Effects of Nonsteroidal Anti-Inflammatory Drugs on Helicobacter pylori-Infected Gastric Mucosae of Mice: Apoptosis, Cell Proliferation, and Inflammatory Activity

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Helicobacter pylori and nonsteroidal anti-inflammatory drugs (NSAIDs) are two well-known important causative factors of gastric damage. While H. pylori increases apoptosis and the proliferation of gastric epithelial cells and is an important factor in peptic ulcer and gastric cancer, NSAIDs induce cell apoptosis and have antineoplastic effects. We investigated the effects of NSAIDs (a nonselective cyclooxygenase [COX] inhibitor [indomethacin] and a selective COX-2 inhibitor [NS-398]) on the apoptosis and proliferation of gastric epithelial cells and gastric inflammation in H. pylori-infected mice. C57BL/6 mice were sacrificed 8 weeks after H. pylori SS1 inoculation. Indomethacin (2 mg/kg) or NS-398 (10 mg/kg) was administered subcutaneously once daily for 10 days before sacrifice. The following were assessed: gastric inflammatory activity, gastric COX protein expression by Western blotting; gastric prostaglandin E2 levels by enzyme immunoassay, apoptosis by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling, and cell proliferation by Ki67 immunostaining. Compared to the controls, Helicobacter pylori infection and/or NSAID treatment increased COX-1 and COX-2 protein expression. Gastric prostaglandin E2 levels, apoptotic index, cell proliferation index, neutrophil activity, and the degree of chronic inflammation were all increased by H. pylori infection, and these effects were significantly decreased by indomethacin treatment. However, NS-398 treatment after H. pylori infection did not induce a significant reduction, although it did result in a tendency to decrease. These results show that NSAIDs can reverse the increased apoptosis and proliferation of epithelial cells and inflammatory activity in the stomachs of H. pylori-infected mice and that, like COX-2 activation, COX-1 induction contributes to the change of gastric mucosal cell turnover and inflammation induced by H. pylori infection.

Helicobacter pylori and nonsteroidal anti-inflammatory drugs (NSAIDs) are two well-known important causative factors of gastric injury such as gastritis and peptic ulcer. However, the interaction between these two factors in terms of their effects on gastric mucosa remains controversial. Some clinical studies have reported no interaction between H. pylori and NSAIDs or of a protective role of H. pylori in patients given NSAIDs (19, 24, 27). Other studies have shown harmful effects of H. pylori in patients given NSAIDs (1, 4, 25). There are many reasons for these controversies, including differences in the characteristics of subjects, differences in methods of approach, relatively small sample sizes, etc. Furthermore, the complicated mechanisms, including cyclooxygenase 2 (COX-2) and/or COX-1 activity, may be involved in the interaction between H. pylori and NSAIDs (nonselective and COX-2 selective) on the gastric mucosa. Therefore, an understanding of processes at the cellular level and large studies comparing COX-2 inhibitors with nonselective COX inhibitors will provide better information.

When one considers cell turnover and the pathogenic effects on the gastric mucosa, it becomes apparent that the dynamic balance between epithelial cell proliferation and apoptosis is essential for maintaining normal mucosal integrity and that an alteration of the balance in either direction could result in ulcerogenesis or carcinogenesis of the stomach (38, 44). H. pylori is associated in vivo with both increased apoptosis and the proliferation of gastric epithelial cells; accordingly, an alteration in the balance of these events may lead to increased injury or neoplastic transformation (2, 7, 34). On the other hand, NSAIDs induce cell apoptosis and have antineoplastic effects, and they reduce the incidence of and mortality from colon adenocarcinoma and gastric cancer (17, 57). However, in human clinical studies on the interaction of these two factors during cell turnover, some controversial aspects remain unresolved. Zhu et al. showed that NSAIDs could abrogate the apoptosis and proliferation enhanced by H. pylori (66). However, Leung et al. reported that the eradication of H. pylori prior to NSAID therapy significantly reduced the level of apoptosis in the gastric mucosa (29).

COX expression and prostaglandin (PG) production may have some mechanistic influence on the interaction between H. pylori and NSAIDs in the gastric mucosa. It is well known that COX-2 is the inducible form, involved in the inflammatory reaction and tumorigenesis, and that COX-1 is the constitutive form, related to physiologic cytoprotection. In H. pylori-infected gastric tissue, upregulated COX-2 expression and increased PGE2 production may be important for cell proliferation (13, 46). In the case of NSAID-induced gastric injury, the
most important mucosal injury factor appears to be the decrease in mucosal PG production by COX inhibition and apoptosis induction (16, 30). It has also been shown that selective COX-2 inhibitors have antineoplastic effects, as do nonselective COX inhibitors, and that they induce less mucosal damage than nonselective COX inhibitors (27, 49, 54). However, it has not been determined whether there are any differences between the effects of nonselective and selective COX-2 inhibitors on gastric epithelial cell kinetics in the H. pylori-infected stomach. Therefore, in this study, we evaluated the interaction between H. pylori and NSAIDs in terms of gastric epithelial cell apoptosis, proliferation, and inflammation in a mouse model. In particular, we investigated the differences between nonselective and selective COX-2 inhibitors on these interactions.

MATERIALS AND METHODS

Animals and H. pylori challenge. Rat and mice models have been used to investigate the NSAID-induced gastric damage and the effects of NSAIDs in ulcer healing in many studies (37, 52, 61). H. pylori infection, inducing chronic gastritis similar to H. pylori infection in humans (28), Therefore, in the present study, we used the C57BL/6 mouse as the animal model. Specific-pathogen-free, inbred C57BL/6 female mice 7 weeks of age were purchased from Jackson Laboratory (Bar Harbor, Maine). All animals were housed in the specific-pathogen-free barrier area of Department of Laboratory Animal Medicine, Yonsei University College of Medicine, for the duration of the experiments. All experiments and procedures involving the animals were reviewed by the Yonsei University Committee for the Care and Use of Laboratory Animals according to university’s the guidelines and regulations for use and care of animals.

The mice were divided into six groups A to F, and treated according to the following schedule: A, control (n = 7); B, H. pylori infection (n = 7); C, H. pylori infection followed by indomethacin treatment (n = 7); D, H. pylori infection followed by NS-398 treatment (n = 7); E, indomethacin treatment (n = 7); and F, NS-398 treatment (n = 5). H. pylori SS1 (vacA* cagA*), obtained from Daewoong Research and Development, Daewoong Pharmaceutical Co., Ltd., was grown on blood agar plates containing sterile sheep blood (5%, vol/vol) in blood agar base (Becton Dickinson and Co., Cockeysville, Md.). The H. pylori colonies were suspended in phosphate-buffered saline and administered intragastrically to the mice. The animals were dosed three times in a 3-day period with 0.1 ml of bacterial suspension (approximately 10^9 organisms/ml). The animals that were not inoculated with H. pylori were inoculated with identical volumes of sterile phosphate-buffered saline alone.

Administration of NSAIDs. Indomethacin and NS-398 were purchased from Sigma Chemical Co. (St. Louis, Mo.) and Calbiochem (La Jolla, Calif.), respectively. In other experiments, NS-398 was used as a selective COX-2 inhibitor in doses of less than 10 mg/kg (14, 37, 52); in our experiment, we found that a 13-mg/kg dose of NS-398 reduced the levels of PGE2 in mouse stomachs (5) and NS-398 showed a significant antineoplastic effect (14, 37, 52); in our experiment, we found that a 13-mg/kg dose of NS-398 reduced the levels of PGE2 in mouse stomachs (5). The PVDF membrane was blocked with 5% nonfat milk in TBST (10 mM Tris-HCl, 150 mM NaCl, 0.5% NP-40, 1 mM phenylmethylsulfonyl fluoride, and 10 mM EDTA in 0.1 M Tris [pH 7.4]). 100 mM NaCl, 0.5% Tween 20) and then probed for 1 h with 1:1000 dilution of goat anti-COX-1 or anti-COX-2 polyclonal immunoglobulin Ig G (IgG; Santa Cruz Biotechnology, Santa Cruz, Calif.). Bound antibodies were detected with horseradish peroxidase-conjugated anti-goat IgG (Santa Cruz Biotechnology), using the enhanced chemiluminescence detection system.

Enzyme immunoassay for the measurement of gastric PGE2 levels. The frozen specimens were weighed and put in a tube containing 100% ethanol plus 10 g of indomethacin per ml. The samples were then minced with scissors, homogenized, and centrifuged for 10 min at 12,000 rpm at 4°C. The supernatant of each sample was used for determination of PGE2, using a Bicycle PGE2 enzyme immunoassay kit (Cayman Chemical Company, Ann Arbor, Mich.), as described by the manufacturer.

Determination of apoptosis and proliferation. A single experienced pathologist blinded to treatment schedule quantitated gastric epithelial cell apoptosis and proliferation by using immunohistochemical stains. Epithelial cell apoptosis was determined in situ from paraffin-embedded tissue sections by the terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling (TUNEL) technique (apoTACS-Basic kit; R&D Systems, Minneapolis, Minn.) as described by the manufacturer. All samples were processed under the same conditions with positive and negative controls. Positive control sections were pretreated with DNase I and negative controls were processed without terminal deoxynucleotidyl-transferase. For each mouse, 50 well-oriented, representative gastric glands were scored in a tissue section, and the results of apoptosis are expressed as apoptotic index, defined as the number of positive cells per 50 glands.

Epithelial cell proliferation was determined by immunohistochemical staining for Ki67. Briefly, sections were deparaffinized, placed in citrate buffer (pH 6.0) (10 mmol/liter), and heated in a 780-W microwave oven for 10 min. Endogenous peroxidase activity was quenched with hydrogen peroxide, and after washing in immunoblotting buffer, the slides were incubated with a monoclonal mouse IgG raised against Ki67 (PharMingen, San Diego, Calif.) in a humidified chamber. Slides then were incubated with biotinylated anti-mouse IgG and streptavidin-horseradish peroxidase (LSAB2 kit; DAKO Corporation, Carpinteria, Calif.), developed in aminoethylcarbazole (AEC; DAKO), and counterstained with hematoxylin. The results of cell proliferation are expressed as proliferation index, defined as the number of positive cells per 50 glands.

Statistics. Two-tailed statistical tests were used to evaluate the data. The Mann-Whitney U test for nonparametric data was used to compare scores between animal groups. The correlation among apoptosis, proliferation, and inflammatory activity was evaluated using Spearman’s rho test for nonparametric data. The data are presented as means in parentheses (range) in Table 1. Significance was defined as a P value of <0.05.

RESULTS

H. pylori infection and chronic gastritis. H. pylori infection in the stomach was confirmed by Giemsa staining in all H. pylori-inoculated mice (Fig. 1A), and organisms were found throughout the glandular stomach, colonizing both the antrum and the body, as found in a previous study (28). Increased levels of polymorphonuclear neutrophils and mononuclear cells were found in both the antrum and the body of the stomachs of H. pylori-infected mice (Fig. 1B), whereas atrophy, intestinal metaplasia, and ulceration were not observed.

Gastric PGE2 levels showed a tendency to increase upon H. pylori infection, although this was not significant, and further
FIG. 1. *H. pylori* infection, chronic gastritis, apoptosis, and proliferation in stomachs of *H. pylori*-infected mice. (A) Light microscopic photograph of gastric tissue from a C57BL/6 mouse colonized with *H. pylori* SS1 showing the S shape of the bacteria. *H. pylori* infections were confirmed by Giemsa staining in all cases of *H. pylori*-inoculated mice (magnification ×730). (B) Findings of chronic gastritis with many inflammatory cells in the stomach of an *H. pylori*-infected mouse (H&E, ×292). (C) Increased positive apoptotic epithelial cells in the superficial portion of the stomach of an *H. pylori*-infected mouse (TUNEL stain, ×292). (D) Increased numbers of positive apoptotic epithelial cells in the proliferation compartment and superficial portion of the antral mucosa of the stomach of an *H. pylori*-infected mouse (TUNEL stain, ×292). (E and F) Increased number of Ki67-positive cells in the proliferation compartment of the body (E) and antrum (F) of the stomach of an *H. pylori*-infected mouse (Ki67 immunostain, ×292).
administration of indomethacin significantly decreased this heightened PGE$_2$ level ($P = 0.002$) (Fig. 2). However, the effect of NS-398 treatment after $H.\, pylori$ infection on PGE$_2$ production was not significant, although it tended to decrease to the level of the controls (Fig. 2). In the absence of $H.\, pylori$ infection, indomethacin treatment markedly decreased the PGE$_2$ level ($P = 0.002$), but NS-398 treatment resulted in no difference compared with the control group (Fig. 2).

We determined COX-1 and COX-2 expression levels by Western blotting to evaluate the effect of NSAIDs and/or NSAID treatment. Levels in mouse stomachs were found to be increased by $H.\, pylori$ infection and/or NSAID treatment (Fig. 3A), and this result was confirmed by densitometric band measurements (Fig. 3B).

**Apoptosis of gastric epithelial cells.** Because $H.\, pylori$ and gastric mucosal inflammation contribute to cell turnover, and NSAIDs have anti-inflammatory and apoptotic effects, we investigated whether epithelial cell apoptosis is altered by $H.\, pylori$ infection and how these apoptotic effects are affected by NSAIDs.

Apoptotic cells were detected by the TUNEL method, and positive staining was found to be confined to the nucleus. In the $H.\, pylori$-negative control group, TUNEL-positive cells were few in number and usually situated at the superficial part of the glands. However, in $H.\, pylori$-infected mice, apoptotic cells in the superficial portion of the body and antrum were increased in number and positive cells were often detected in the proliferating basal zone of the antral mucosa, which is an important site for epithelial replication and the regeneration of mucosal glands and surface epithelium (Fig. 1C and D).

To investigate the effect of NSAIDs on apoptosis in $H.\, pylori$-infected gastric mucosa, we measured the apoptotic index in the gastric mucosa of $H.\, pylori$-infected mouse with or without NSAID treatment. The apoptotic index was significantly increased upon $H.\, pylori$ infection ($P = 0.003$), and this effect was significantly decreased after further treatment with indomethacin ($P = 0.025$) (Fig. 4). The apoptotic index was also lower in $H.\, pylori$-infected mice after NS-398 treatment than in nontreated $H.\, pylori$-infected mice, but this difference was not statistically significant (Fig. 4). When noninfected mice were treated with indomethacin or NS-398, no significant change in the apoptotic indices was observed compared to the control group (Fig. 4).

**Proliferation of gastric epithelial cells.** To determine whether $H.\, pylori$ infection increases proliferation in mouse mucosa and how NSAIDs affect the gastric epithelial cell hyperproliferation induced by $H.\, pylori$, epithelial cell proliferation was quantified after challenge with $H.\, pylori$ and/or NSAIDs. Immunostaining of Ki67 was localized in the nuclei, and
virtually all epithelial cells that stained positively for Ki67 were located within the proliferating compartment, which is located in the upper one third, the glandular neck of the body, and the basal zone of the antrum (Fig. 1E and F). The cell proliferation index was significantly increased after H. pylori infection (P = 0.002), and this effect was significantly decreased by indomethacin (P = 0.011) (Fig. 5). On the other hand, NS-398 treatment after H. pylori infection led to lower cell proliferation indices compared to the H. pylori-infected mice, but this difference was not significant (Fig. 5). When indomethacin or NS-398 was administered in the absence of H. pylori infection, no significant changes in cell proliferation were observed compared to the controls (Fig. 5).

**Inflammation of gastric mucosa.** To determine how NSAIDs affect H. pylori infection-induced inflammatory activity in the stomach, we quantified neutrophils as indicators of active inflammatory activity and mononuclear cells as indicators of chronic inflammatory activity, based on the updated Sydney system (9).

The neutrophil score was significantly increased after H. pylori infection (P = 0.004), and this effect was decreased significantly by indomethacin (P = 0.012) (Table 1). The mononuclear cell score showed a similar pattern, increasing after H. pylori infection (P = 0.001) and decreasing after further treatment with indomethacin (P = 0.007) (Table 1). NS-398 treatment after H. pylori infection did not induce significant reductions in the neutrophil and mononuclear cell scores compared to the H. pylori-infected mice (Table 1).

When indomethacin or NS-398 treatment was used in the absence of H. pylori infection, no significant change was observed in the neutrophil and mononuclear cell scores, except in the case of the indomethacin-treated mice, which showed a significant increase in the mononuclear cell score (P = 0.018), compared to the controls (Table 1).

**Correlations between cell apoptosis, proliferation, and inflammatory activity.** To determine whether increased apoptosis and proliferation may be related in a compensatory manner and whether the effect of H. pylori on mucosal cell cycle events in vivo may be associated with mucosal inflammation, we examined the relationships between apoptosis, proliferation indices, and inflammatory activity. In no group of mice was a correlation found between increased apoptosis and proliferation indices of epithelial cells or between increased apoptosis or proliferation index and inflammatory activity.

### Discussion

According to epidemiological studies (40, 56), H. pylori-infected subjects have a significantly higher risk of developing peptic ulcer or gastric malignancy. It has been hypothesized that the alteration of the balance between apoptosis and the proliferation of gastric epithelial cells, induced by H. pylori infection, contributes to either gastric injury such as gastritis and peptic ulcer or carcinogenesis of the stomach (2, 7, 34). Although H. pylori decreases epithelial cell proliferation and induces apoptosis in vitro (5, 45, 47, 59), H. pylori infection in vivo is associated with both increased apoptosis and proliferation (12, 41, 42). These differences between the in vivo and in vitro results suggest that in vivo, factors including inflammatory cells, extracellular matrix proteins, cytokines, and adhesion molecules may also contribute to epithelial cell turnover. The present study also confirmed that H. pylori infection increases the rates of gastric epithelial cell apoptosis and proliferation, as reported previously (12, 23, 34, 39, 41, 42).

Epidemiological, clinical, and animal studies have shown that NSAIDs have an antineoplastic effect in colon cancer, and NSAIDs also appear to reduce the incidence of and mortality from gastric cancer (17, 57). The mechanisms responsible for these remarkable antineoplastic effects are not understood, but emerging data indicate that NSAIDs are likely to target several steps along the carcinogenic pathway. These include NSAID-induced inhibition of cell turnover, such as the induction of apoptosis and inhibition of cell proliferation, stimulation of immune surveillance, and antiangiogenic effects (22, 51, 55). In the case of NSAID-induced gastric damage, it has been also shown that NSAIDs induce the apoptosis of epithelial cells and delay ulcer healing (52), and in combination with decreased

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**Table 1. Neutrophil activity and degree of chronic inflammation in stomachs of H. pylori-infected mice with or without NSAID treatment**

<table>
<thead>
<tr>
<th>Groupa</th>
<th>Median score (range)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, control</td>
<td>Neutrophil 0 (0–0.5)</td>
</tr>
<tr>
<td>B, H. pylori infection</td>
<td>1.5 (0.5–2.0)c</td>
</tr>
<tr>
<td>C, H. pylori infection followed by indomethacin treatment</td>
<td>0.5 (0–1.0)d</td>
</tr>
<tr>
<td>D, H. pylori infection followed by NS-398 treatment</td>
<td>0.5 (0–1.5)</td>
</tr>
<tr>
<td>E, indomethacin treatment</td>
<td>0.5 (0–1.0)c</td>
</tr>
<tr>
<td>F, NS-398 treatment</td>
<td>0 (0–1–0)</td>
</tr>
</tbody>
</table>

a A, control; B, H. pylori infection; C, H. pylori infection followed by indomethacin (2 mg/kg) treatment; D, H. pylori infection followed by NS-398 (10 mg/kg) treatment; E, indomethacin treatment; F, NS-398 treatment.

b Graded from 0 to 3 by a single pathologist.

c Significantly different (P < 0.05) from controls.

d Significantly different (P < 0.05) from group B as calculated by the Mann-Whitney U test.
gastric PG production, this apoptosis may serve as an important prerequisite for the gastric damage caused by NSAIDs (34, 44, 53). In the present study, NSAID administration without H. pylori infection induced no significant changes in the proliferative and apoptotic activities of gastric epithelial cells. In other studies, the gastric mucosal cell proliferation rates in subjects given NSAIDs have been reported to be enhanced (31) or to be unaffected (48), and the increased turnover of gastric mucosa has been hypothesized to represent an adaptive response, which is a process that gradually resolves early gastric mucosal damage, despite continued exposure to NSAIDs (15, 31). Therefore, we believe that our results regarding effects of NSAIDs on the epithelial cell turnover rate of gastric mucosa may be explained in part by the adaptive response of gastric mucosa.

Considering the combined effects of H. pylori and NSAIDs on cell turnover in the stomach, interestingly, indomethacin suppressed the apoptotic activities enhanced by H. pylori infection and reversed H. pylori-mediated cell proliferation in our study. Similar findings were reported in a clinical human study (66). From these results, although the mechanism underlying this effect is unclear and complicated, it appears that NSAID administration can suppress uncontrolled apoptosis and proliferation in H. pylori-infected subjects and that this effect might be extended to subsequent processes involving neoplastic changes. We also found that both of the inflammatory activities increased by H. pylori infection were also significantly reduced by indomethacin, suggesting the anti-inflammatory activity of NSAIDs in the H. pylori-infected stomach. These interesting results on cell turnover and inflammation lead to speculation on the complicated mechanisms involving inflammatory cells, cytokines, direct H. pylori stimuli, etc., in the interaction between H. pylori and NSAIDs in the gastric mucosa.

Our results also suggest that apoptosis and proliferation may be compensatory mechanisms, counteracting excessive cell proliferation and apoptosis, respectively (35). However, we found no correlation between cell proliferation and apoptosis in H. pylori-infected and/or NSAID-treated mice, in agreement with the results of other studies (41, 66). This observation supports the notion that the induction of apoptosis and proliferation in vivo are independent events regulated by different factors, including the direct effects of H. pylori stimuli and host inflammatory and immune responses, involving cytokines, nitric oxide, gastrin, etc. (8, 20, 36, 50).

In addition, because chronic inflammation may be a recognized risk factor for epithelial carcinogenesis (63), we can speculate about the association between cell turnover and inflammatory activity. However, in many other reports, inflammatory cytokines, oxygen free radicals, direct H. pylori stimuli, and other factors following H. pylori infection are believed to contribute to the increased gastric epithelial cell turnover (11, 32, 33) rather than the inflammation itself. Although the number of subjects involved was limited, our data also show that there is no correlation between the degree of inflammation and apoptosis or proliferation, as reported previously (33, 39, 66).

In a clinical report on the interaction between NSAIDs and H. pylori, Hawkey et al. showed that H. pylori eradication in long-term users of NSAIDs, with past or current peptic ulcer or troublesome dyspepsia, led to a impaired healing of the gastric ulcers (19), implying that under certain circumstances some patients with H. pylori are less prone to NSAID-induced ulceration than noninfected patients. This may be due to the opposing effects of H. pylori and NSAIDs on PG synthesis in the gastric mucosa (18). However, other studies have shown that the eradication of H. pylori prior to NSAID therapy reduces the occurrence of peptic ulcers (4) and the level of apoptosis in gastric mucosae (29), and that NSAID users infected with H. pylori carry a greater risk of peptic ulcer than noninfected NSAID users (1). It was also reported that H. pylori infection may reduce the adaptation threshold and that the eradication of H. pylori restored the ability of the gastric mucosa to adapt to aspirin (26). However, in our results showed no significant difference between the proliferative, apoptotic, and inflammatory activities of NSAID-treated mice with H. pylori infection and NSAID-treated mice without H. pylori infection, suggesting that H. pylori infection may not induce significant additive harmful effects in the stomachs of subjects administered NSAIDs, as reported previously (27, 64).

The aforementioned antineoplastic and anti-inflammatory effects of NSAIDs are commonly attributed to the inhibition of COX-2, the inducible form of COX (62). The levels of PGs and COX-2 gene expression are elevated in colon cancer tissue (10, 65), and the overexpression of COX-2 in epithelial cells inhibits apoptosis (54, 58). Although some NSAIDs were shown to induce apoptosis by a mechanism that may be unrelated to the ability of these drugs to inhibit PG synthesis (43), PG and COX-2 have an important role in cell proliferation and carcinogenesis and are elevated in H. pylori-infected gastric mucosa (13). In terms of COX expression as measured by Western blotting in our mouse model, both COX-2 and COX-1 expression levels were found to be elevated in the gastric tissue of H. pylori-infected mice compared with control mice. Wallace et al. showed that an inflammatory response could be elicited in COX-2−/− mice as well as wild-type mice and that this response could be reduced in both groups by indomethacin but not by a selective COX-2 inhibitor, which strongly suggests that COX-1 plays a role in the inflammatory response (60). Furthermore, other studies have suggested that like COX-2, COX-1 can be induced by H. pylori (3, 21) or by stressful stimuli such as radiation injury (6). In addition, in our present study, along with the increased expression of both COX-1 and COX-2 in the gastric tissue of H. pylori-infected mice, indomethacin treatment after H. pylori infection induced more significant decreases in the enhanced apoptotic, proliferative, and inflammatory activities of the H. pylori-infected stomach than NS-398 treatment after H. pylori infection. Therefore, it is possible that like COX-2 activation, some degree of COX-1 induction may also contribute to increased cell turnover and the inflammatory activity induced by H. pylori infection in gastric tissue. Although it is questionable that these effects translate to human disease, recently Jackson et al. showed the importance of COX-1 in H. pylori-infected humans (21). Therefore, from the viewpoint of both gastric injury and carcinogenesis, it will be interesting to compare the effects of nonselective and selective COX-2 inhibitors on gastric events including epithelial cell kinetics, gastric injury, and its molecular mechanism in H. pylori-infected humans.

In summary, NSAIDs can reverse the increased apoptosis
and proliferation of epithelial cells and the inflammatory activity induced by *H. pylori* in the mouse stomach, and *H. pylori* does not potentiate significantly the effects on cell turnover and inflammation induced by NSAID treatment. In addition, like COX-2 activation, some degree of COX-1 induction might also contribute to the changes in gastric mucosal cell turnover and inflammation induced by *H. pylori* infection.

**REFERENCES**


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