Induction of Cyclooxygenase 2 in Gastric Mucosal Lesions and Its Inhibition by the Specific Antagonist Delays Healing in Mice

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Background & Aims: The role of two forms of cyclooxygenase (COX-1 and COX-2) in gastric mucosal lesions is not well understood. The regulation of both forms of COX and the effect of COX-2 on the repair process of gastric mucosal lesions in mice were investigated.

Methods: Gastric mucosal erosions and ulcers were induced experimentally in mice. The level of COX messenger RNA (mRNA) was determined by reverse-transcription polymerase chain reaction. COX proteins were detected by Western blot analysis, and COX activity was determined in the presence or absence of NS-398, a specific COX-2 antagonist. The effects of long-term administration of NS-398 on gastric ulcers were examined.

Results: COX-2 mRNA levels were not detected in control conditions but were high during the acute stages of gastric erosions and ulcers. COX-2 protein was detected 5 days after ulcer induction but not in control mice. Gastric ulceration was not associated with a change in COX-1 mRNA and protein levels. Administration of NS-398 to mice with ulcers at acute stages impaired the healing of ulcers.

Conclusions: High levels of COX-2 mRNA and protein during the acute stages of gastric mucosal lesions may be involved in the repair process of these lesions in mice.

Prostaglandins (PGs) are known to protect the gastric mucosa against injury caused by a variety of necrotizing agents. Furthermore, PGE_2 stimulates the secretion of gastric mucus, bicarbonate, and surfactant-like phospholipid. Nonsteroidal anti-inflammatory drugs (NSAIDs) may cause gastric mucosal injury in humans and experimental animals by inhibiting the biosynthesis of PGs, thus acting as cyclooxygenase (COX) inhibitors. These data suggest that PGs play a physiological role in maintaining the integrity of gastric mucosa. However, only limited information is available regarding the site of synthesis and regulation of endogenous PGE_2 in the stomach. The regulation of PG production during the acute stage and the subsequent repair process of gastric lesions are also not understood at present.

The rate-limiting steps in eicosanoid biosynthesis are not only the liberation of arachidonic acid (AA) from membrane glycerophospholipids but also the conversion of AA to PG by PG endoperoxide synthase/COX. Several recent studies have confirmed the presence of two forms of COX, a constitutively produced COX-1 and an inducible COX-2.

Abbreviations used in this paper: AA, arachidonic acid; CHAPS, 3-(3-cholamidopropyl)-dimethylammonio)-1-propane-sulfonate; COX, cyclooxygenase; dCTP, deoxycytidine triphosphate; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse-transcription polymerase chain reaction; TGF, transforming growth factor.
Preparation of Mice With Gastric Erosions

Male mice, weighing 30–35 g, were fasted for 24 hours and administered pentobarbital (10% of body weight) subcutaneously. After anesthetization, the abdomen was opened through a midline incision, and 50 mL of 20% acetic acid was injected in the subserosa of the anterior wall of the stomach. The mice were killed 1, 3, 6, 12, and 24 hours after the administration, and the extent of gastric mucosal lesions was estimated by measuring the total length of all erosions. Total cellular RNA was extracted as described below. For histological examination, the stomach was fixed with 10% buffered formalin followed by H&E staining.

Preparation of Mice With Gastric Ulcers

Gastric ulcers were induced experimentally in mice according to the method described by Wang et al. Briefly, after anesthetization with pentobarbital (5 mg/100 g body wt), the abdomen was opened through a midline incision, and 50 µL of 20% acetic acid was injected in the subserosa of the anterior wall of the stomach. In control mice, the abdomen was opened and closed without injection. The stomach was opened and frozen immediately in liquid nitrogen until further analysis or fixation as described above.

The experimental protocol was approved by the Animal Care Committee of our institution.

Northern Blot Analysis for Accumulation of COX mRNA

Gastric tissue samples of 10-mm diameter with or without ulcers were trimmed, and the total cellular RNA was extracted using the standard guanidine thiocyanate method. mRNA was extracted from the total RNA using oligo(dT) latex. The mRNA (3 µg) was electrophoresed in 1.0% agarose gel and transferred onto a nylon filter. A 1.8-kilobase fragment of mouse COX-1 cDNA and a 1.2-kilobase fragment of mouse COX-2 cDNA, kindly donated by Dr. David L. DeWitt, Michigan State University, were 32P-labeled by the random primer method and used as cDNA probes for hybridization. Prehybridization for 2 hours and hybridization for 18 hours were performed at 42°C for both COX-1 and COX-2. Autoradiography was performed at −70°C using XAR-5 film.

Reverse-Transcription PCR Analysis of COX-1 and COX-2 mRNA

To compare the level of mRNA in stomachs with erosions or ulcers at different time intervals, we used the reverse-transcription (RT)-PCR method as described previously. Briefly, a variable amount of total RNA (0.125, 0.25, 0.5, 1, and 2 µg) was dissolved in 20 µL of a reaction mixture of cDNA synthesis kit containing deoxynucleotide triphosphate mixture, 100 pmol random primer, and 40 U of murine leukemia virus reverse transcriptase and was then reverse transcribed. The resulting cDNA product was precipitated with 100% ethanol and resolved in 10 µL of water. For amplification, 1.0-µL aliquot of the cDNA corresponding to cDNA prepared from either 12.5, 25, 50, 100, or 200 ng of total RNA was incubated in a total volume of 10 µL of Gene Amp PCR reagent kit containing a primer pair of either COX-1, COX-2, or glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA as well as 1.0 U of Taq polymerase and deoxynucleotide triphosphates in the presence or absence of [32P]dCTP. The thermal profile used in the Perkin-Elmer Cetus thermal cycler consisted of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension temperature at 72°C for 1 minute. The cDNA was amplified at 20, 25, or 30 cycles to determine the amplification cycle at which PCR products increased linearly with increases in total RNA used at cDNA synthesis reaction. The products were electrophoretically separated on agarose gels, the bands corresponding to either COX-1, COX-2, or G3PDH were cut off, and the radioactivity of the bands was determined.

Oligonucleotides Used for PCR

Mouse COX-1 cDNA was amplified by 24-mer oligonucleotides with the following sequences: 5’-AGTGGGCCATACCACTGTCATGC-3’ as a sense primer (bases 40–63) and 5’-CAGGAAAAATGGTGAACGAGGGCT-3’ as an antisense primer (bases 318–295). This yielded an amplification product of 279 base pairs in the length of mouse COX-1 cDNA. COX-2 cDNA was amplified by two pairs of primer oligonucleotides with the following sequences. One primer pair consisted of 5’-GGCCACCACTGCACATCCATGTC-3’ as a sense primer (bases 259–280) and 5’-CTTGGATCCCCATGTCCTTG-3’ as an antisense primer (bases 594–571) (primer pair A). Another primer pair consisted of 5’-TCAAAGAAGTCTGGAAGGAGTT-3’ as a sense primer (bases 603–626) and 5’-TCTACCTGAGTGTCCTGTGACT-
GTG-3' as an antisense primer (bases 898–875) (primer pair B). These primer pairs yielded amplification products of 336 base pairs in the former and 296 base pairs in the latter of mouse COX-2 cDNA. The sense primer for the detection of mouse G3PDH cDNA spanned oligonucleotide bases 51–76 (5'-TGAAGGGTGGTGAACGGGATTGGC-3'), and the antisense primer spanned bases 1033–1010 (5'-CATGTA-GGCCATGAGGTCCACAC-3'). This yielded an amplification product of 983 base pairs in the length of mouse G3PDH cDNA.

The amplified fragments were subcloned and sequenced by the dideoxy chain termination method. The sequences of the PCR fragments were identical to the published mouse COX-1, COX-2, and G3PDH sequences (data not shown).

**Western Blot Analysis of COX-1 and COX-2 Proteins**

COX protein was purified partially as described by Gierse et al. Stomachs with or without ulcers were homogenized in 25 mmol/L Tris-HCl (pH 8.1), 0.25 mol/L sucrose containing 1.0 mmol/L phenylmethylsulfonyl fluoride, 1.0 µmol/L pepstatin A, and 1.0 mmol/L ethylenediaminetetraacetic acid. The pellet was collected by centrifugation at 10,000 g for 2 minutes and resuspended in the same buffer. CHAPS was added to 1% (wt/vol), and the mixture was stirred for 2 hours at 4°C. After centrifugation at 50,000 g for 20 minutes, the supernatant was loaded onto an anion-exchange column equilibrated with 20 mmol/L Tris-HCl (pH 8.1) plus 0.4% CHAPS. Fraction eluted at 10,000 g for 2 minutes and resuspended in the same buffer. CHAPS was added to 1% (wt/vol), and the mixture was stirred for 2 hours at 4°C. After centrifugation at 50,000 g for 20 minutes, the supernatant was loaded onto an anion-exchange column equilibrated with 20 mmol/L Tris-HCl (pH 8.1) plus 0.4% CHAPS. Fraction eluted at 150 mmol/L NaCl was concentrated to 10% of the initial volume by centrifugation at 10,000 g for 5 minutes, 4 mL of organic phase was dried, resolved in 50 L methanol, and then spotted on silica gel 60 plates.

The plates were developed in a solvent system consisting of chloroform/acetic acid/methanol/water (vol/vol, 90:1:8:0.8), the bands corresponding to PGA2, PGE2, and PGF2α, and AA were scraped, and radioactivity was counted. Protein concentration was determined by the method of Bradford.

**Administration of NS-398 to Mice With Gastric Ulcers**

To examine the effect of NS-398 on the healing and repair process of gastric ulcers, NS-398 was administered intraperitoneally to mice with or without ulcers. NS-398 at a single dose of 1.0 mg/100 g body wt suspended in 0.3 mL of 5% gum arabic solution was administered in the morning. The compound was injected 1 day (group A), 5 days (group B), or 10 days (group C) after ulcer induction and continued until 30 days after ulcer induction. Gum arabic solution only was administered intraperitoneally to a group of mice with ulcers (control). The mice were later killed, and the maximal diameter of the ulcers, representing the ulcer index, was measured 5, 10, 20, and 30 days after ulcer induction.

**Statistical Methods**

Data are expressed as means ± SEM. Statistical comparison of group data was performed using analysis of variance followed by Student’s t test. Differences were considered significant at P values of <0.05.

**Results**

**COX mRNA Level in Normal Gastric Tissue**

In the first step, we examined the levels of COX-1 and COX-2 mRNAs in normal gastric tissues. Northern blot analysis using COX-1 and COX-2 cDNA probes showed a 2.8-kilobase band hybridized with COX-1 cDNA probes (Figure 1). Accumulation of mRNA hybridized with COX-2 cDNA probe was not observed, suggesting that only COX-1 mRNA is present in normal gastric tissue.

**Experimentally Induced Gastric Erosions**

Oral ingestion of acidi®ed ethanol produced multiple linear erosions in the stomach within 1 hour. Figure 2 shows sections of normal gastric mucosa and mucosa with erosions 3 and 24 hours after ingestion of acidi®ed ethanol. Although the lesion extended to the deep part of the mucosal layer, it did not penetrate the muscularis mucosa.
Northern blot analysis for COX-1 and COX-2 mRNA accumulation. mRNA from mouse gastric mucosa was separated on 1.0% agarose gel and transferred onto a nylon filter. The mRNA was hybridized with 32P-labeled COX-1 (left) or COX-2 cDNA (right) probes as described in Materials and Methods.

**Figure 1.**

COX mRNA Levels Determined by RT-PCR

We also compared the level of COX-2 mRNA in tissue samples from mice with erosions at different time intervals using RT-PCR. In this process, we first assessed whether COX-1 and COX-2 mRNA could be measured semiquantitatively. RT-PCR was performed using varying amounts of template RNA obtained from areas of gastric erosions induced by ingestion of acidified ethanol 6 hours earlier. A comparison of agarose gel densities stained by ethidium bromide showed that PCR products increased proportionately with template RNA (Figure 3A). The radioactivity of the band corresponding to COX-1, COX-2, and G3PDH cDNA produced by RT-PCR indicated that this approach could be used for semiquantitative measurement, as described previously, when cDNA products reverse-transcribed using total RNA of <200 ng were amplified at 25 cycles (Figure 3B).

**Gastric Erosions and COX mRNA Levels**

The severity of gastric erosions was estimated quantitatively by measuring the total length of all erosions at 1, 3, 6, 12, and 24 hours after ingestion of acidified ethanol. Erosions were detected at 1 hour, became more extensive at 3 hours, diminished at 12 hours, and were almost absent at 24 hours (Figure 4A).

PCR was also performed using cDNA products prepared at 100 ng total RNA to semiquantitate COX-1 and COX-2 mRNA levels. COX-2 cDNA fragment was not amplified by primer pair A in PCR when RNA from control gastric tissue was reverse-transcribed. However,
primer pair B also strongly amplified a 296–base pair fragment of COX-2 cDNA in a manner similar to primer pair A. These results suggest a rapid accumulation of COX-2 mRNA that was dependent on the induction and severity of gastric mucosal damage.

Specific primer pairs for COX-1 and G3PDH amplified a 279–base pair and a 983–base pair fragment of COX-1 and G3PDH cDNA, respectively, even when RNA from control gastric tissue was reverse-transcribed (Figure 4B). The amplified levels of cDNA remained stable throughout the observation period, suggesting that gastric mucosal damage did not influence the level of COX-1 and G3PDH mRNAs.

**COX mRNA Levels in Gastric Ulcers Prepared by Injection of Acetic Acid**

Histological examination of acetic acid–induced gastric ulcers at 5 days showed a typical gastric ulcer at acute stage consisting of a necrotic ulcer bed with leukocyte infiltration, whereas examination of ulcers at 20 days showed reepithelialized granulation tissues (Figure 5).

Tissue samples from gastric ulcers of 10-mm diameter at days 5 and 10 were obtained for PCR. COX-2 primer pair A amplified a 336–base pair COX-2 cDNA fragment (Figure 6). The primer pair B specific for COX-2 cDNA also strongly amplified a 296–base pair fragment of COX-2 cDNA when RNA from gastric ulcer tissues was reverse-transcribed. The two sets of primer pairs for COX-2 cDNA showed that the level of COX-2 mRNA in gastric ulcer tissues 20 days after ulcer induction was less than in tissues at 5 or 10 days. Similar to experiments using tissue from gastric erosions, a primer pair specific
Figure 7. Serial changes in ulcer indexes and COX mRNA levels in gastric tissues with ulcers. (A) The maximum diameter of the ulcer expressed as the ulcer index was measured and plotted against indicated time intervals. (B) RT-PCR was performed in the presence of \(^{32}\)P-dCTP, the bands corresponding to COX-1, COX-2, and G3PDH cDNA products were cut off, and the radioactivity was measured. Each value is expressed as percent of the control value, and the data are expressed as the means ± SEM of four separate experiments. \(^{+}\)P < 0.01 compared with controls. △, COX-1 cDNA product; ○, COX-2 cDNA product; □, G3PDH cDNA product.

Figure 6. Serial changes in COX mRNA levels in gastric tissues with ulcers. RT-PCR was performed using RNA extracted from gastric ulcer tissues at different time intervals. PCR products were separated on 1.8% agarose gels and stained with ethidium bromide. COX-2 A and COX-2 B indicate a 336- and a 296-base pair fragment of COX-2 cDNA, respectively.

PCR primers Days

for COX-1 cDNA amplified a 279-base pair fragment of COX-1 cDNA even when RNA from control gastric tissue was reverse-transcribed. Furthermore, the level of COX-1 mRNA remained stable between days 5 and 20 in these tissue samples. A primer pair specific for G3PDH cDNA also amplified a 983-base pair fragment of mouse G3PDH cDNA. However, the level of G3PDH mRNA remained stable in gastric tissues with or without ulcers.

Comparison of Ulcer Indexes With COX mRNA Levels

We measured the maximum diameter of ulcers at 5, 10, 20, and 30 days representing the ulcer index. The index was highest at day 5 (4.3 ± 0.5 mm) but decreased gradually to 3.6 ± 0.3 mm and 0.8 ± 0.5 mm on days 10 and 20, respectively. No open ulcer was observed at day 30 (Figure 7A). We also examined the level of radioactivity corresponding to the amplified COX-1, COX-2, and G3PDH cDNA fragments at different time intervals (Figure 7B). The analysis showed that increased ulcer index was associated with increases in COX-2 mRNA levels but not COX-1 and G3PDH.

Western Blot Analysis of COX-1 and COX-2 Proteins

Western blot analysis of eluates from anion exchange chromatography of gastric mucosal lysates showed that anti-COX-2 antibody recognized one major immu-
noreactive protein band in samples prepared from stomachs with ulcers at day 5, whereas this antibody did not recognize any protein band in samples from control gastric tissue. The relative molecular size of the protein was estimated to be 72 kilodaltons. Purified sheep COX-2 protein closely corresponding to the molecular size of the immunoreactive protein was specifically recognized by the antibody (Figure 8). On the other hand, reblotting using anti-mouse COX-1 monoclonal antibody of the same sheet showed a single immunoreactive protein band in both samples prepared from stomachs with or without ulcers. The relative molecular size of the protein was smaller than that of the major immunoreactive protein detected by the anti-COX-2 antibody and was estimated to be 70 kilodaltons (Figure 8). These results suggest that the increased accumulation of COX-2 mRNA was associated with increased expression of COX-2 protein in stomachs with ulcers.

**COX Activity in Gastric Tissues Before and After Gastric Ulceration**

We also determined COX activity in gastric tissue samples with or without ulcers. Autoradiography of a thin-layer chromatography plate showed faint bands corresponding to PGA₂, PGE₂, and PGF₃α when control gastric tissue homogenates were analyzed (Figure 9A). The calculated COX activity in control gastric tissue was 3.99 ± 0.50 pmol · min⁻¹ · mg protein⁻¹. On the other hand, when samples from gastric tissues with ulcers at days 5 and 10 were analyzed, clear bands corresponding to PGA₂, PGE₂, and PGF₃α were observed. The COX activity at days 5 and 10 was greater at 9.15 ± 1.25 and 7.15 ± 1.30 pmol · min⁻¹ · mg protein⁻¹, respectively. Thereafter, the activity declined and reached almost the baseline level at day 20 (Figure 9B).

Because the increased COX activity paralleled that of COX-2 protein in gastric ulcer tissues, we also examined the effect of NS-398 on COX activity. NS-398 inhibited COX activity in a dose-dependent manner in homogenates prepared from ulcer tissues at day 5 with the maximal effective dose at 100 μmol/L (data not shown). Although NS-398 at 100 μmol/L significantly inhibited COX activity in these tissues at day 5, it did not affect the basal COX activity in control tissues. On the other hand, indomethacin, a known nonselective COX antagonist, inhibited COX activity in both control and gastric ulcer tissues at 100 μmol/L (Figure 10). These results indicate that the elevated COX activity in gastric ulcer tissues is primarily caused by elevated expression of COX-2 protein that can be inhibited effectively with NS-398.

**Effect of NS-398 on Gastric Ulcer Healing**

To examine the effect of COX-2 protein–dependent increased COX activity on the ulcer repair process, we determined the influence of intraperitoneally administered NS-398 on ulcer index at different stages after ulcer induction. NS-398 administered just after ulcer induction (group A mice) did not influence the ulcer index at day 5. However, the same treatment significantly increased the ulcer indexes at days 10, 20, and 30 in the same group of mice (day 10: before, 3.6 ± 0.3 mm; after, 4.7 ± 0.7 mm; P < 0.05; day 20: before, 0.8 ± 0.5 mm; after, 2.4 ± 0.4 mm; P < 0.05; and day 30: before, 0 mm; after, 0.9 ± 0.5 mm; P < 0.05). Similarly,
the ulcer indexes in group B mice at days 10, 20, and 30 were also significantly greater than in controls (Figure 11). Although the indexes were greater in group A mice than in group B mice, only the ulcer index at day 20 in group A mice was significantly different from that in group B mice. On the other hand, ulcer indexes in group C mice were not significantly different from controls, suggesting that treatment with NS-398 after the acute stage of gastric ulceration does not delay ulcer healing. We examined also the long-term effect of NS-398 on the induction of gastric ulcers and gastric erosions. Gastric ulcers did not develop in mice when NS-398 was administered at the same dose (10 mg/kg) for 30 days. Therefore, the repair process of ulcers seems to be impaired when COX-2 activity of the ulcer tissue was inhibited by NS-398 in the acute stage.

**Discussion**

In this study, we describe for the first time a marked accumulation of COX-2 mRNA in gastric mucosal erosions and ulcers. COX-2 protein expression detected by specific anti–COX-2 antibody was also observed in partially purified lysates prepared from stomachs with ulcers. Although COX-1 mRNA and protein were clearly observed in control tissues, their levels did not change during the healing process of gastric ulceration. Furthermore, we detected increased COX activity in ulcer tissues that was proportionate with increased levels of COX-2 mRNA and protein. NS-398, a specific antagonist for COX-2 enzyme, inhibited COX activity in vitro in homogenates of gastric ulcer tissues but not in the control tissue. Our results suggest that the increased production of PG in tissues surrounding ulcerated gastric mucosa in animal models may be caused by increased accumulation of COX-2 mRNA and protein.

Our results also indicated that COX-2 enzyme may play an important role during gastric ulcer healing and repair. Daily administration of NS-398 beginning with the early stage of ulcer induction (days 1–5) caused significant impairment of healing. However, late treatment (day 10 after ulcer induction) failed to influence the ulcer healing process. The dose of NS-398 used in the present

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**Figure 10.** Effect of NS-398 on COX activity in ulcer tissues. Basal COX activity and COX activity in ulcer tissues at 5 days were measured as described in Materials and Methods in the presence or absence of either NS-398 (100 μmol/L) or indomethacin (100 μmol/L). Data are expressed as the means ± SEM of four separate experiments. *P < 0.05 compared with the respective control value without inhibitors. C, control group; Ind, indomethacin-treated group; NS, NS-398–treated group; prot., protein.

**Figure 11.** Effect of daily administration of NS-398 on gastric ulcer healing. NS-398 suspended in 5% gum arabic was administered intra-peritoneally after ulcer induction as described in Materials and Methods. The maximal diameter of the ulcer was measured at the indicated time intervals. Data are expressed as the means ± SEM of six separate experiments. *P < 0.05; **P < 0.01 compared with the respective control value. A, group A mice; B, group B mice; C, group C mice; Co, control mice treated with 5% gum arabic solution only.
experiment has no effect on basal PGE2 contents in the gastric mucosa of rats. Thus, the delayed healing of gastric ulcers may be solely caused by the inhibition of COX-2 enzyme activity by NS-398, suggesting that the increased activity of COX-2 enzyme during the acute stage may be important for subsequent ulcer repair process.

Although acetic acid-induced ulceration of the stomach in the rat and mouse is a model for gastric ulcer in humans, the pathogenesis of gastric ulcers in the animal model may not be equivalent to that in humans. The present results together with those of other investigators suggest that endogenous PGE2 levels around the ulcerated area in the animal model may be significantly greater than those in the surrounding normal mucosa 5 and 10 days after ulcer induction. In contrast, the most plausible hypothesis of gastric ulceration in humans is that defects in PG synthesis and action on gastric mucosa weaken mucosal resistance or impair mucosal repair, leading to the development of chronic ulcers. 35,36 However, defects in PG production are not likely to be the only mechanism by which gastric ulceration is induced because COX-1 gene disruption in mice does not lead to any gastric pathology. Nevertheless, administration of PGE2 enhances natural healing even in animals with gastric ulcers. 26 These effects observed with exogenous PGE2 do not seem to be the result of replacement of PGE2 because elevated PGE2 levels are usually present around ulcerated areas. In addition, repeated administration of indomethacin, a compound known to inhibit the activity of COX-1 and COX-2, is known to delay spontaneous healing of gastric ulcers in the rat induced by the same method used in the present study. 26,38 Administration of PGE2 combined with indomethacin prevents the delay in ulcer healing. 26 Although it is not clear whether accumulation of COX-2 mRNA and protein around the ulcerated area is stimulated in humans, our results together with those of other investigators suggest that PGE2, even if it is attributed to the activity of either COX-1 or COX-2 enzyme, seems to be important for gastric mucosal resistance and mucosal repair process.

In the present study, we did not examine whether an increased rate of COX-2 gene transcription was directly stimulated in gastric mucosal ulcers or erosions. COX-2 mRNA has a shorter half life than COX-1 mRNA because of the presence of several copies of AUU motif conferring mRNA instability in the 3′-untranslated region of COX-2 mRNA. 40,41 However, TGF-β causes accumulation of COX-2 mRNA by stabilizing COX-2 mRNA in osteoblastic MC3T3-E1 cells. 42 Because TGF-β is thought to play a role in wound repair, e.g., ulcer healing, it may be involved in the accumulation of COX-2 mRNA in gastric ulceration. Further work is necessary to understand the mechanism of enhanced COX-2 mRNA accumulation in gastric erosions and ulcers.

We did not determine the exact location of COX-2 mRNA and enzyme in gastric ulcers. However, it is reasonable to speculate that macrophages, monocytes, and fibroblasts infiltrating the ulcer bed contribute to the high levels of COX-2 mRNA and enzyme in response to a variety of stimuli because accumulation of COX-2 mRNA is induced in these cells. In addition to such inflammatory cells, DuBois et al. have shown recently that TGF-α or a tumor promoter, tetradecanoyl phorbol acetate, enhance the accumulation of a 4.5-kilobase mRNA, which hybridizes with mouse COX-2 cDNA probe in rat intestinal epithelial cells cultured in vitro. These investigators also suggest that the rat intestinal epithelial cells overexpressing COX-2 protein are resistant to butyrate-induced apoptosis and reduced TGF-β2 receptor levels. We have also found recently that the epidermal growth factor enhances the accumulation of a 5.1-kilobase mRNA that hybridizes with mouse COX-2 cDNA probe in guinea pig gastric epithelial cells in vitro. Epidermal growth factor further stimulates the expression of a 70-kilodalton protein in the guinea pig gastric epithelial cells blotted by anti-COX antiserum that immunologically recognizes both COX enzymes. Thus, it is of interest and remains to be clarified whether gastric epithelial cells produce COX-2 enzyme during the ulcer repair process.

Recently, the specificity of NSAIDs for COX-2 was examined to develop nonulcerogenic drugs that cause a selective inhibition of COX-2 activity. Nonspecific inhibition of PG production in organs such as the stomach and kidney by NSAIDs can result in gastric lesions and nephrotoxicity as reported previously. In fact, certain drugs, such as NS-398, 5-bromo-2-(4-fluorophenyl), 3-(4-methylsulfonylphenyl) thiophene (DuP 697), and SC58125, reduce the activity of COX-2 enzyme in vitro with a median infective dose of 3, 10, and 0.05 μmol/L, respectively, and complete inhibition
at 100, 100, and 10 μmol/L, respectively, without affecting COX-1 enzyme activity. With respect to PG production in rats in vivo, the median infective dose of NS-398 in the inflammatory exudate and gastric mucosa was 0.18 and 62.2 mg/kg, respectively. Oral administration of NS-398 at 10 mg/kg completely blocks PGE2 production in exudate of air-pouch inflammation, but this dose of NS-398 does not seem to inhibit gastric PGE2 content. Furthermore, NS-398 in a single dose of 1000 mg/kg fails to cause gastric ulceration in rats. In addition, in the present study, we found that long-term administration of NS-398 at 10 mg/kg for 30 days also failed to cause gastric lesions. On the other hand, indomethacin reduces PG production in the stomach with a median infective dose of 0.3–0.5 mg/kg in gastric lesions 6 hours after administration of a single dose at 10 mg/kg. These results suggest that a drug specifically targeted against COX-2 enzyme should produce better effects than a nonselective NSAID in the treatment of acute and chronic inflammatory disorders. However, NS-398 was found to inhibit COX activity in gastric ulcer tissues expressing COX-2 protein but not in normal gastric tissues in which COX-2 protein was not expressed. Furthermore, NS-398 impaired the healing of experimentally induced gastric ulcers. It is not known at present whether COX-2 mRNA and protein levels are elevated in ulcer-containing human gastric tissues. If accumulation of COX-2 mRNA and enzyme are similarly induced in the acute stage of gastric ulcer in humans in a manner similar to our animal experiments, it is possible that drugs specific for COX-2 enzyme may reduce PG production by inhibiting the enzyme. Therefore, even if COX-2–selective inhibitors are available, our results suggest that COX-2 antagonists may delay ulcer healing if used in the acute stage of gastric ulceration in humans.

Clearly, further studies are necessary to understand whether NSAIDs specific for COX-2 enzyme are nonulcerogenic and do not delay ulcer healing in humans.

References


