Anti-inflammatory effect of two isoforms of COX in \textit{H. pylori}-induced gastritis in mice: possible involvement of PGE$_2$

Tetsuya Tanigawa, Toshio Watanabe, Masaki Hamaguchi, Eiji Sasaki, Kazunari Tominaga, Yasuhiro Fujiwara, Nobuhide Oshitani, Takayuki Matsumoto, Kazuhide Higuchi, and Tetsuo Arakawa  
Department of Gastroenterology, Osaka City University Graduate School of Medicine, Osaka 545-8585, Japan  
Submitted 24 March 2003; accepted in final form 28 August 2003

PGE$_2$ for 1 wk. selective COX-2 inhibitor NS-398 (10 mg/kg), or nonselective COX were given selective COX-1 inhibitor SC-560 (10 mg/kg), accompanied by a significant increase in PGE$_2$ production by gastric stomach. SC-560 augmented MPO activity and epithelial cell apoptosis in the tissue, a marker of neutrophil infiltration, and epithelial cell apoptosis in the stomach. SC-560 and NS-398 resulted in a stronger increase in MPO activity both COX-1 and -2 by indomethacin or concurrent treatment with indomethacin, a nonselective COX inhibitor as well as a selective COX-2 inhibitor and a nonselective COX inhibitor indomethacin (2 mg/kg) with or without 16,16-dimethyl PGE$_2$ for 1 wk. \textit{H. pylori} infection increased levels of mRNA for COX-1 and -2 in gastric tissue by 1.2-fold and 3.3-fold, respectively, accompanied by a significant increase in PGE$_2$ production by gastric tissue. \textit{H. pylori} infection significantly elevated MPO activity, neutrophil infiltration, and epithelial cell apoptosis in the stomach. SC-560 augmented MPO activity and epithelial cell apoptosis with associated reduction in PGE$_2$ production, whereas NS-398 had the same effects without affecting PGE$_2$ production. Inhibition of both COX-1 and -2 by indomethacin or concurrent treatment with SC-560 and NS-398 resulted in a stronger increase in MPO activity and apoptosis than inhibition of either COX-1 or -2 alone. \textit{H. pylori} infection elevated TNF-$\alpha$ mRNA expression in the stomach, which was further increased by indomethacin. Effects of COX inhibitors on neutrophil infiltration, apoptosis, and TNF-$\alpha$ expression in \textit{H. pylori}-infected mice were abolished by exogenous 16,16-dimethyl PGE$_2$. In conclusion, PGE$_2$ derived from either COX-1 or -2 is involved in regulation of gastric mucosal inflammation and contributes to maintenance of mucosal integrity during \textit{H. pylori} infection via inhibition of TNF-$\alpha$ expression.

neutrophil; tumor necrosis factor-$\alpha$; gastric mucosal integrity

\textit{HELIcobacter pylori} is a major cause of chronic gastritis and peptic ulcer diseases. In response to the bacterial pathogenetic factors, levels of various types of inflammatory mediators including cytokines and inducible enzymes are increased in gastric mucosa. Some of these mediators injure gastric mucosa, whereas others are involved in the regulation of host immune responses and gastric mucosal integrity.

PGs, which are synthesized by cyclooxygenase (COX) from arachidonic acid liberated from membrane phospholipids, have a variety of biological effects. In the stomach, PGs, especially PGE$_2$, play an important role in maintenance of gastric mucosal integrity via several mechanisms including regulation of gastric mucosal blood flow, kinetics of epithelial cells, synthesis of mucus, and inhibition of gastric acid secretion (44).

COX exists in at least two isoforms: 1) COX-1 is constitutively expressed in the gastrointestinal tract and most other organs, and 2) COX-2 is undetectable or expressed at very low levels in most tissues but is significantly induced at sites of inflammation such as \textit{H. pylori}-induced gastric mucosal inflammation (11, 13, 42). It is believed that COX-1 is regularly expressed to maintain gastric mucosal integrity, primarily under physiological conditions, as a housekeeping enzyme. In contrast, COX-2 has been reported to contribute to gastrointestinal mucosal defense and repair under certain pathophysiological conditions. COX-2 protects against noxious agents such as high concentrations of ethanol on gastric mucosa pretreated with a mild irritant (14, 27). Treatment with a selective COX-2 inhibitor delays healing of gastric and esophageal ulcers (2, 9, 37).

However, the role of COX-2 in \textit{H. pylori}-induced gastritis remains unclear. Some studies (12, 40) have reported that administration of a selective COX-2 inhibitor results in exacerbation of gastritis induced by \textit{H. pylori} in Mongolian gerbils. Another study (20) has shown that a selective COX-2 inhibitor reverses increases in infiltration of inflammatory cells into gastric mucosa and apoptosis of gastric epithelial cells in the stomach of \textit{H. pylori}-infected mice. Furthermore, it has been reported that inhibition of COX-2 did not affect gastric mucosal inflammation in Mongolian gerbils (5). Thus, the role of COX-2 in \textit{H. pylori}-induced gastritis is still unclear. Similarly, the involvement of COX-1 in \textit{H. pylori}-induced gastritis has yet to be definitively clarified, although there is some evidence suggesting involvement of COX-1 in the regulation of \textit{H. pylori}-induced gastritis. In \textit{H. pylori}-infected Mongolian gerbils, the stimulative effect of indomethacin, a nonselective COX inhibitor, on inflammatory response in gastric mucosa was more potent than that of a selective COX-2 inhibitor (40).

Gastric mucosal PGE$_2$ production is higher in \textit{H. pylori}-positive patients than in \textit{H. pylori}-negative patients (1, 15, 18), and this increase has been demonstrated to be responsible for decreased secretion of gastric acid. However, the contribution of each of the COXs to increase in PGE$_2$ production is still unclear, and roles of PGE$_2$ in the gastric mucosal inflammation induced by \textit{H. pylori} have not been elucidated in detail.

The present study aimed to elucidate the precise roles of two COX isoforms in the gastric mucosal inflammation induced by \textit{H. pylori} by determining the effects of a selective COX-1 inhibitor as well as a selective COX-2 inhibitor and a nonselective...
lective COX inhibitor on gastritis induced by *H. pylori* infection in mice. In addition, to clarify the role of PGE2 in gastric mucosal inflammation induced by *H. pylori*, we assessed the effect of oral administration of PGE2 on *H. pylori*-induced gastritis in mice treated with COX inhibitors.

**MATERIALS AND METHODS**

*Animals.* Specific pathogen-free female C57BL/6 mice (4 wk old, 10–15 g) were obtained (Charles River Japan, Atsugi, Japan). Animals were housed in polycarbonate cages with paper chip bedding in an air-conditioned biohazard room with a 12-h light-dark cycle. All animals had free access to food and water. All experimental procedures were approved by the Animal Care Committee of the Osaka City University Graduate School of Medicine.

*H. pylori preparation and inoculation of mice.* The *H. pylori* Sydney Strain (SS)-1 (cagA+ and vacA+), which readily colonizes the stomach and induces gastritis in C57BL/6 mice (23), was used in this study. *H. pylori* was grown to a concentration of \( 2 \times 10^8 \) colony-forming units (CFUs)/ml in Brucella broth (BBL, Cockeysville, MD) containing 10% sterile heat-inactivated horse serum. The flasks were incubated at 37°C for 24 h under microaerophilic conditions with agitation on a rotary shaker at 160 rpm. Mice were orally given 0.3 ml of the broth culture of *H. pylori* (6 \( \times \) 10^5 CFUs/animal) with a feeding needle after being fasted for 18 h on three successive occasions within a 5-day period. Animals of the uninfected groups were administered broth medium alone.

*Experimental design.* Four months after inoculation, mice with or without inoculation of *H. pylori* were given COX-1 selective inhibitor SC-560 (10 mg/kg; Cayman Chemical, Ann Arbor, MI), COX-2 selective inhibitor NS-398 (10 mg/kg; Cayman Chemical), SC-560 (10 mg/kg) together with NS-398 (10 mg/kg), nonselective COX inhibitor indomethacin (2 mg/kg; Sigma, St. Louis, MO), or vehicle (1% carboxymethyl cellulose solution, Wako Pure Chemical Industries, Osaka, Japan) alone subcutaneously once a day for 1 wk. Additionally, some mice treated with each of the COX inhibitors were orally given 16,16-dimethyl PGE2 (16,16-dmPGE2; Cayman Chemical) at a dose of 5–100 \( \mu \)g/kg twice a day for 1 wk. Animals were killed under ether anesthesia 4 h after final administration of drugs. Their stomachs were incised along the greater curvature and cut into fragments. Samples of gastric tissue were immediately frozen in liquid nitrogen and stored at \(-80^\circ\)C.

*Determination of selectivity of COX inhibitors.* A dose of 10 mg/kg of SC-560 or NS-398 had been shown to selectively inhibit the activity of COX-1 or COX-2, respectively (3, 13, 17, 25, 26, 39). In this study, we confirmed that doses of SC-560 and NS-398 produced significant and selective inhibition of COX-1 and COX-2, respectively.

Blood thromboxane (TX) production is an index of COX-1 activity (30), whereas the PGE2 in the exudates of carrageenan-induced air pouch inflammation is almost entirely derived from COX-2 (45). Therefore, we measured the levels of blood TX and PGE2 in the inflammatory exudates to determine the selectivity and effectiveness of COX inhibitors. Blood was collected 4 h after the administration of the tested drugs and was incubated at 37°C for 45 min and then centrifuged. The concentration of TXB2 was measured by enzyme immunoassay (TXB2 EIA kit; Cayman Chemical).

Carrageenan (1.5 ml of a 1% solution) was injected into the air pouch of mice 1 h after the administration of the tested drugs. Six hours later, the exudates were collected, and PGE2 concentration was measured by enzyme immunoassay (PGE2 EIA kit, Cayman Chemical).

*Determination of *H. pylori* colonization of the stomach.* Specimens were weighed and homogenized with 0.3 ml of PBS, pH 7.6, and further diluted, and aliquots (0.1 ml) were inoculated onto Helicobacter-selective agar plates (plate Helicobacter agar; Nissui Pharmaceutical, Tokyo, Japan) and incubated at 37°C for 7 days under microaerophilic conditions. Bacterial colonies were identified by the rapid urease test (CLO test; Ballard Medical Products, Draper, UT) and gram stained for morphology. The number of colonies was counted, and viable *H. pylori* expressed as log CFUs per gram tissue.

*Determination of expression of COX-1, COX-2, and TNF-α mRNA in gastric tissue by real-time quantitative RT-PCR.* Total RNA was isolated from gastric tissue by using an ISOGEN kit (Nippon Gene, Tokyo, Japan) according to the manufacturer’s protocol. After precipitation, the RNA was resuspended in RNase-free Tris/HCl EDTA buffer and concentration was measured by absorbance at a wavelength of 260 nm.

PCR primers and TaqMan probes for COX-1, COX-2, and TNF-α were designed by using Primer Express, a software program (PE Applied Biosystems, Foster City, CA). TaqMan probes were labeled with a reporter fluorescent dye (6-carboxyfluorescein) at the 5’ end and a fluorescent dye quencher (6-carboxytetramethylrhodamine) at the 3’ end. For the mouse COX-1, the sense primer was 5'-CAT-CAAGGAGTCCGAGAGAT-3’, the antisense primer was 5’-TA-AGGCTTCAAGC8AAACCTC-3’, and the TaqMan probe was 5’-CGCCTACAGCCCTTCAATACCCA-3’. For mouse COX-2, the sense primer was 5'-AAGCCCTCTCACTGTACATCGA-3’, the antisense primer was 5’-ATGGTCTCCCAAAAGTAAGCAT-3’, and the TaqMan probe was 5’-CTGGTCTGGGAAATGCTCCTCCA-3’. For mouse TNF-α, the sense primer was 5’-CATGACACCATCATAAGGA-3’, the antisense primer was 5’-GAGGCCAACCCT-GACCACCTCCTC-3’, and the TaqMan probe was 5’-AATGCGTCTC- TCGAATTACTCGGAGC-3’. Real-time quantitative RT-PCR analyses were performed by using an ABI PRISM 7700 Sequence Detection System instrument and software (PE Applied Biosystems). The reaction mixture was prepared according to the manufacturer’s protocol by using the Platinum qRT-PCR ThermoScript One-Step System (Invitrogen, Carlsbad, CA). Thermal cycling conditions comprised 50°C for 30 min and 95°C for 5 min, followed by 45 cycles of amplification at 95°C for 15 s and 60°C for 1 min. Total RNA was subjected to real-time quantitative RT-PCR for measurement of target genes and GAPDH as an internal standard using TaqMan GAPDH control reagents (PE Applied Biosystems). Expressions of mRNAs for COX-1, COX-2, and TNF-α in gastritis tissue and normal gastric tissue were standardized to GAPDH mRNA, and mRNA levels of COX-1 and COX-2, and TNF-α in gastritis tissue are expressed as ratios to the mean value for normal gastric tissue.

*Western blot analysis.* Expression of COX-1 and COX-2 proteins was examined by Western blotting. Gastric tissues were homogenized and lysed on ice in buffer containing 0.5% Nonidet P-40, 40 mM Tris/HCl, pH 8.0, 120 mM NaCl, 1 mM PMSF, and 10 μg of leupeptin per milliliter. Protein of the lysate was measured with a modified bicinchoninic acid method (BCA) protein assay reagent kit, (Pierce, Rockford, IL). Proteins were denatured with SDS sample buffer and subjected to 10% SDS-PAGE and transferred to the polyvinylidene difluoride membrane. Membranes were blocked in 5% skim milk for 1 h and then incubated with anti-COX-1, anti-COX-2, and anti-TNF-α monoclonal antibodies at 4°C overnight.

Table 1. Colonization of Helicobacter pylori in the stomach of mice four months after inoculation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Colonies, log CFUs/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5.36 ± 0.16</td>
</tr>
<tr>
<td>SC-560 (10 mg/kg)</td>
<td>5.61 ± 0.14</td>
</tr>
<tr>
<td>NS-398 (10 mg/kg)</td>
<td>5.22 ± 0.16</td>
</tr>
<tr>
<td>SC-560 (10 mg/kg) + NS-398 (10 mg/kg)</td>
<td>4.93 ± 0.16</td>
</tr>
<tr>
<td>Indomethacin (2 mg/kg)</td>
<td>4.76 ± 0.26</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 8–10. Test drugs or vehicle were administered subcutaneously for 1 wk. Pieces of stomach were weighed and homogenized with 0.3 ml PBS and further diluted. Aliquots were inoculated onto Helicobacter-selective agar plates and incubated at 37°C for 7 days under microaerophilic conditions. Number of colonies was counted, and viable *H. pylori* expressed as log CFUs/g tissue.
TBS buffer (10 mM Tris·HCl, pH 7.5, 100 mM NaCl, 0.1% Tween-20) containing 5% BSA and were then incubated with the specific polyclonal antibody against COX-1 and COX-2 (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:1,000 overnight. The bound antigen-antibody complexes were detected with anti-rabbit IgG-HRP by using enhanced chemiluminescence in accordance with the manufacturer’s instructions (Amersham, Arlington Heights, IL).

**Determination of PGE2 production by gastric tissue.** PGE2 production by gastric tissue was assessed by using a vortex method, developed from that originally described by Whittle et al. (47). Each specimen was minced with a clean pair of scissors in 2.0-ml polypropylene tubes containing 1 ml of Tris·HCl buffer (150 mM, pH 8.2) for 1 min, and then centrifuged at 9,000 rpm for 40 s. Supernatant was discarded, and 1.5 ml of fresh Tris·HCl buffer was added. Tubes were then simultaneously vortexed for 1 min, and 30 µl of 10 mM indomethacin (Sigma) was added immediately to inhibit excessive COX activity. Tubes were centrifuged at 9,000 rpm for 40 s. Supernatants were collected and stored at −80°C. The amount of PGE2 in the supernatant was determined by enzyme immunoassay (PGE2 EIA kit, Cayman Chemical). PGE2 production was expressed as nanograms of PGE2 per gram of tissue per 1 minute.

**Measurement of myeloperoxidase activity.** MPO activity of gastric tissue, a marker of neutrophil infiltration (21), was assayed by the method of Bradley et al. (6). In brief, the specimens were homogenized in 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (Wako Pure Chemical Industries). Each suspension was then centrifuged and MPO in the resulting supernatant was assayed with a spectrophotometer (Beckman Instruments, Fullerton, CA). One unit of MPO activity was defined as that degrading 1 µmol of peroxide per minute at 25°C. Proteins were measured with a modified BCA method (BCA protein assay reagent kit; Pierce, Rockford, IL). Results are expressed as units per gram tissue protein.

**Determination of apoptosis of gastric epithelium.** Rabbit polyclonal antibody to single-stranded DNA (ssDNA) was used for

---

**Fig. 1.** Effect of Helicobacter pylori infection on expression of COX-1 and COX-2 in gastric tissue of mice. Expression of COX-1 and COX-2 mRNA in gastric tissue was determined by real-time quantitative RT-PCR (A and B). mRNA level is expressed as a ratio to the mean value for gastric tissue of H. pylori-uninfected mice treated with vehicle. Expression of COX-1 and COX-2 proteins was determined by Western blot analysis (C). Each column represents the mean ± SE. #P < 0.05 vs. H. pylori-uninfected mice treated with vehicle; n = 8–10.

**Fig. 2.** Effects of COX inhibitors on blood thromboxane B2 production and inflammatory PGE2 production. Production of blood thromboxane B2 and inflammatory PGE2 in carrageenan-air pouch model was assessed by enzyme immunoassay. SC, SC-560 (10 mg/kg); NS, NS-398 (10 mg/kg); SC + NS; SC-560 (10 mg/kg) together with NS-398 (10 mg/kg); IM, indomethacin (2 mg/kg). #P < 0.001 vs. vehicle-treated group; n = 8–10.

**Fig. 3.** Effects of COX inhibitors on PGE2 production by gastric tissue in H. pylori-uninfected mice. Test drugs or vehicle were administered subcutaneously for 1 wk as in Fig. 2. PGE2 production by gastric tissue in mice was determined by enzyme immunoassay. Each column represents the mean ± SE. **P < 0.001 vs. vehicle-treated mice; n = 8–10.
Gastritis induced by *H. pylori* in mice. All mice inoculated with broth culture of *H. pylori* were successfully infected, as assessed by bacterial culture. About $10^8$ viable bacteria per gram tissue were present in the stomach of mice inoculated with *H. pylori*. None of the COX inhibitors affected the viability of *H. pylori* (Table 1).

Macroscopically, edematous thickening of gastric mucosa was observed in *H. pylori*-infected mice, especially in the fundus near the antrum. None of the COX inhibitors caused macroscopic lesions such as hemorrhagic erosions in these mice. Histologically, infiltration of neutrophils and mononuclear cells in the lamina propria and hyperplastic change of the gastric glands were observed in infected animals.

**Effect of *H. pylori* infection on expression of COX-1 and COX-2 in gastric mucosa of mice.** As shown in Fig. 1A, the level of COX-1 mRNA in gastric tissue was higher in *H. pylori*-infected mice than in uninfected mice, although the difference between the two groups did not reach statistical significance ($P = 0.079$ vs. *H. pylori*-uninfected mice treated with vehicle). The level of COX-2 mRNA significantly increased in *H. pylori*-infected mucosa (Fig. 1B, $P < 0.001$ vs. *H. pylori*-uninfected mice treated with vehicle).

COX-1 proteins in the gastric mucosa were expressed in mice with and without *H. pylori* infection, and the levels of COX-1 expression were not different between the two groups (Fig. 1C). COX-2 protein was not detected in the gastric mucosa of *H. pylori*-uninfected mice by Western blotting. *H. pylori* infection induced COX-2 protein in gastric mucosa, but the level of COX-2 expression was low.

**RESULTS**

**Fig. 4.** Effects of COX inhibitors on PGE$_2$ production by gastric tissue in *H. pylori*-infected mice. Test drugs or vehicle were administered subcutaneously for 1 wk as in Fig. 2. PGE$_2$ production by gastric tissue was determined by enzyme immunoassay. Each column represents the mean ± SE. *#P < 0.05 vs. *H. pylori*-uninfected mice treated with vehicle; **P < 0.001 vs. *H. pylori*-infected mice treated with vehicle; n = 8–10.

Fig. 5. Effects of COX inhibitors on gastric epithelial cell apoptosis in mice infected or uninfected with *H. pylori*. Test drugs or vehicle were administered subcutaneously for 1 wk in the body (A) or antrum (B) as in Fig. 2. Gastric epithelial cell apoptosis was evaluated by immunohistochemical staining for single-stranded DNA (ssDNA). For each mouse, 50 well-oriented, representative gastric glands were scored in a tissue section, and the number of positive cells per gland was used as an index of apoptosis.

**Statistical analysis.** Values are expressed as means ± SE. Sample groups for multiple-treatment experiments were analyzed by using Kruskal-Wallis one-way ANOVA, followed by pairwise comparisons of treatments. When two independent sample groups were compared, data were analyzed by using the Mann-Whitney U-test with Bonferroni correction. Differences with *P* values < 0.05 were considered significant.
Selectivity of COX inhibitors. SC-560 at a dose of 10 mg/kg inhibited blood TX production by 79%, but had no effect on PGE₂ production in the carrageenan-air pouch model (Fig. 2). In contrast, NS-398 at a dose of 10 mg/kg did not affect blood TX synthesis, but inhibited inflammatory PGE₂ production by 60%. Thus the doses of SC-560 and NS-398 selectively inhibited COX-1 and COX-2, respectively. Indomethacin (at a dose of 2 mg/kg) caused marked reduction of both blood TX production and inflammatory PGE₂ production by 98 and 85%, respectively.

Effect of *H. pylori* infection and administration of COX inhibitors on PGE₂ production by gastric tissue in mice. PGE₂ production by gastric tissue of *H. pylori*-uninfected mice was 334.6 ± 39.3 ng·g tissue⁻¹·min⁻¹ (Fig. 3). SC-560 inhibited gastric PGE₂ production by 67% (*P* < 0.001 vs. *H. pylori*-uninfected mice treated with vehicle). In contrast, NS-398 did not affect gastric PGE₂ production. Concurrent treatment with SC-560 and NS-398 reduced PGE₂ production to the same level as in animals given SC-560 alone. Administration of indomethacin nearly abolished PGE₂ production, with inhibition of 96% (*P* < 0.001 vs. *H. pylori*-uninfected mice treated with vehicle).

*H. pylori* infection significantly increased PGE₂ production by 1.3-fold (*P* = 0.036 vs. *H. pylori*-uninfected mice treated with vehicle). Similarly, SC-560, concurrent treatment with SC-560 and NS-398, and indomethacin inhibited gastric PGE₂ production of *H. pylori*-infected mice by 68, 54, and 93%, respectively. Increased production of PGE₂ by *H. pylori*-infection was not affected by NS-398 (Fig. 4).

Effect of COX inhibitors on MPO activity in gastric mucosa of mice. Treatment with each of the COX inhibitors resulted in slight, but not significant, increase in MPO activity in the gastric mucosa of uninfected animals. *H. pylori* infection significantly elevated MPO activity by 2.1-fold (*P* = 0.02 vs. *H. pylori*-uninfected mice treated with vehicle). In *H. pylori*-infected mice, administration of either SC-560 or NS-398 significantly elevated MPO activity by 2.5-fold and 2.8-fold, respectively (*P* = 0.001 and = 0.011 vs. *H. pylori*-infected mice treated with vehicle, respectively). Concurrent treatment with SC-560 and NS-398 further increased MPO activity to the same extent as indomethacin, the increases in MPO activity being 3.8-fold and 3.9-fold in the groups given SC-560 together with NS-398 and indomethacin, respectively (*P* < 0.001 vs. *H. pylori*-infected mice treated with vehicle).

Effect of *H. pylori* infection and COX inhibitors on gastric epithelial cell apoptosis in mice. In *H. pylori*-uninfected mice, a few epithelial cells were stained for ssDNA, with indices of apoptosis in the body and antrum of the stomach of 1.1 ± 0.4/gland and 1.1 ± 0.2/gland, respectively. None of the COX inhibitors affected gastric epithelial cell apoptosis in uninfected animals.

The number of apoptotic cells in the body of the stomach was significantly increased by *H. pylori* infection. SC-560 and NS-398 each significantly increased the number of apoptotic cells in the antrum and body of the stomach. Concurrent treatment with SC-560 and NS-398 as well as indomethacin further increased the number of apoptotic cells, especially in the body of the stomach (Fig. 5).

Fig. 6. Histological findings of gastric mucosa in *H. pylori*-infected mice given indomethacin with or without prostaglandin E₂. *H. pylori* infection induced infiltration of inflammatory cells including neutrophils and mononuclear cells in the lamina propria (A). Treatment with indomethacin enhanced this inflammatory response (B), which was counteracted by oral administration of 16,16-dmPGE₂ (C).
Effect of treatment with 16,16-dmPGE₂ on enhancement of gastric inflammatory response by COX inhibitors in *H. pylori*-infected mice. A: effect of exogenous administration of 16,16-dmPGE₂ on increases in MPO activity in gastric tissue in mice treated with COX inhibitors. Test drugs or vehicle were administered subcutaneously for 1 wk as in Fig. 2, and 16,16-dmPGE₂ (5 μg/kg) was administered orally for 1 wk. MPO activity in gastric tissue was assayed by the method of Bradley et al. (6). Each column represents the mean ± SE. #P < 0.05 vs. *H. pylori*-uninfected mice treated with vehicle; *P < 0.05 and **P < 0.001 vs. *H. pylori*-infected mice treated with vehicle; n = 8–10.

B: indomethacin (2 mg/kg) or vehicle was administered subcutaneously and 16,16-dmPGE₂ (5–100 μg/kg) or vehicle was administered orally for 1 wk. Each column represents the mean ± SE. #P < 0.05 vs. *H. pylori*-uninfected mice treated with vehicle; *P < 0.05 vs. *H. pylori*-infected mice treated with vehicle; n = 8–10.

C: effect of 16,16-dmPGE₂ on TNF-α mRNA expression in gastric tissue of indomethacin-treated mice infected with *H. pylori*. Indomethacin (2 mg/kg) or vehicle was administered subcutaneously, and 16,16-dmPGE₂ (5–100 μg/kg) or vehicle was administered orally for 1 wk. Expression of TNF-α was determined by real-time quantitative RT-PCR. mRNA level is expressed as a ratio to the mean value of gastric tissue of *H. pylori*-uninfected mice treated with vehicle. Each column represents the mean ± SE. #P < 0.05 vs. *H. pylori*-uninfected mice treated with vehicle; *P < 0.05 vs. *H. pylori*-infected mice treated with vehicle; n = 8–10.

D: effect of 16,16-dmPGE₂ on gastric epithelial cell apoptosis in the body of the stomach in indomethacin-treated mice infected with *H. pylori*. Indomethacin (2 mg/kg) or vehicle was administered subcutaneously and 16,16-dmPGE₂ (5–100 μg/kg) or vehicle was administered orally for 1 wk. Gastric epithelial cell apoptosis was evaluated by immunohistochemical staining for ssDNA. Apoptosis was expressed by an index of apoptosis, defined as the number of positive cells per 50 glands. Each column represents the mean ± SE. #P < 0.05 vs. *H. pylori*-uninfected mice treated with vehicle; *P < 0.05 vs. *H. pylori*-infected mice treated with vehicle; n = 8–10. Test drugs or vehicle were administered subcutaneously for 1 wk. The pieces of the stomach were weighed and homogenized with 0.3 ml of PBS and further diluted, and aliquots were inoculated onto *Helicobacter*-selective agar plates and incubated at 37°C for 7 days under microaerophilic conditions. Number of colonies was counted, and viable *H. pylori* was expressed as log colony-forming units per gram tissue. Values are means ± SE; n = 8–10.

Effect of treatment with 16,16-dmPGE₂ on enhancement of gastric inflammatory response by COX inhibitors in *H. pylori*-infected mice. Histologically, infiltration of inflammatory cells in the lamina propria induced by *H. pylori* infection was enhanced by the COX inhibitors, which was counteracted by oral administration of 16,16-dmPGE₂ (Fig. 6).

In *H. pylori*-infected mice, oral administration of 16,16-dmPGE₂ at 5 μg/kg attenuated the increase in MPO activity in gastric tissue by treatment with any of the COX inhibitors (Fig. 7A).

In indomethacin-treated mice, administration of 16,16-dmPGE₂ at doses of 5, 20, and 100 μg/kg diminished increases in MPO activity and TNF-α mRNA levels by 28, 69, and 66%, respectively and 55, 73, and 76%, respectively (Fig. 7B and C). Similarly, administration of 16,16-dmPGE₂ at doses of 5, 20, and 100 μg/kg diminished increases in number of gastric
epithelial apoptotic cells in a dose-dependent manner (Fig. 7D).

DISCUSSION

Some clinical and experimental studies have demonstrated that nonsteroidal anti-inflammatory drugs enhanced gastric mucosal inflammation and injury in *H. pylori*-associated gastritis (7, 10, 34, 36, 48). However, the factors responsible for these drug’s effects are unclear. In the present study, we have demonstrated that inhibition of either COX-1 or COX-2 enhanced inflammatory responses including neutrophil infiltration and TNF-α expression in *H. pylori*-infected mice. Administration of PGE 2 counteracted these effects of COX inhibitors, resulting in reduction of apoptosis in gastric epithelial cells. These results suggest that both COX-1 and COX-2 may play crucial roles in maintenance of gastric mucosal integrity in *H. pylori*-induced gastritis via regulation of inflammation, and that PGE 2 derived from COXs is a key mediator of the anti-inflammatory effects of COXs.

*H. pylori*-induced gastritis is histologically characterized by infiltration of inflammatory cells including neutrophils into gastric mucosa (9). TNF-α has been suggested as one of the key proinflammatory cytokines promoting neutrophil infiltration into gastric mucosa induced by *H. pylori* infection (8, 24, 32). In the present study, inhibition of COX aggravated neutrophil infiltration, accompanied by increase in expression of TNF-α mRNA induced by *H. pylori* infection. We found that this effect of COX inhibitors was abolished by oral administration of 16,16-dmPGE 2. This finding indicates that PGs regulate neutrophil infiltration caused by *H. pylori* infection through suppressing expression of TNF-α. PGs are reported to have anti-inflammatory effects; PGE 2 inhibits production of TNF-α and its mRNA by monocytes or macrophages stimulated by *Escherichia coli* lipopolysaccharide (22) or water extract of *H. pylori* (41). PGE 2 may therefore attenuate excessive host-inflammatory responses to *H. pylori* and minimize gastric mucosal injury.

Previous studies (12, 40) demonstrated that inhibition of COX-2 enhanced gastric mucosal injury induced by *H. pylori* infection in animal experimental models. In physiological conditions, suppression of both COX-1 and COX-2 is required for induction of gastric injury and inhibition of COX-2 contributes to neutrophil adherence in mesenteric venules, one of the steps of neutrophil infiltration into gastric mucosa (46). In the current study, we demonstrated that inhibition of COX-1 as well as COX-2 enhanced neutrophil infiltration into gastric mucosa in *H. pylori*-infected mice, and that inhibition of both COX-1 and COX-2 resulted in stronger enhancement of neutrophil infiltration than inhibition of either of the two COXs alone, suggesting that COX-1 and COX-2 independently or cooperatively contribute to the regulation of gastric mucosal inflammation in *H. pylori*-induced gastritis through PGE 2 production.

In the present study, inhibition of COX-1 enhanced neutrophil infiltration, and was accompanied by a reduction of PGE 2 production by gastric tissue in *H. pylori*-infected mice. In contrast, inhibition of COX-2 enhanced neutrophil infiltration without reducing gastric PGE 2 production. NS-398 at the same dose used in the present study inhibited PGE 2 production by 90% in gastric ulcerated tissue in which there is a marked increase in COX-2 expression (Watanabe, unpublished data). We demonstrated induction of COX-2 in *H. pylori*-infected gastric mucosa by RT-PCR and Western blotting, but the level of COX-2 expression assessed by Western blotting was low. Therefore, a possible explanation for these results is that COX-1 contributes much more than COX-2 to gastric PGE 2 production, and that reduction of PGE 2 by COX-2 inhibitors cannot be detected with present assay methods. Similar findings have been observed in other experimental and clinical studies (4, 14, 15, 27, 46). Nevertheless, the present study and other previous reports (14, 27) suggest that local expression of COX-2 plays an important role in maintenance of gastric mucosal integrity.

Previous clinical studies have reported 1) induction of apoptosis by *H. pylori* infection in patients with gastritis (16), and 2) the extent of gastric epithelial cell apoptosis is correlated with the grade of gastritis (24). We found that inhibition of COXs was associated with increases in neutrophil infiltration and gastric epithelial cell apoptosis in *H. pylori*-infected mice. Various types of inflammatory mediators are involved in gastric epithelial cell apoptosis in *H. pylori* infection. Free radicals and elastase derived from neutrophils and TNF-α are thought to be the key factors in gastric epithelial cell apoptosis in *H. pylori*-induced gastritis (19, 28, 49). These results suggest that increases in neutrophil infiltration and/or TNF-α expression may be responsible for enhancement of gastric epithelial cell apoptosis by COX inhibitors in *H. pylori*-infected mice.

Previous in vitro studies demonstrated that overexpression of COX-2 inhibits apoptosis (43), and *H. pylori* can induce COX-2 in gastric epithelial cells, which is dependent on genes within the cag pathogenicity island (35). Additionally, apoptosis of gastric epithelial cells in patients infected with cagA + *H. pylori* strains was significantly decreased compared with that in patients infected with cagA – strains (33). Because the *H. pylori* SS-1 strain used in the present study possesses cagA, these findings support our notion that COX-2 induced by cagA + *H. pylori* strain plays an important role in prevention of gastric epithelial apoptosis.

Contrary to the present findings, Kim et al. (20) reported that indomethacin and NS-398 decreased the gastric inflammation and epithelial cell apoptosis induced by *H. pylori* infection in mice. This discrepancy might be due to differences among the studies in the duration of *H. pylori* infection, the period of treatment with COX inhibitors, and the methods of assessment used.

In summary, COX inhibitors aggravate neutrophil infiltration and epithelial cell apoptosis in gastric mucosa in *H. pylori*-infected mice. Our findings indicate that PGE 2 derived from either COX-1 or COX-2 has an anti-inflammatory effect on *H. pylori*-induced gastritis, possibly by suppressing the expression of TNF-α. Although clinical studies have suggested that selective COX-2 inhibitors cause less gastric injury than nonselective COX inhibitors (17, 38), selective COX-2 inhibitors may impair gastric mucosal integrity in some pathophysiological conditions such as *H. pylori* infection.

REFERENCES

COX AND PG IN H. PYLORI-INDUCED GASTRITIS


10. Lehmann FS, Terracciano L, Carena I, Baeriswyl C, Drewe J, Tor-