE$_2$ production.

Gastric specimens (the fundus near the antrum) were taken from normal and H. pylori-infected animals. After being washed with PBS, the tissues were minced and then incubated in 1 ml of Dulbecco’s modified Eagle’s medium supplemented with 2.5% fetal bovine serum and the above antibiotics at 37°C for 20 h under 5% CO$_2$ in air. Thereafter, the tissues were homogenized in the culture medium containing 0.1 mM phenylmethylsulfonyl fluoride and 1 $\mu$g/ml leupeptin. The homogenates were centrifuged at 10,000 $g$ for 20 min. The amounts of CINC/KC, IFN-$\gamma$, and IL-10 in the resulting supernatants were determined by enzyme immunoassay (Cayman EIA kit; Immuno Biological Laboratories, Fujioka, Japan) and ELISA (IFN-$\gamma$ ELISA kit and IL-10 ELISA kit; Biosource International, Camarillo, CA), respectively. CINC/KC, IFN-$\gamma$, and IL-10 production was expressed as picograms per milligram tissue per hour.

Evaluation of H. pylori-induced gastritis.

Gastric pathology was blindly evaluated. Normal and H. pylori-infected animals were killed, and their stomachs were excised. The stomachs were incised along the greater curvature and spread out with pins on a cork board. Gastric mucosal hemorrhagic lesions were examined under a dissecting microscope (magnification, $\times 10$). Thereafter, four gastric specimens were cut off from the fundus near the antrum and fixed in 4% paraformaldehyde in PBS. Frozen sections (12 $\mu$m thick) were prepared, and neutrophil-specific myeloperoxidase activity-dependent staining was carried out (11). In brief, the sections were incubated in 50 mM Tris-$\cdot$HCl (pH 7.6) containing 0.2 mg/ml 3’,3’-diaminobenzidine tetrahydrochloride (Dojindo, Kumamoto, Japan) in the presence of 0.005% H$_2$O$_2$ at room temperature. After being washed with PBS, the sections were successively stained with hematoxylin. Brown- or black-stained cells in the mucosa were identified as neutrophils when their morphological features were confirmed under a light microscope at high magnification and the cells were not stained in the absence of H$_2$O$_2$ or 3’, 3’-diaminobenzidine. Histological features of mucosal inflammation, epithelium damage, and lymphoid follicle formation were graded on a scale of 0–3 for each specimen under a light microscope (magnification, $\times 25$), and the median score was used. According to the Sydney system (27), neutrophil infiltration into the mucosa was evaluated as follows: 0, none; 1, mild; 2, moderate; and 3, severe. As described by Atherton et al. (1), epithelium damage was graded as follows: 0, no exfoliation; 1, exfoliation of $<30$% of epithelium; 2, 30–70% exfoliation; and 3, $>70$% exfoliation. Similarly, lymphoid follicle formation was also graded as follows: 0, no follicle; 1, follicles of $<30$% under the muscularis mucosa; 2, 30–70% follicles; and 3, $>70$% follicles.

Determination of viable H. pylori.

Viable H. pylori in the stomach was assayed according to our previously reported method (34). The animals were killed, and their stomachs were excised. The stomachs were homogenized in 20 ml PBS. The diluted homogenates were applied onto Brucella agar (GIBCO BRL) plates supplemented with 10% horse blood (Nippon Bio-Test, Tokyo, Japan) and (in $\mu$g/ml) 2.5 amphotericin B (Sigma, St. Louis, MO), 9 vancomycin (Sigma), 0.32 polymyxin B (Sigma), 5 trimethoprim (Sigma), and 50 2,3,5-triphenyltetrazolium chloride (Wako Pure Chemicals). The plates were incubated at 37°C under a microaerophilic atmosphere for 7 days. The number of colonies was counted, and viable H. pylori was expressed as CFU per stomach.

Determination of neutrophil chemokine, interferon-$\gamma$, and interleukin-10 production.

The production of cytokine-induced neutrophil chemotactant (CINC/KC; interleukin (IL)-8 family chemokine in rodents), interferon (IFN)-$\gamma$, and IL-10 was assayed according to the method of Noach et al. (26) with slight modifications. Gastric specimens (the fundus near the antrum) were taken from normal and H. pylori-infected animals. After being washed with PBS, the tissues were minced and then incubated in 1 ml of Dulbecco’s modified Eagle’s medium supplemented with 2.5% fetal bovine serum and the above antibiotics at 37°C for 20 h under 5% CO$_2$ in air. Thereafter, the tissues were homogenized in the culture medium containing 0.1 mM phenylmethylsulfonyl fluoride and 1 $\mu$g/ml leupeptin. The homogenates were centrifuged at 10,000 $g$ for 20 min. The amounts of CINC/KC, IFN-$\gamma$, and IL-10 in the resulting supernatants were determined by enzyme immunoassay (Cayman EIA kit; Immuno Biological Laboratories, Fujioka, Japan) and ELISA (IFN-$\gamma$ ELISA kit and IL-10 ELISA kit; Biosource International, Camarillo, CA), respectively. CINC/KC, IFN-$\gamma$, and IL-10 production was expressed as picograms per milligram tissue per 20 h.

Drugs. NS-398 (kindly synthesized by Nippon Chemiphar, Tokyo, Japan) and indomethacin (Sigma) were finely dispersed in Tween 80 (10 mg/50 $\mu$l) and then suspended by adding saline to the desired concentrations. In our preliminary experiment, NS-398 at 20 mg/kg, but not at 10 mg/kg, had inhibited PGE$_2$ production in normal mucosa, indicating that 10 mg/kg NS-398 was ineffective in COX-1 activity. In addition, indomethacin even at 2 mg/kg had potently reduced PGE$_2$ levels in normal mucosa, but repeated administration of 5 mg/kg indomethacin had been lethal to H. pylori-infected gerbils. Therefore, the doses of 10 mg/kg and 2 mg/kg were
selected for NS-398 and indomethacin, respectively. The drugs were subcutaneously administered once daily for 4 wk from 2 wk of H. pylori infection. The animals were given the drugs in a volume of 10 ml/kg body wt. Control animals received the vehicle alone.

Statistical analysis. Data are presented as means ± SE of 3–6 animals/group. Statistical differences were evaluated by Student’s t-test or Mann-Whitney U-test. P values of <0.05 were considered significant.

RESULTS

COX-2 induction in the gastric mucosa by H. pylori infection. In our model of H. pylori infection, H. pylori is colonized for at least 10 mo in the gastric mucosa of all gerbils given the bacteria (20, 34). The number of viable H. pylori in the stomach reached a plateau level from 2 wk after the inoculation. In addition, gastritis with hemorrhagic mucosal lesions and gastric ulcer were generated only in the fundus near the antrum from 4 wk and 20 wk of the infection, respectively (20, 34). Thus the region is highly sensitive to H. pylori, and the following parameters, except for H. pylori viability, were measured in the region.

At first, we examined the expression of COX proteins and PGE2 production in the gastric mucosa during H. pylori infection (Fig. 1). On Western blot analysis, COX-1 protein was detected both in normal mucosa and in the mucosa with H. pylori infection. The level of COX-1 protein remained nearly constant during the infection. In contrast, COX-2 protein was not found in normal or H. pylori-infected (2 wk) mucosa, but H. pylori infection for >4 wk induced the expression of COX-2 protein. PGE2 production in normal mucosa was 36.7 ± 5.6 pg·mg⁻¹·h⁻¹. At 2 wk of H. pylori infection, there was no difference in PGE2 production compared with the normal level. However, PGE2 production was significantly elevated from 4 wk of the infection compared with that in normal mucosa. There was a correlation between the increase in PGE2 production and the intensity of the COX-2 protein band.

Effects of NS-398 and indomethacin on H. pylori-induced PGE2 production and gastritis. As shown in Fig. 1, the increase in PGE2 production was associated with COX-2 induction in the H. pylori-infected mucosa. To investigate the role of COX-2 in H. pylori-induced gastritis, we examined the effects of NSAIDs on PGE2 production and gastric pathology caused by H. pylori.

NS-398 (a COX-2-selective inhibitor) at 10 mg/kg or indomethacin (a nonselective COX inhibitor) at 2 mg/kg was administered for 4 wk to normal and H. pylori-infected animals. NS-398 failed to inhibit PGE2 production in normal mucosa but significantly reduced the H. pylori-increased PGE2 production (Fig. 2). In contrast, indomethacin potently inhibited PGE2 production in both normal and H. pylori-infected mucosa. Significant differences were observed between the effects of NS-398 and indomethacin in the H. pylori-infected animals.

In the control animals with H. pylori infection, there were hemorrhagic mucosal lesions of 1.0 ± 0.4 mm². Erosive lesions were apparently enlarged by treatment.
with NS-398 and indomethacin, although there were no significant differences between the drug-treated and control groups. Erosive area was $1.7 \pm 0.6 \text{ mm}^2$ and $2.0 \pm 0.6 \text{ mm}^2$ in NS-398- and indomethacin-treated groups, respectively.

Neutrophil infiltration, epithelium damage, and lymphoid follicle formation were also evaluated as shown in Figs. 3 and 4. *H. pylori* infection caused marked infiltration of neutrophils into the gastric mucosa and epithelium damage. Furthermore, lymphoid follicles were generated in the submucosa. Both NS-398 and indomethacin significantly aggravated the *H. pylori*-induced neutrophil infiltration, epithelium damage, and lymphoid follicle formation. The aggravation of neutrophil infiltration appeared to be more severe in indomethacin-treated animals than in NS-398-treated ones. Epithelium damage was also more significant and lymphoid follicles more enlarged by indomethacin than by NS-398. However, repeated administration of NS-398 for 4 wk induced no inflammation or damage in normal animals. Similarly, indomethacin did not cause appreciable pathological changes in normal animals.

**Effects of NS-398 and indomethacin on *H. pylori* infection and the production of CINC/KC, IFN-γ, and IL-10.** Since NS-398 and indomethacin aggravated *H. pylori*-induced gastritis, we determined the number of viable *H. pylori* in the stomach after treatment with the drugs. The number of *H. pylori* colonized in the stomach was $2.19 \pm 0.56 \times 10^5 \text{ CFU}$ in the control group. Both NS-398 ($1.92 \pm 0.42 \times 10^5 \text{ CFU}$) and indomethacin ($1.51 \pm 0.38 \times 10^5 \text{ CFU}$) tended to reduce *H. pylori* number, but there were no significant differences between the drug-treated and control groups.

We next examined the effects of NS-398 and indomethacin on the production of CINC/KC (a predominant neutrophil chemokine in rodents) and IFN-γ in the gastric mucosa (Fig. 5). *H. pylori* infection for 6 wk significantly increased CINC/KC and IFN-γ production by ∼23- and ∼8-fold, respectively, compared with the production in normal mucosa. Both NS-398 and indomethacin significantly potentiated the *H. pylori*-increased CINC/KC and IFN-γ production. Treatment with NS-398 and indomethacin further promoted CINC/KC production by 2.0- and 2.8-fold, respectively, compared with the *H. pylori*-infected control group. The IFN-γ production in the NS-398- and indomethacin-treated groups was also increased by 1.6- and 2.0-fold, respectively, compared with the control with *H. pylori* infection.

IL-10 was not detected in normal mucosa, whereas its production was slightly induced by *H. pylori* infection ($0.28 \pm 0.09 \text{ pg mg}^{-1} \cdot \text{h}^{-1}$). However, the IL-10 production in the *H. pylori*-infected mucosa was not affected by either NS-398 or indomethacin.

Neither NS-398 nor indomethacin increased the cytokine production in normal animals. Similar to the effects on gastric pathology, indomethacin had a stronger effect on *H. pylori*-induced CINC/KC and IFN-γ production than NS-398.

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**Fig. 3.** Histological appearance of pathological changes in the gastric mucosa of *H. pylori*-infected gerbils. Vehicle (control), 10 mg/kg NS-398, or 2 mg/kg indomethacin was administered for 4 wk to normal and *H. pylori*-infected gerbils. A: normal mucosa. B: *H. pylori*-infected mucosa in control animals. C: *H. pylori*-infected mucosa in NS-398-treated animals. D: *H. pylori*-infected mucosa in indomethacin-treated animals. *H. pylori*-induced neutrophil infiltration, lymphoid follicles, and epithelium damage were aggravated by NS-398 and indomethacin. Magnification, ×25.
In the present study, *H. pylori* infection for >4 wk induced COX-2 expression in the gastric mucosa of Mongolian gerbils. Recent studies with human gastric specimens also revealed that COX-2 expression is absent in normal mucosa but is profoundly induced in *H. pylori*-positive gastritis (22, 31, 33). In contrast, COX-1 protein was constitutively expressed in both normal and *H. pylori*-infected mucosa of gerbils. PGE$_2$ produc-
tion was elevated with COX-2 protein expression, and the increased production was significantly inhibited by NS-398 at 10 mg/kg, at which dose PGE₂ production in normal mucosa was not affected. These results indicate that COX-2 functionally contributes to the increased PGE₂ production in H. pylori-infected mucosa. Similarly, COX-1 also produces PGE₂ in H. pylori-infected mucosa, because 2 mg/kg indomethacin reduced PGE₂ production in normal mucosa and the inhibitory effect of 2 mg/kg indomethacin on PGE₂ production in H. pylori-infected mucosa was more potent than that of 10 mg/kg NS-398. Romano et al. (29) reported that adhesion of H. pylori on cultured gastric cancer MKN28 cells results in COX-2 mRNA expression. However, it is unclear whether the direct effect of H. pylori on gastric cells is crucial for COX-2 expression in vivo, because COX-2 protein was not expressed even at 2 wk of H. pylori infection in our model. COX-2 expression may result from H. pylori-associated mucosal inflammation, because 4 wk of H. pylori infection was required for gastritis and COX-2 expression. In fact, it is also known that H. pylori infection increases the expression of cytokines such as IL-1 and tumor necrosis factor-α, which serve as potent COX-2 inducers (13, 23, 37). Further investigation is needed to clarify the relationship between H. pylori infection and COX-2 induction in vivo.

In H. pylori infection, both bacterial and host factors are believed to contribute to gastric mucosal damage. Regarding host factors, it is suspected that inflammatory responses may be involved in H. pylori-induced gastritis (3, 5, 10, 43). Blaser (3) speculated that severe inflammatory responses may be involved in H. pylori-associated mucosal inflammation, i.e., PGs might exert anti-inflammatory effects in H. pylori-infected gastric mucosa. Consequently, aggravation of H. pylori-induced epithelial damage and mucosal erosions by the NSAIDs might result from potentiation of mucosal inflammation.

In response to H. pylori infection, mucosal defense mechanisms might be activated. Because PGs are important defensive factors in the gastric mucosa (9, 28), COX-2 induction is reasonable for protection of the mucosa against H. pylori. PGE₂ increases blood flow and secretion of mucus and bicarbonate, inhibits acid secretion, and directly protects gastric cells against toxic stimuli (9). We reported that H. pylori infection for 2 and 4 wk causes an increase in mucus synthesis in the gastric mucosa of gerbils and that both NS-398 and indomethacin suppress the increased mucus synthesis (35). The decreases in these PG defensive responses to H. pylori infection are suggested to contribute to aggravation of mucosal inflammation caused by COX inhibition.

In addition, downregulation of proinflammatory cytokine expression by PGs is also likely to be important for mucosal protection against H. pylori infection. In the patients with H. pylori-positive gastritis, the IL-8 level in the gastric mucosa is significantly higher than in ones with H. pylori-negative gastritis (6, 42). Similarly, in our gerbil model, CINC/KC production was induced by H. pylori infection. NS-398 and indomethacin significantly potentiated CINC/KC production in H. pylori-infected mucosa. CINC/KC belongs to the IL-8 chemokine family and is considered to play a crucial role in neutrophil infiltration in rodents because rodent counterparts of IL-8 have not been identified (40). The increased CINC/KC production accounts for enhancement of neutrophil infiltration by the NSAIDs in H. pylori-infected mucosa. These results suggest that PGs, derived from COX-2 as well as COX-1, downregulate CINC/KC production, resulting in suppression of neutrophil infiltration in H. pylori-infected mucosa.

Gastric mucosal T-lymphocyte response to H. pylori infection is dependent predominantly on type 1 helper T-lymphocytes, which are characterized as IFN-γ-secreting cells (8, 10, 19). Mohammadi et al. (25) reported that in vivo neutralization of IFN-γ by administration of anti-IFN-γ antibody causes a significant reduction of gastric inflammation induced by Helicobacter felis in mice. Irisawa et al. (17) reported that H. pylori-induced mucosal damage is augmented by the overexpression of IFN-γ in the stomach of mice. Furthermore, Sawai et al. (30) reported that there is no inflammatory pathology in the gastric mucosa of H. pylori-infected IFN-γ gene-deficient mice, whereas inflammatory cell infiltration and erosions are observed in the mucosa of H. pylori-infected wild-type mice. Consequently, it has been widely accepted that IFN-γ is one of the important causal factors for H. pylori-associated diseases. We also confirmed that H. pylori infection increases IFN-γ production in gerbils and found that the increase in IFN-γ production is potentiated by NS-398 and indomethacin. Similar to the case of CINC/KC production, these results suggest that PGs, derived from both COX-1 and COX-2, downregulate IFN-γ production, resulting in an attenuation of mucosal inflammation induced by H. pylori infection.

In contrast, IL-10 production was induced by H. pylori infection, as observed in human patients (42), but was not affected by NS-398 or indomethacin. IL-10 has been shown to inhibit the production of cytokines, including IL-8 and IFN-γ (2, 7). The failure to promote IL-10 level did not attenuate the NSAID-increased production of CINC/KC and IFN-γ in H. pylori-infected mucosa. In addition, the fact that NSAIDs had no effect on IL-10 production suggests that the NSAID-induced increases in CINC/KC and IFN-γ levels are due to the increased production and do not result from the inhibition of peptidase activity during culturing.

The number of viable H. pylori in the stomach was reduced by NS-398 and indomethacin, although there
were no significant differences between the drug-treated and control groups. This result indicates that the deleterious effects of the NSAIDs on the gastric mucosa do not result from the increase in the number of viable *H. pylori*. Caselli et al. (4) reported the similar result that, in *H. pylori*-positive patients with rheumatoid arthritis, the *H. pylori*-positive rate is significantly reduced by NSAID treatment compared with the non-treated group. To our knowledge, there have been no reports that NSAIDs possess an anti-*H. pylori* activity, and we confirmed that the minimal inhibitory concentration of indomethacin toward *H. pylori* is 50 μg/ml and is quite a bit higher than that of clarithromycin (0.06 μg/ml). An attenuation of mucus synthesis may be related to the decrease in *H. pylori* number. The gastric mucus layer is known to serve as the major habitat of *H. pylori*, and NSAIDs inhibit the increased mucus synthesis in *H. pylori*-infected mucosa of gerbils. Alternatively, the increase in IFN-γ production may result in the decrease in *H. pylori* number. In a recent study by Sawai et al. (30), the number of *H. pylori* in the stomach in IFN-γ gene-deficient mice was higher than that in wild-type mice, suggesting that IFN-γ plays a protective role in *H. pylori* infection. Interestingly, we found that IFN-γ, in concert with *H. pylori*, potently reduces mucus secretion by cultured gastric epithelial cells (36).

COX-2-selective inhibitors are expected as new NSAIDs without ulcerogenic effect. In fact, COX-2-selective NSAIDs such as nimesulide and celecoxib are clinically used as anti-inflammatory drugs, but the incidences of their gastric side effects are significantly lower than those of conventional NSAIDs. However, our results suggest the possibility that COX-2-selective NSAIDs may have an injurious effect on the gastric mucosa of *H. pylori*-positive patients. Wallace et al. (39) reported that, to achieve a desirable anti-inflammatory effect, COX-2-selective NSAIDs needed to be given at high doses in which both COX-1 and COX-2 activities are inhibited. If so, the safety of COX-2-selective NSAIDs may be lowered, especially in *H. pylori*-infected patients. Overall, independent of type of NSAID, NSAID users should be aware of these side effects if they are infected with *H. pylori*. In the case of application of COX-2-selective NSAIDs to preexisting gastric ulcers, the drugs also exert an unfavorable effect. We previously reported that gastric ulcer healing is significantly impaired by NS-398 at low doses in which COX-2 activity only is inhibited in rats (32).

We conclude that COX-2 as well as COX-1 has an anti-inflammatory effect in *H. pylori*-induced gastritis in Mongolian gerbils. COX-2-selective inhibitors may aggravate gastritis if used in *H. pylori*-positive patients.

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