Involvement of cyclooxygenase-2 in hyperplastic gastritis induced by Helicobacter pylori infection in C57BL/6 mice

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SUMMARY
Background and aims: The hyperplastic changes observed in Helicobacter pylori-associated gastritis have been considered to increase the risk of gastric cancer. The aim of this study was to determine whether cyclooxygenase-2 is involved in the hyperplastic changes in mice infected with H. pylori.
Methods: Seven-week-old, male C57BL/6 mice (n = 40) were inoculated with the Sydney strain of H. pylori. Control mice (n = 40) were treated with vehicle only. Half of the infected and control mice were fed an experimental diet containing etodolac (10 mg/kg/day) from 1 week after inoculation until the end of the experiment. The thickness of gastric pits, COX-2 mRNA and protein levels, and prostaglandin E2 (PGE2) levels in the gastric mucosa were determined before and 12, and 24 weeks after inoculation.
Results: The thickness of gastric pits, COX-2 mRNA and protein levels, and PGE2 levels were significantly increased at 24 weeks after inoculation of H. pylori compared with the control groups. Treatment with etodolac resulted in significant decreases in PGE2 production and in the thickness of gastric pits in the infected groups at 24 weeks after inoculation.
Conclusions: Our findings suggest that COX-2 is involved in the development of hyperplastic gastritis caused by H. pylori infection via the production of PGE2.

INTRODUCTION
Helicobacter pylori infection is associated with many gastroduodenal disorders such as chronic gastritis, peptic ulceration, gastric mucosa-associated lymphoid tissue lymphoma, and gastric carcinoma.1–5 However, the precise pathological process involved in the development of these disorders remains unclear. It has been reported that H. pylori increases proliferation of gastric epithelial cells and curing of the infection returns cell proliferation to normal levels.6–8 The hyperplastic changes observed in H. pylori-induced gastritis are thought to be associated with pre-cancerous changes.9,10 The mechanisms involved in these changes are not yet clear.

Cyclooxygenase (COX) is the rate-limiting enzyme in the production of prostanoids [prostaglandins (PGs) and thromboxanes] from arachidonic acid. Several recent studies have confirmed that there are two forms of cyclooxygenase: a constitutively produced COX-1 and inducible COX-2.11–13 COX-1 is thought to participate in the production of tissue prostaglandin under normal physiological conditions, whereas COX-2 is induced by cytokines, growth factors and tumour promoters, and its expression has been characterized as an immediate–early response in inflammation.14–16 Recently, elevated COX-2 expression has been demonstrated in human and rodent colorectal adenomas and carcinomas.17–20 Importantly, inhibition of COX by non-steroidal anti-inflammatory drugs (NSAIDs) such as sulindac and piroxicam results in both regression of neoplastic...
polyps and prevention of their development in individuals with familial adenomatous polyposis, as well as in murine models of this disease such as the Min mouse and APC knockout mouse. In humans, increased COX-2 expression is observed in H. pylori-induced gastritis and gastric adenocarcinomas. Epidemiological studies have revealed that the use of aspirin and other NSAIDs decreases the risk of gastric cancer. However, limited information is available regarding the role of COX-2 in such H. pylori-associated disorders.

Recently, an H. pylori-infected C57BL/6 mouse model was established using the Sydney strain of H. pylori isolated from a human subject. In this model, a large number of bacteria are visible on gastric mucosa, as observed in H. felis-infected animals and humans infected with H. pylori. Chronic active gastritis are also observed after H. pylori inoculation. On the basis of the backgrounds, as discussed above, we examined whether COX-2 is involved in the development of hyperplastic gastritis in this H. pylori-infected C57BL/6 mouse model.

MATERIALS AND METHODS

Animals

Ninety, 7-week-old specific pathogen-free male C57BL/6 mice with an average body weight of 25 g were purchased from Seiwa Experimental Animal Ltd (Fukuoka, Japan) and housed in polycarbonate cages in isolators under a 12-h light/12-h dark cycle. The animals used in this study were cared for in accordance with our institutional guidelines.

Bacteria

The strain SS1 of H. pylori was kindly provided by Lee et al., who originally established the H. pylori-induced C57BL/6 mouse gastritis model. This strain is positive for both cytotoxin-associated protein (CagA) and vacuolating cytotoxin (VacA). The bacteria were inoculated onto plated agar media supplemented with vancomycin (10 mg/L), polymyxin B (2500 I.U./L) and amphotericin B (2 mg/L) for isolation of H. pylori (Poamedia, Eikenkagaku Co., Ltd, Tokyo, Japan). The plates were incubated under microaerobic conditions at 37 °C for 4–5 days. Colonies were then transferred into Brucella broth (BBL, Becton Dickinson and Co., Cockeysville, MD) liquid medium supplemented with 10% horse serum and incubated for a further 24 h with agitation under the same conditions as described above.

Bacterial inoculation

The culture of H. pylori (0.5 mL, 2.5 × 10^8 colony-forming units) was administered orally to C57BL/6 mice three times in a 5-day period using a metal stomach catheter. The control groups were inoculated with Brucella broth liquid medium without H. pylori.

Treatment of C57BL/6 mice with inhibitor of COX-2 and experimental protocol

Etodolac was used as a selective COX-2 inhibitor in this study. The selectivity of etodolac for COX-2:COX-1 is 10:1. The experimental diet was prepared monthly by mixing etodolac 10 mg/kg/day with the pellet diet and stored at 4 °C until feeding. Eighty C57BL/6 mice were randomly distributed into uninfected (H. pylori-negative) or infected (H. pylori-positive) groups (40 for each group). Both control and experimental groups were divided into vehicle-treated sub-groups (H. pylori-negative/etodolac-negative, H. pylori-positive/etodolac-negative) and etodolac-treated groups (H. pylori-negative/etodolac-positive, H. pylori-positive/etodolac-positive). The etodolac-treated groups were administered etodolac diet from 1 week after inoculation. The regimen was continued until the end of the experiment. Ten mice in each group were weighed and sacrificed under anaesthesia with ether before, or 12 and 24 weeks after inoculation. The stomach was excised and weighed. It was then cut along the greater curvature, and the surface of the mucosa was inoculated onto the H. pylori isolation agar plate as described above. The plates were incubated for 4–5 days at 37 °C under the same conditions as described above. The bacterium cultured was identified as H. pylori on the basis of results of urease, catalase and oxidase assays.

Half of the stomach was placed in 10% buffered formalin and embedded in paraffin, and 4-μm sections were cut from the body to the antrum. The sections were stained with haematoxylin-eosin for histological evaluation and with Giemsa to assess bacterial colonization. The other half was used for extraction of total RNA and proteins for competitive reverse transcription-
polymerase chain reaction (RT-PCR) and western blotting analyses, as described below.

**Histology and quantification of mucosal thickness**

H&E- and Giemsa-stained sections were carefully screened for the presence of spiral *H. pylori*-like organisms. The infiltration of polymorphonuclear cells and lymphocytes was also evaluated.

The thickness of pits and glands of gastric mucosa was measured with a micrometer (OCM 10 × 10 SG; Olympus, Tokyo, Japan) on H&E-stained sections. Briefly, the gastric mucosa was scanned along the middle zone of the body, which was sectioned vertically to the surface of the gastric mucosa, where hyperplastic changes were predominantly observed. The pathologist choosing the section to scan was blinded to the identity of the treatment group. Values are expressed as the means of measurements along three or more foveolae. Total mucosal thickness was calculated by adding the thickness of the pit to that of the gland.

**Competitive reverse transcription-polymerase chain reaction analysis for COX-2 mRNA**

Total RNA was extracted from the gastric mucosa of C57BL/6 mice immediately after the removal of the stomach. RNA extraction was performed using a commercial kit (RNA zoll Tel. Test, Inc., Friendwood, TX). RNA content was quantified by measuring optical density (OD) at 260 nm (1.0 OD_{260nm} = 40.0 μg/mL). Aliquots of 5 μg of RNA solution were subjected to reverse transcription. RNA was deposited by ethanol and dried under vacuum. The RNA pellet was dissolved in a reverse transcription mixture containing, in a final volume of 100 μL, 20 μL of 5× First Strand Buffer, 10 μL of 0.1 M DTT, 1 μL of Oligo (dT)_{12–18} (Gibco BRL, Life Technologies, Inc., Rockville, MD), 5 μL of mixed dNTP Stock (10 μM each of dATP, dGTP, dCTP, and dTTP at neutral pH) (Takara Shuzo Co., Ltd, Shiga, Japan), and 2.5 μL of RNasin Ribonuclease Inhibitor (Promega, Madison, WI). The mixture was heated at 70 °C for 5 min, and rapidly chilled in ice. The RNA mixture was converted to

![Figure 1. Oligonucleotide sequences of competitive templates and primers for the quantitative analysis of COX-2 and β-actin. The primer pair for C57BL/6 mouse COX-2 amplifies a 276-bp band. The primer pair for β-actin amplifies a 540-bp band. The competitive template for COX-2 was 200 bp in length and that for β-actin was 345 bp.](attachment:image.png)
cDNA with 200 units of reverse transcriptase (Super-Script RNase H-Reverse Transcriptase; Gibco BRL, Life Technologies, Inc., Rockville, MD) by incubation at 42 °C for 60 min. The product was labelled as cDNA solution.

The oligonucleotide primers for C57BL/6 mouse COX-2 designed by Williams et al. amplified a 276-bp band from the cDNA (Figure 1). The competitor was constructed (Figure 1) using a commercial Competitive DNA Construction Kit (Takara Shuzo, Co., Ltd., Shiga, Japan) according to the manufacturer’s instructions. Eight precise 100.5-fold serial dilutions ranging from 1.0 × 10^–0.5 pg/μL to 1.0 × 10^–4 pg/μL of competitor were prepared in relatively large volumes so that the same dilution series could be used for measurement of many samples under identical conditions.

We prepared a master mixture containing, in a final volume of 50 μL, 5 μL of 10× PCR buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM MgCl₂, 0.1% gelatin], dNTPs (each at a final concentration of 200 μM), oligonucleotide primers (COX-2 forward primer and COX-2 reverse primer, each at a final concentration of 0.5 μM), 1.25 units of Taq polymerase (Ex Taq, Takara Shuzo, Shiga, Japan), and 1.0 μL of cDNA solution. An aliquot of 48 μL of this mixture was added to 2.0 μL of previously prepared competitor of known concentration in a dilution series. PCR was performed with an automatic thermal cycler (DNA Thermal Cycler P|2000, Perkin-Elmer, Norwalk, Conn., USA). The amplification consisted of an initial denaturation at 94 °C for 5 min, followed by denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s, and polymerization at 72 °C for 1 min. The final cycle included extension for 7 min at 72 °C to ensure full extension of the product. COX-2 cDNA was amplified through 35 consecutive cycles. PCR products (10 μL) were electrophoretically separated on 3.0% (wt/vol) agarose gels containing 0.5 μg/mL ethidium bromide in 1× TAE (Tris Acetone EDTA) buffer. The bands were visualized by excitation with UV light, and bands of 276 bp (COX-2) and 200 bp (competitor) were detected (Figure 2A). The gels were photographed and the intensity of ethidium bromide luminescence was measured with a CCD image sensor (Densitograph AE-6900-F, Atto, Tokyo, Japan), and the ratio of the 276 bp (COX-2) band to the 200 bp band (competitor) was plotted for each dose of competitor added to the reaction tube. The point of equivalence (that is, where there was a 1:1 ratio) was where COX-2 equalled the competitor and represented the concentration of COX-2 mRNA in the unknown sample (Figure 3A).

The amount of β-actin mRNA was also measured by a similar method (Figures 2B and 3B). The sequences of oligonucleotide primers for β-actin (Clontech, Inc., USA) and the competitive template are shown in Figure 1. The amount of COX-2 mRNA in the gastric mucosa was standardized relative to β-actin and expressed as the COX-2:β-actin ratio.

Western blotting for COX-2

The gastric mucosa was homogenized at 4 °C in lysis buffer [9.1 mM dibasic sodium phosphate, 1.7 mM monobasic sodium phosphate, 150 mM NaCl, 1% Igepal CA-630 (Sigma Chemicals), 0.5% sodium deoxycholate, 0.1% SDS], containing 30 μL/mL aprotinin (Sigma Chemicals), 1 mM sodium orthovanadate, and 100 μg/mL phenylmethylsulphonyl fluoride. The homogenate was centrifuged twice at 14 000 r.p.m. for 20 min at 4 °C. The supernatant was the protein lysate. The protein concentration was measured by Lowry’s assay with BSA as the standard. The protein lysate was mixed with an equal volume of sample buffer and denatured at 94 °C for 2 min. Equal amounts of

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**Figure 2.** Electrophoresis of products of competitive PCR for quantitative analysis of COX-2 (A) and β-actin (B). Lane M is the size marker: e X174 digested with Hinf I. Lanes 1–8 contained eightfold serial dilutions of competitive templates for COX-2 ranging from 2.0 × 10^–0.5 pg to 2.0 × 10^–4.0 pg/tube (A) and those for β-actin ranging from 2.0 × 10^–1.5 pg to 2.0 × 10^–3.5 pg/tube (B) against a fixed amount of cDNA.

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protein (40 lg/lane) were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and then transferred on to Hybond ECL nitrocellulose membranes (Amer sham Inc., Buckinghamshire, UK) by electroblotting. The transfer of protein and equal loading in all lanes were verified using reversible staining with Ponceau S.31 The membranes were incubated for 1 h at room temperature in blocking solution [Tris-buffered saline (10 mM Tris-HCl, pH 8.0; 150 mM NaCl) containing 5% non-fat dried milk and 0.05% Tween 20], followed by overnight incubation with a polyclonal goat anti-mouse COX-2 antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) at 1:250 dilution in blocking solution. Filters were washed three times for 5 min each with blocking solution, then probed with alkaline phosphatase-conjugated donkey anti-goat immunoglobulin (Santa Cruz Biotechnology Inc., Santa Cruz, CA) as the secondary antibody at 1:1000 dilution in blocking solution for 30 min. After three additional washes with blocking solution followed by one wash for 5 min with AP buffer (100 mM Tris-HCl, pH 9.5; 100 mM NaCl; 5 mM MgCl2), filters were treated with BM Purple AP substrate (Boehringer Mannheim GmbH, Germany). One sample from gastric mucosa of a C57BL/6 mouse infected by H. pylori for 24 weeks was used as a standard for measurement of other different samples. Bands were detected with a CCD image sensor (Densitograph AE-6900-F, Atto, Tokyo, Japan). The amount of COX-2 protein in gastric mucosa was standardized relative to the positive control infected with H. pylori for 24 weeks and expressed as the sample COX-2/positive control ratio. Representative results are shown in Figure 4. The right lane (H. pylori inoculation for 24 weeks) was used as a standard for measurement of different samples.

**Measurement of PGE2**

The gastric mucosa was homogenized at 4 °C in lysis buffer. Next, the sample was vortexed. Homogenates were centrifuged at 14 000 r.p.m. for 20 min at 4 °C. An aliquot of 10 l from each sample was used to determine protein concentration with Lowry’s assay.

![Figure 3](image1.png)

**Figure 3.** (A) The ratio of the 276 bp band (COX-2) : 200 bp band (competitor) vs. the dose of competitor added to the reaction tube. The points of equivalence (i.e. where there was a 1 : 1 ratio) were where COX-2 equalled competitor, and represented the concentration of COX-2 in the unknown sample. (B) The ratio of the 540 bp band (β-actin) : 345 bp band (competitor) vs. the dose of competitor added to the reaction tube.

![Figure 4](image2.png)

**Figure 4.** COX-2 protein (70 kilodalton band) detected by immunoblotting in representative gastric mucosa of C57BL/6 mice at 24 weeks after inoculation. The intensity of the band in the right lane derived from the H. pylori-positive/etodolac-negative group was the highest, and was used as a standard.
The supernatant from each sample was subjected to a PGE\textsubscript{2} assay. Determination of PGE\textsubscript{2} levels by enzyme immunoassay was accomplished using a Prostaglandin-E\textsubscript{2}-Monoclonal Enzyme Immunoassay Kit (Caymen Chemical, Ann Arbor, MI), according to the manufacturer’s protocol. Plates were read at 410 nm with a plate reader (Delta SOFT 4.0B BioMetallics, Inc., Japan).

**Statistical analysis**

All numerical data are presented as means ± S.E.M. Whether body and stomach weights, COX-2 mRNA, COX-2 protein, and PGE\textsubscript{2} levels in gastric mucosa, and thickness of gastric pit differed among the four groups (\textit{H. pylori}-negative/etodolac-negative, \textit{H. pylori}-negative/etodolac-positive, \textit{H. pylori}-positive/etodolac-negative, \textit{H. pylori}-positive/etodolac-positive) was determined by one-way ANOVA followed by Fisher’s multiple comparison test. Whether body and stomach weights, COX-2 mRNA, COX-2 protein, and PGE\textsubscript{2} levels in gastric mucosa, and thickness of gastric pit changed from before to 12 or 24 weeks after inoculation in each study group was determined by the Student’s \textit{t}-test. Pearson’s correlation coefficient was calculated to analyse the relationship between thickness of the gastric pits and the gastric mucosa. Statistical calculations were carried out with SAS software (SAS Institute, Cary, North Carolina). All \textit{P}-values are two-sided and \textit{P} < 0.05 was considered significant.

**RESULTS**

\textit{H. pylori} infection, body and stomach weight of C57BL/6 mice

\textit{H. pylori} was detected in all infected animals throughout the study. Bacterial colonization was detectable on Giemsa staining, and the bacteria cultured were identified as \textit{H. pylori} by urease, catalase and oxidase assays.

The mean body weight of each of the four study groups increased in a time-dependent manner from before to 12 and 24 weeks after inoculation. No significant intergroup differences were observed during the study period (data not shown).

The mean stomach weight of the \textit{H. pylori}-positive/etodolac-negative group at 24 weeks after inoculation was significantly increased compared with those before and 12 weeks after inoculation (\textit{P} < 0.001 and \textit{P} < 0.05), and was significantly heavier than those of the \textit{H. pylori}-negative/etodolac-negative (\textit{P} < 0.001), and \textit{H. pylori}-positive/etodolac-positive (\textit{P} < 0.05) groups (Figure 5). Differences in mean stomach weights observed at 12 weeks after inoculation were not statistically significant.

**Histopathological changes**

At 12 weeks after inoculation, mild infiltration of polymorphonuclear cells and lymphocytes was observed in both the antrum and body, mainly in the submucosa in the \textit{H. pylori}-positive/etodolac-negative group. However, the inflammation was clearly more severe in the transitional zone between the antral and body mucosa (Figure 6F). At 24 weeks after inoculation, infiltration of lymphocytes and plasma cells became prominent, and multifocal elongation of the gastric pits was observed in the gastric body with variable sparing of the antrum (Figure 6G). The thickness of the gastric pits was significantly increased in the \textit{H. pylori}-positive/etodolac-negative group at 24 weeks after inoculation in comparison with that in the \textit{H. pylori}-negative/etodolac-negative group (268.8 ± 37.2 vs. 64.0 ± 7.5, \textit{P} < 0.0001). The thickness of the gastric pits in the \textit{H. pylori}-positive/etodolac-positive group was signifi-
cantly decreased compared with that in the *H. pylori*-positive/etodolac-negative group (128.0 ± 13.6 vs. 268.8 ± 37.2, \( P < 0.001 \)), but was greater than that in the *H. pylori*-negative/etodolac-negative group (128.0 ± 13.6 vs. 64.0 ± 7.5, \( P < 0.05 \)) (Figures 6 and 7A). There was an increase in thickness of both gastric pits and glands with multifocal erosions on the pit surface in the *H. pylori*-infected groups. The increase in total mucosal thickness was associated with an apparent increase in gastric pit thickness (\( r = 0.918, \ P < 0.0001 \); Figure 8), but not with an increase in gastric gland thickness. Densities of *H. pylori* on Giemsa stained specimens did not differ between etodolac-positive and etodolac-negative groups.

Figure 6. Histopathological changes in H&E-stained sections at before, 12 and 24 weeks after inoculation in the four study groups. In the *H. pylori*-negative/etodolac-negative (A, B and C) and *H. pylori*-negative/etodolac-positive (A, D and E) groups, no histological changes were observed throughout the study period. In the *H. pylori*-positive/etodolac-negative, mild (F) and moderate (G) infiltration of mono- and poly-nuclear cells was observed and the thickness of the gastric mucosa gradually increased from before to 12 (F) and 24 (G) weeks after *H. pylori* inoculation. In the *H. pylori*-positive/etodolac-positive group at 24 weeks after inoculation (I), mucosal thickening was observed, but to a lesser extent than that of the *H. pylori*-positive/etodolac-negative group at 24 weeks after inoculation. At 12 weeks after inoculation, no mucosal thickening was observed in the *H. pylori*-positive/etodolac-positive group (H). Original magnification, \( \times 10 \).
**COX-2 mRNA expression**

Competitive reverse transcription-polymerase chain reaction analysis showed that COX-2 mRNA levels in the gastric mucosa in the *H. pylori*-positive/etodolac-negative group 24 weeks after inoculation were significantly increased compared with those before and 12 weeks after inoculation (*P* < 0.01 and *P* < 0.05, respectively), and were significantly higher than that in the *H. pylori*-negative/etodolac-negative group.

Before inoculation and at 12 weeks after inoculation, COX-2 mRNA levels in the gastric mucosa in the *H. pylori*-positive/etodolac-negative group were not significantly different from those in the *H. pylori*-positive/etodolac-positive group or between *H. pylori*-positive/etodolac-positive and *H. pylori*-positive/etodolac-negative groups.
etodolac-negative and *H. pylori*-negative/etodolac-positive groups. Etodolac dosing had no effect on COX-2 mRNA levels in *H. pylori*-infected or -uninfected groups. COX-2 protein

As shown in Figure 7(C), western blotting analysis demonstrated that the amount of COX-2 protein in the gastric mucosa in the *H. pylori*-positive/etodolac-negative group at 24 weeks after inoculation was significantly increased compared with that before inoculation (P < 0.05) and was significantly higher than that of the *H. pylori*-negative/etodolac-negative group (P < 0.05), consistent with the levels of COX-2 mRNA. Before inoculation and at 12 weeks after inoculation, COX-2 protein levels in the gastric mucosa in the *H. pylori*-positive/etodolac-negative group were not significantly different from those of the *H. pylori*-negative/etodolac-negative group. No significant changes were observed in COX-2 protein levels following etodolac treatment in either *H. pylori*-infected or -uninfected groups at 24 weeks after inoculation, indicating that etodolac does not inhibit COX-2 expression.

**PGE_2 levels**

In the *H. pylori*-uninfected groups (*H. pylori*-negative/etodolac-negative and *H. pylori*-negative/etodolac-positive groups), PGE_2 levels in the gastric mucosa were not changed significantly from before to 12 and 24 weeks after inoculation.

At 12 weeks after inoculation, the PGE_2 level in the infected group was not significantly different from that in the control group. However, the PGE_2 level in the gastric mucosa in the *H. pylori*-positive/etodolac-negative group at 24 weeks after inoculation was significantly higher than those in the *H. pylori*-negative/etodolac-negative and the *H. pylori*-negative/etodolac-positive groups at 24 weeks after inoculation and before inoculation (30.4 ± 4.1 vs. 16.4 ± 2.1, 12.7 ± 1.9, and 14.2 ± 2.1; P < 0.05, P < 0.001, and P < 0.05, respectively). The PGE_2 level in the gastric mucosa in the *H. pylori*-positive/etodolac-positive group was significantly lower than that in the *H. pylori*-positive/etodolac-negative group (19.1 ± 1.9 vs. 30.4 ± 4.1, P < 0.05; Figure 7D).

**DISCUSSION**

*H. pylori*-induced gastritis has recently been reported to be associated with increased epithelial proliferation.6–8 NSAIDs were shown to reverse *H. pylori*-induced proliferation in gastric epithelial cells in human subjects.32 However, the precise mechanisms by which *H. pylori* infection induces hyperplastic gastritis have not been elucidated. The present study, using C57BL/6 mice infected with the strain SS1 of *H. pylori*, revealed that COX-2 plays an important role in the development of hyperplastic changes in *H. pylori*-infected gastric mucosa.

In humans, a possible association of gastric hyperplastic changes (e.g. hyperplastic gastritis, hyperplastic polyps) with *H. pylori* infection has been reported, and in this study hyperplastic gastric polyps disappeared after cure of *H. pylori* infection in more than 40% of patients.13 Stolte et al. examined 138 patients with hyperplastic gastritis and found that 90% were infected with *H. pylori*.34 In their study, there seemed to be marked *H. pylori* colonization and a higher degree of gastritis in the corpus of the stomach compared with the antrum, as reported in *H. felis*-infected C57BL/6 mice.29 In the present study, we determined changes in gastric mucosal thickness after *H. pylori* inoculation in C57BL/6 mice, and showed that *H. pylori* infection caused hyperplastic gastritis with marked colonization by this bacterium.

COX-2 is involved in various inflammatory responses such as *H. pylori* gastritis, inflammatory bowel diseases...
and gastric cancer in humans.\textsuperscript{23, 25, 35} NSAIDs were shown to abrogate the proliferation effects induced by \textit{H. pylori} infection in gastric epithelial cells in humans.\textsuperscript{32} Epidemiological and experimental studies have revealed that NSAIDs, particularly aspirin, sulindac and piroxicam, are promising candidates as chemopreventive agents against the development of colorectal cancer and gastric cancer.\textsuperscript{22, 26, 27, 36–38} The target enzyme of NSAIDs is assumed to be COX-2, an inducible isotype of COX.\textsuperscript{12–14} The detailed roles of COX-2 in \textit{H. pylori}-associated hyperplastic gastritis have not, however, been clarified.

In previous reports, COX-2 expression was thought to be induced under pathological conditions. Our results indicated that COX-2 was, however, constitutively expressed in the normal gastric mucosa of C57BL/6 mice, which coincided with the observations of Zimmermann et al. in the healthy human and rabbit gastric mucosa.\textsuperscript{39} Using immunoblotting analysis, low but detectable levels of COX-2 protein were demonstrated in microsomes prepared from gastrointestinal tissues of normal rats.\textsuperscript{40} Similarly, low levels of COX-2 mRNA and protein expression were observed in endothelial cells of the microvessels and basement membranes, and mucosal macrophages of normal human gastric mucosa.\textsuperscript{41} COX-2 was also detected in myofibroblasts and endothelial cells of unstimulated human gastric mucosa and was expressed constitutively in human gastric endothelial cells in culture.\textsuperscript{42} These findings, taken together with our results, suggest that COX-2 is constitutively expressed in normal gastric tissue as well as COX-1. However, the role of constitutively expressed COX-2 is unclear. In the present study, the selective COX-2 inhibitor etodolac had no effect on gastric mucosa in relation to mucosal thickness and PGE\textsubscript{2} levels in gastric tissue in the \textit{H. pylori}-uninfected groups. Therefore, the role of constitutively expressed COX-2 in normal gastric mucosa is assumed to be of limited significance.

On the other hand, in the present study, expression of COX-2 was markedly up-regulated in the mRNA level in the gastric mucosa of C57BL/6 mice infected with \textit{H. pylori} compared with those of the control groups at 24 weeks after inoculation and was corroborated by the finding of an increase in COX-2 protein, along with the appearance of hyperplastic gastritis. Moreover, when the selective COX-2 inhibitor etodolac was continuously administered soon after inoculation, PGE\textsubscript{2} levels in gastric tissue were significantly decreased and the hyperplastic changes were significantly suppressed. This suggests that COX-2 induced by \textit{H. pylori} infection might play an important role in the development of hyperplastic gastritis through an increase in PGE\textsubscript{2} level, and that this process might be inhibited by etodolac. However, as shown in Figures 6 and 7, the selective COX-2 inhibitor did not completely inhibit hyperplastic changes. This suggests that other factors such as gastrin and several growth factors [e.g. transforming growth factor-alpha (TGF-\alpha), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and fibroblast growth factor (FGF)], may be also involved in hyperplastic changes in \textit{H. pylori} infection.\textsuperscript{43, 44} Previously, a slight increase (less than twofold) in antral PGE\textsubscript{2} levels was reported in patients infected with \textit{H. pylori}, but this was not statistically significant.\textsuperscript{45} Patients taking no NSAIDs in this previous study showed a threefold increase in PGE\textsubscript{2} levels with \textit{H. pylori} infection in comparison with those taking NSAIDs, and this effect was statistically significant. Significant increases in expression of mRNA and protein of COX-2 were also observed in \textit{H. pylori}-positive gastritis.\textsuperscript{23} In a previous immunohistochemical study, up-regulated expression of COX-2 was shown to be reduced after \textit{H. pylori} eradication.\textsuperscript{24} Therefore, expression of COX-2 in epithelial cells was assumed to be strongly correlated with the intensity of chronic inflammatory cell infiltration. Several studies have indicated that COX-2 expression is induced in several cell lines by growth factors and cytokines such as transforming growth factor-\alpha, transforming growth factor-\beta and interleukin-1,\textsuperscript{15, 46, 47} In the present study, increased expression of COX-2 was observed at 24 weeks after \textit{H. pylori} inoculation, when apparent chronic active gastritis was observed, but not at 12 weeks after \textit{H. pylori} inoculation, when only mild infiltration of polymorphonuclear cells and lymphocytes were seen. Thus, increased expression of COX-2 was correlated with chronic inflammatory cell infiltration in the gastric mucosa of C57BL/6 mice infected with \textit{H. pylori}.

Tsuji et al. suggested a possible link between COX-2 and epithelial cell growth.\textsuperscript{48} NSAIDs, agents that block the activity of COX-2, were shown to be associated with a decreased incidence of colon cancer, which is mediated by a decrease in COX-2 expression in colonic tumours, in a human study.\textsuperscript{39} The preventive mechanisms have been postulated to involve inhibition of COX-2, and thus, production of eicosanoids such as prostaglandins, which influence tumour growth either
by directly participating in the signal cascade for cell proliferation or by disturbing immunological surveillance.\textsuperscript{36, 37} In a murine model of familial adenomatous polyposis, the NSAIDs such as sulindac decreased COX-2 and PGE\textsubscript{2} levels to baseline in the small bowel and inhibited tumour formation.\textsuperscript{38} Takahashi et al. reported that \textit{H. pylori} increased the PGE\textsubscript{2} and HGF releases from human gastric fibroblasts and that these increased releases were inhibited by NSAIDs including a COX-2 specific inhibitor.\textsuperscript{39} Several studies showed that \textit{H. pylori} infection can cause increased gastric cell proliferation, which is assumed to play an important role in gastric carcinogenesis.\textsuperscript{6–8} NSAIDs were shown to reverse \textit{H. pylori}-induced proliferation in gastric epithelial cells.\textsuperscript{32} In the present study, continuous administration of a selective COX-2 inhibitor, etodolac, significantly inhibited the production of PGE\textsubscript{2}. This was suggested to result in a significant reduction of hyperplastic changes in gastric mucosa of C57BL/6 mice at 24 weeks after \textit{H. pylori} inoculation, compared with the controls. These observations indicate that increased expression of COX-2 plays a crucial role in the hyperplastic changes in the gastric mucosa induced by \textit{H. pylori} infection.

CONCLUSION

In summary, our results suggest that increased expression of COX-2 has a crucial role in the hyperplastic changes associated with gastritis induced by \textit{H. pylori} infection, which is mediated by PGE\textsubscript{2}. The COX-2-selective inhibitor etodolac may be useful for the prevention and treatment of \textit{H. pylori}-associated gastric hyperplasia, which may be related to the chemopreventive effect of NSAIDs against gastric carcinogenesis.

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REFERENCES


42 Donnelly MT, Hull MA, Jenkins D, Hawkey CJ. Immunohistochemical distribution of constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2) in benign and gastric mucosa. Gastroenterology 1997; 112: A105(Abstract).


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