Cyclooxygenase (COX)-1 and COX-2 Both Play an Important Role in the Protection of the Duodenal Mucosa in Cats

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ABSTRACT

Although nonsteroidal anti-inflammatory drugs often cause ulcers in the duodenum in humans, the role of cyclooxygenase (COX) isoforms in the pathogenesis of duodenal ulcers has not been fully elucidated. We examined in cats the 1) ulcerogenic effects of selective COX-1 (SC-560, ketorolac) and COX-2 (celecoxib, meloxicam) inhibitors on the gastrointestinal mucosa, 2) effect of feeding and cimetidine on the expression of COX isoforms and prostaglandin (PG) E2 (PGE2) level in the duodenum, and 3) localization of COX isoforms in the duodenum. COX inhibitors were administered after the morning meal in cats once daily for 3 days. Gastrointestinal lesions were examined on day 4. Localization and expression of COX isoforms (by immunohistochemistry, Western blot) and PGE2 level (by enzyme immunoassay) were examined. Results were as follows. First, selective COX-1 or COX-2 inhibitors alone produced marked ulcers in the duodenum but did not cause obvious lesions in the small intestine. Coadministration of SC-560 and celecoxib produced marked lesions in the small intestine. Second, feeding increased both the expression of COX isoforms and PGE2 level in the duodenum, and the effects were markedly inhibited by pretreatment with cimetidine. Third, COX-1 was localized in goblet and Brunner's gland cells, Meissner's and Auerbach's plexus, smooth muscle cells, and arterioles; and COX-2 was observed in capillaries, venules, and basilar granulated cells. The expression of COX isoforms in the duodenum is up-regulated by feeding, and inhibition of either COX-1 or COX-2 causes ulcers in the duodenum, suggesting that both isoforms play an important role in the protection of the duodenal mucosa.

Introduction

There are two isoforms of cyclooxygenase (COX) in the gastrointestinal (GI) tract: COX-1, which maintains the integrity of the GI mucosa under physiologic conditions, and COX-2, which is implicated in invasive events such as inflammation (Mitchell et al., 1993; Masferrer et al., 1994; Takeuchi et al., 2010a). Therefore, it has been commonly believed that adverse effects on the GI tract caused by conventional nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (IND) are due to their inhibitory effect on COX-1 (Vane and Botting, 1995; Warner et al., 1999). This contention is based on the finding that selective COX-2 inhibitors such as celecoxib and rofecoxib neither decreased prostaglandin (PG) levels nor produced lesions in the GI mucosa in experimental animals (Putaki et al., 1993; Seibert et al., 1994; Laudanno et al., 2001). Morham et al. (1995) also reported that COX-2 deficiency did not cause spontaneous ulceration in the stomachs of COX-2 knockout mice. Conversely, Langenbach et al. (1995) proposed a query on the role of COX-1 in maintaining the integrity of the gastric mucosa, since COX-1 knockout mice were shown to have no damage to the stomach despite a marked decrease in gastric PG levels (<1% of wild-type mice). Wallace et al. (2000) also reported that the selective COX-1 inhibitor SC-560 did not cause any visible lesions in the rat stomach despite a marked decrease in PG levels, but that the coadministration of SC-560 and celecoxib induced obvious lesions in the stomach. Based on these results, it was proposed that simultaneous inhibition of both COX-1 and COX-2 is necessary for the formation of gastric lesions induced by NSAIDs. This notion was supported by studies (Gretzer et al., 2001; Tanaka et al., 2001) using SC-560 and the selective COX-2 inhibitor rofecoxib in a rat gastric lesion model. Similarly, inhibition of both COX isoforms is required to produce lesions in the small intestine in rats (Tanaka et al., 2002a). Sigthorsson et al. (2002) reported that short-term deficiency or inhibition of COX-1 or COX-2 alone did not cause any damage to the small intestine of mice, but that dual inhibition of COX isoforms led to damage similar to that seen with IND.

Although the ability of NSAIDs to cause ulcers in the duodenum of humans is well known (Hawkey 1990; Allison et al., 1992), it has not been widely reported whether inhibition of both COX isoforms is necessary for induction of

ABBREVIATIONS: COX, cyclooxygenase; GI, gastrointestinal; IND, indomethacin; IR, immunoreactivity; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; PGE2, prostaglandin E2; PO, by mouth; TLA, total lesion area.
NSAID-induced duodenal ulcers, probably due to the lack of suitable animal models for duodenal ulcers induced by NSAIDs. Recently, we reported that the nonselective COX inhibitor IND produced obvious lesions in the duodenum of cats (Satoh et al., 2009). Therefore, in the present study, we examined in cats the 1) ulcerogenic effects of selective COX-1 (SC-560 and ketorolac) and COX-2 (celecoxib and meloxicam) inhibitors (Smith et al., 1998; Warner et al., 1999) on the GI mucosa, 2) effects of feeding and cinetidine on the expression of COX isoforms and the prostaglandin E2 (PGE2) level in the duodenum, and 3) localization of COX isoforms in the duodenum. Our results showed that the expression of COX isoforms in the duodenum is up-regulated by feeding, and that inhibition of either COX-1 or COX-2 alone causes lesions in the duodenal mucosa in cats, suggesting that both isoforms play an important role in the protection of the duodenal mucosa.

Materials and Methods

Ethics Approval

Experimental protocols were approved by the Animal Research Committees, Division of Pathologic Sciences, Kyoto Pharmaceutical University, Kyoto Japan.

Animals

Male and female mongrel cats bred for the experiments in the animal house of Kyoto Pharmaceutical University were used after feeding for >8 months. During the experiments, the animals were given dry food (Canet; Petline, Gifu, Japan) twice daily between 9–10 AM and 5–6 PM.

Drugs

The following drugs were used: atropine sulfate (Tanabe, Tokyo, Japan), celecoxib (Cellex; Pfizer, Tokyo, Japan), cinmetidine (Wako, Osaka, Japan), indomethacin (Wako), ketorolac (Sigma, St. Louis, MO), meloxicam (Metacam; Boehringer Ingelheim, Hyogo, Japan), pentobarbital sodium (Nembutal; Dainippon-Sumitomo, Osaka, Japan), SC-560 (Alexis, Lausen, Switzerland), and xylazine (Ceractal; Bayer, Tokyo, Japan). Drugs for subcutaneous injection were dissolved in physiologic saline, whereas drugs for oral administration were suspended in 1% carboxymethylcellulose (Wako); 0.5 ml/kg of each drug was administered.

Antibodies

The primary antibodies used for immunohistochemistry and Western blot analysis were a polyclonal rabbit anti-ovine COX-1 antibody (Cayman Chemical, Ann Arbor, MI) and a polyclonal anti-COX-2 (COX-2) human C term (Oxford Biomedical Research, Oxford, MI). These antibodies have been used in immunohistochemical studies in cats (Hayes et al., 2006).

Macroscopic Examinations of Mucosal Damage in the GI Tract

Each NSAID was administered by mouth (PO), except for meloxicam (s.c.), 1 hour before or just after the morning meal once daily for 3 days, and the GI lesions were evaluated 24 hours after the final dose of NSAIDs. Cats were sacrificed by bleeding from the carotid arteries under deep anesthesia as described previously, and a 6-cm length of the proximal duodenum was removed from each animal and cut into two samples. The samples were either frozen in acetone/dry ice and stored at −80°C until Western blotting, or transferred to a tube containing 99.8% methanol plus 0.1 M IND and stored at −80°C until the assay of PGE2 by EIA.

Histologic Examinations

Staining with Hematoxylin and Eosin or Periodic Acid–Schiff. Serial sections (4 μm) of formalin-fixed, paraffin-embedded samples of each tissue were mounted on slides. According to conventional methods, either hematoxylin and eosin or periodic acid–Schiff (PAS) staining was performed.

Measurement of the Number of PAS-Positive Goblet Cells in the Villi. Photographs of the proximal duodenum stained with PAS in the normal control cats or in the cats given COX inhibitors were taken under a microscope (40×). Ten villi (>400 μm in length) were selected in each sample, and both the length of the villus and the number of PAS-positive cells were measured in each villus. The number of positive cells per 100-μm length of villus was calculated for each villus. The mean number of PAS-positive cells per 100 villi was then obtained for each cat.

Immunohistochemical Staining. According to the method described by Hayes et al. (2006), localization of COX isoforms in the duodenum was examined in normal control cats. The animals were sacrificed as described previously (fasted) or just after a 1-hour feeding period (fed). The proximal duodenum was removed and immersed in 20% formalin. Serial sections (4 μm) of formalin-fixed, paraffin-embedded samples of each tissue were mounted on MAS (Matsutani, Tokyo, Japan)-coated glass slides. Antigen retrieval was performed at 90°C for 20 minutes in HistoVT One Retrieval Solution (Nacalai Tesque, Kyoto, Japan). After deactivation of endogenous peroxidase with 0.3% H2O2 and blockade of nonspecific binding sites with 0.5% goat serum, the sections were reacted with anti-COX-1 or anti-COX-2 antibody 1:400 dilution over night at 4°C. COX-1 and COX-2 proteins were visualized using a Vectastain ABC peroxidase kit (Vector, Burlingame, CA). Identical samples incubated with the primary antibody omitted were used as negative controls. Slides were counterstained with hematoxylin.

Biochemical Examinations

In this study, four cats were used in each group, and duodenal samples were obtained before and 0.5 hour after a 1-hour feeding of diet after a 16-hour fast. In another study, cimetidine (40 mg/kg, PO) was administered 1 hour before feeding, and duodenal samples were obtained 0.5 hour after a 1-hour feeding period. The animals were sacrificed by bleeding from the carotid arteries under deep anesthesia as described previously, and a 6-cm length of the proximal duodenum was removed from each animal and cut into two samples. The samples were either frozen in acetone/dry ice and stored at −80°C until Western blotting, or transferred to a tube containing 99.8% methanol plus 0.1 M IND and stored at −80°C until the assay of PGE2 by EIA.

Western Blot Analysis for COX-1 and COX-2. The expression of COX proteins in the samples was assessed by Western blot analysis as previously described (Takeuchi et al., 2010b). Samples were homogenized with protease inhibitor cocktail tablets (Complete; Roche, Penzberg, Germany) and centrifuged at 20,000 g for 30 minutes at 4°C. Supernatants were used for protein determination. The protein concentration of each sample was measured using a BCA protein assay kit (Pierce, Rockford, IL). Fifty micrograms of protein underwent electrophoresis on sodium dodecyl sulfate (SDS)–12% polyacrylamide slab gels, and were electrothermically transferred to...

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a Polyvinylidene fluoride membrane (Immuno-Blot; Bio-Rad, Hercules, CA). The membrane was incubated with primary antibody for COX-1, COX-2, or β-actin at 4°C overnight. The membranes were then incubated with horseradish peroxidase–conjugated anti-rabbit or anti-mouse IgG (Santa Cruz, Santa Cruz, CA), or anti-mouse IgG horseradish peroxidase (Santa Cruz) as a secondary antibody for 2 hours. The immunoreactive bands were visualized by an enhanced chemiluminescence system (Western Blot Chemiluminescence Reagent Plus; NEN, Boston, MA), and developed films were scanned and analyzed densitometrically. The integrated density of the bands was quantified using Quantity One analysis software (Bio-Rad, Hercules, CA). Values are expressed as the relative intensity of the mean density (per β-actin) for four fasted cats.

**Determination of PGE2 Levels.** PGE2 levels in the duodenum were determined as previously described (Tanaka et al., 2002b). In brief, the duodenal samples were homogenized using a Polytron homogenizer (Kinematica, Sweden) and centrifuged at 10,000 g for 10 minutes at 4°C. After the supernatant of each sample had been evaporated under N2 gas, the residue was resolved in assay buffer and used for determination of PGE2. The concentration of PGE2 in the sample of duodenal homogenates was measured using a PGE2 EIA kit (Cayman Chemical). The protein concentrations of each sample were measured using BCA protein assay kits (Pierce).

**Statistics**

All data are expressed as the mean ± S.E.M. Differences between groups were analyzed by Student's t test for paired group comparisons, or analysis of variance (Dunnett’s multiple range test) if more than two variables were considered, with the significance level set at \( P < 0.05 \).

**Results**

**Effects of Selective COX Inhibitors on GI Mucosa**

**Macroscopic Observation.** In normal control cats (four fasted and four fed), no lesions were observed in the GI tract. When COX inhibitors were administered after feeding of the diet, none of the cats produced visible lesions in the stomach.

**Effects of Drugs Given after Feeding of Diet.** SC-560, a selective COX-1 inhibitor, at doses of 3 and 10 mg/kg PO produced severe lesions in the duodenum in a dose-dependent manner; the TLAs in the duodenum were 0.4 ± 0.1 cm² and 1.2 ± 0.1 cm², respectively (Fig. 1). Two or three large lesions were often observed in the anterior and posterior regions of the proximal duodenum just caudal to the pyloric ring (Fig. 2A). SC-560 at a dose of 3 mg/kg did not cause any visible lesions in the small intestine, but at 10 mg/kg, SC-560 caused mild lesions in the small intestine in two of the cats (Fig. 1). Another COX-1 inhibitor, ketorolac, at a dose of 3 mg/kg PO produced marked lesions in the duodenum (TLA: 0.6 ± 0.3 cm², \( n = 4 \)) in all cats but did not cause lesions in the small intestine (Fig. 1).

Celecoxib, a selective COX-2 inhibitor, at a dose of 10 mg/kg PO produced severe lesions in the duodenum in all four cats (Fig. 2B), and caused mild lesions in the small intestine in one cat. The TLAs in the duodenum and small intestine were 0.6 ± 0.2 cm² and 0.5 ± 0.3 cm², respectively. Another COX-2 inhibitor, meloxicam (0.6 mg/kg, s.c.), produced marked lesions in the duodenum in all four animals but did not cause any visible lesions in the small intestine (Fig. 1).

**Effects of Simultaneous Inhibition of Both COX-1 and COX-2.** Coadministration of SC-560 (3 mg/kg, PO) and celecoxib (10 mg/kg, PO) caused severe lesions not only in the duodenum but also in the small intestine (Fig. 1). The TLAs in the duodenum and small intestine were 0.7 ± 0.2 cm² and 4.1 ± 1.0 cm², respectively. The TLA of the small intestine was significantly (\( P < 0.01 \)) larger than that with administration of SC-560 or celecoxib alone (Fig. 1). As reported in our previous paper (Satoh et al., 2009), the nonselective COX inhibitor IND (3 mg/kg, PO) produced overt lesions in both the duodenum and the lower half of the small intestine in all four cats (Fig. 1). The TLAs in the duodenum and small intestine were 0.8 ± 0.2 cm² and 7.7 ± 2.0 cm², respectively.

**Effects of Drugs Given before Feeding of Diet.** When a low dose (3 mg/kg) of SC-560 was administered 1 hour before the morning meal once daily for 3 days, it produced severe lesions in the duodenum. The TLAs in the duodenum and small intestine were 1.0 ± 0.2 cm² and 0.1 ± 0.0 cm² (\( n = 4 \)), respectively. The TLA of the small intestine was significantly (\( P < 0.01 \)) larger than that with administration of SC-560 or celecoxib alone (Fig. 1). As reported in our previous paper (Satoh et al., 2009), the nonselective COX inhibitor IND (3 mg/kg, PO) produced overt lesions in both the duodenum and the lower half of the small intestine in all four cats (Fig. 1). A similar result was also observed in the duodenum after the administration of celecoxib, i.e., the TLA in the group given celecoxib (10 mg/kg, PO) before feeding was significantly (\( P < 0.05 \)) larger than that given celecoxib after feeding (Table 1).

**Histologic Examination**

No pathologic lesion was detected in the duodenal mucosa in normal control cats (Fig. 2C). The duodenal lesions caused by SC-560 or celecoxib had very similar changes, i.e., changes were observed just below the pyloric ring, and were UL-II–grade ulcers, in which destruction of the Lamina muscularis mucosa and degenerative and necrotic damage of Brunner's glands were observed (Figs. 2D and 2E).

**Effects of NSAIDs on the Number of PAS-Positive Goblet Cells in the Duodenal Villi**

The effect of various COX inhibitors on the distribution of PAS-positive cells in the duodenal mucosa was examined in...
Effect of Feeding and Cimetidine on Expression of COX Isoforms and PGE₂ Levels in the Duodenum

Expression of both isoforms of COX protein was observed in fasted cats, and their expression was markedly increased in fed cats; such an increase was prevented by pretreatment with cimetidine (Fig. 4A). The relative intensity values of COX-1 expression in cats that were fasted, fed, or fed and pretreated with cimetidine were 1.0 ± 0.1, 2.4 ± 0.6 (P < 0.05 versus fasted cats), and 1.3 ± 0.1 fold (n = 4), respectively (Fig. 4B). Those of COX-2 expression were 1.0 ± 0.4, 4.7 ± 1.4 (P < 0.05 versus fasted cats), and 1.1 ± 0.4 (P < 0.05 versus fed cats) fold (n = 4), respectively (Fig. 4B).

PGE₂ levels in the duodenum are shown in Fig. 4C. The level in fasted cats was 179.5 ± 8.1 pg/mg protein (n = 4), and was markedly higher in fed cats (289.9 ± 19.0 pg/mg protein, n = 4, P < 0.01 versus fasted cats). The PGE₂ level was markedly decreased by pretreatment with cimetidine (181.2 ± 27.5 pg/mg protein, n = 4, P < 0.01 versus fed cats).

Localization of COX-1 and COX-2 in the Duodenum

As shown in Fig. 5, COX-1 or COX-2 immunoreactivity (IR) was recognized in various areas and cells in the duodenum of fed cats. COX-1 IR was observed markedly in goblet cells, Brunner’s gland cells, and ganglion cells in both Meissner’s and Auerbach’s plexus, and moderately in smooth muscle cells, arterioles, and villous columnar cells (Fig. 5B). In most of the positive cells, except for goblet cells, COX-1 IR was observed predominantly in the cytoplasm and nuclear areas. In contrast, COX-2 IR was observed in the cytoplasm of endothelial cells of capillaries and venules, and basal granulated cells in both the villi and crypts (Fig. 5C). As in fed cats, COX-1 IR and COX-2 IR were observed in fasted cats, although the staining was weak (not shown).

Discussion

Although it is well known that NSAIDs cause ulcers in the duodenum of humans, the role of COX isoforms in the pathogenesis of duodenal ulcers has not been well studied, probably due to the lack of suitable experimental animal models for duodenal ulcers induced by NSAIDs. In the previous study, we reported that IND produced marked ulcers in the duodenum of cats (Satoh et al., 2009), suggesting that cats would be a good animal model to elucidate the role of COX isoforms in the pathogenesis of duodenal ulcers induced by NSAIDs.

It has been reported that NSAID-induced gastric and small intestinal lesions in rats and mice require simultaneous

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**TABLE 1**

Comparison of the ulcerogenic effect of selective COX inhibitors given before or after feeding of diet

<table>
<thead>
<tr>
<th>Drug</th>
<th>Timing of Drug Administration</th>
<th>No. of Cats</th>
<th>Lesion Index in the Duodenum</th>
<th>Lesion Index in the Small Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg, PO</td>
<td></td>
<td>cm²</td>
<td>cm²</td>
</tr>
<tr>
<td>SC-560 (3)</td>
<td>After feeding</td>
<td>4</td>
<td>0.4 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>SC-560 (3)</td>
<td>Before feeding</td>
<td>4</td>
<td>1.2 ± 0.3*</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Celecoxib (10)</td>
<td>After feeding</td>
<td>4</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Celecoxib (10)</td>
<td>Before feeding</td>
<td>4</td>
<td>1.3 ± 0.2*</td>
<td>0.8 ± 0.3</td>
</tr>
</tbody>
</table>

* P < 0.05 versus “after feeding,” SC-560 or celecoxib, respectively (Student’s t test).

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**Fig. 2.** Duodenal lesions induced by selective COX inhibitors. Either SC-560 (10 mg/kg) or celecoxib (10 mg/kg) was administered PO just after the morning meal once daily for 3 days. Macroscopic observation of duodenal lesions of cats given SC-560 (A) or celecoxib (B). Microscopic observation of normal duodenum (C), duodenal lesions induced by SC-560 (D), or celecoxib (E) (hematoxylin and eosin staining).

the same cats used for the ulcer experiments. In the cat duodenum, two types of mucus-containing cells (goblet cells and Brunner’s gland cells) were observed. Pretreatment with SC-560 and IND decreased the number of PAS-positive goblet cells in the villi, but celecoxib did not (Fig. 3A). The number of PAS-positive goblet cells in the villi in control cats was 5.46 ± 0.35 per 100 µm villus (n = 4). Pretreatment with SC-560 and IND significantly (P < 0.01 versus control) decreased the number of goblet cells, i.e., 1.93 ± 0.22 and 2.71 ± 0.13 per 100 µm villus (n = 4), respectively (Fig. 3B). Celecoxib had no effect on the number of goblet cells, i.e., 4.49 ± 0.62 per 100 µm villus (n = 4).
inhibition of both COX-1 and COX-2 (Wallace et al., 2000; Gretzer et al., 2001; Tanaka et al., 2001, 2002a; Sigthorsson et al., 2002). In the present study, we examined the effect of selective COX-1 inhibitors (SC-560 and ketorolac) and COX-2 inhibitors (celecoxib and meloxicam) on the GI mucosa in cats, and found that all of these drugs alone, unexpectedly, produced marked lesions in the duodenum, although they did not produce obvious lesions in the small intestine. However, coadministration of low doses of SC-560 and celecoxib produced marked lesions in the small intestine as well as nonselective COX inhibitor IND. These results support the previous notion that simultaneous inhibition of both COX-1 and COX-2 is necessary for the induction of small intestinal lesions by NSAIDs, and suggest that the role of COX isoforms in the formation of duodenal ulcers is different from that in the small intestine. Also suggested is that inhibition of either COX-1 or COX-2 alone will be sufficient to produce ulcers in the duodenum in cats.

Gretzer et al. (2001) found that inhibition of both COX isoforms is necessary to cause lesions in a normal rat stomach, while COX-1 inhibition produces lesions in the stomach in the presence of 0.2 N HCl, indicating that COX-1 inhibition can produce gastric lesions in the presence of noxious substances. In cats, as well as in humans, the duodenal mucosa is periodically exposed to noxious substances such as strong gastric acid and bile acids after a meal. This suggests that the duodenal mucosa of cats is sensitive to gastric acid or a rapid postprandial response exists, and has a specific protective mechanism against the noxious effects of gastric acid. We demonstrated that both the expression of COX isoforms and the levels of PGE₂ in the duodenum were increased after a meal and that the effect was suppressed by pretreatment with cimetidine, suggesting that the influx of gastric acid into the duodenum stimulates the local expression of COX isoforms and that the increase in COX isoforms contributes to the protection of mucosa through the enhanced PG synthesis during the postprandial period. We previously reported that the formation of IND-induced duodenal lesions in cats was markedly prevented by pretreatment with cimetidine or misoprostol (Satoh et al., 2009, 2010). This finding is consistent with the present results. Furthermore, in the present study, the duodenal lesions induced by SC-560 and celecoxib were significantly increased when the drugs were administered 1 hour before a feeding. Therefore, up-regulation of both COX-1 and COX-2 by luminal acid exposure after feeding or by other stimuli during the postprandial period may enhance duodenal mucosal protection, possibly through different mechanisms, since our results showed the differences in the distribution of COX-1 and COX-2 positive cells. Inhibition of either COX-1 or COX-2 before or after feeding decreased the protective activity of the mucosa, and thereby caused lesions in the duodenum, although we did not examine the effect of each COX inhibitor on the increase of PGE₂ levels induced by feeding.

To elucidate the mechanism of lesion formation by selective COX inhibitors, we examined the effect of COX inhibitors on the distribution of mucus, an important factor in the protection of duodenal mucosa (Akiba et al., 2000, 2001). We found that the number of PAS-positive goblet cells in the villi
was significantly decreased by SC-560 and IND, but not by celecoxib, suggesting that production of mucus in goblet cells depends on PGs produced by COX-1 but not by COX-2. We previously reported that SC-560, but not rofecoxib, a selective COX-2 inhibitor, increased intestinal motility in rats (Tanaka et al., 2002a). Wallace et al. (2000) found that SC-560, but not celecoxib, decreased mucosal blood flow in a rat stomach. Furthermore, COX-1 is involved in the local regulation of acid-induced duodenal HCO₃⁻ secretion in rats (Takeuchi et al., 2002). In the present study, COX-1 IR was observed markedly in goblet cells, Brunner's gland cells, and ganglion cells in both Meissner's and Auerbach's plexus, and moderately in smooth muscle cells, arterioles, and villous columnar cells. Taken together, these findings suggest that inhibition of COX-1 causes lesions in the duodenum by increasing motility and decreasing mucus production, mucosal blood flow, and HCO₃⁻ secretion in the presence of noxious substances such as gastric acid and bile acids during the postprandial period.

It has been reported that the ulcerogenic activity of selective COX-2 inhibitors in the GI tract is weaker than that of nonselective COX inhibitors in both rats (Futaki et al., 1993; Seibert et al., 1994; Laudanno et al., 2001) and humans (Laine et al., 1999; Simon et al., 1999; Silverstein et al., 2000; Bombardier et al., 2000, Goldstein et al., 2005). However, Cheung et al. (2010) recently reported that the incidence of duodenal ulcer by the administration of celecoxib (100 mg, twice daily) and diclofenac (50 mg, twice daily), which was examined in 880 patients with osteoarthritis and rheumatoid arthritis, did not differ significantly between those taking celecoxib and diclofenac (2.3% versus 1.5%). Thus, it was shown that the selective COX-2 inhibitor celecoxib produces duodenal ulcers to the same degree as the nonselective COX inhibitor diclofenac in humans. In the present study, the selective COX-2 inhibitors tested, as well as IND, invariably produced severe lesions (ulcers) in the duodenum. Laudanno et al. (2001) reported that the selective COX-2 inhibitors rofecoxib and celecoxib did not produce lesions in the GI mucosa in normal rats, but that the drugs aggravated GI lesions induced by various ulcerogenic stimuli such as water-immersion stress, IND, and cysteamine, and delayed the healing of gastric ulcers induced by acetic acid. Gretzer et al. (1998) reported that selective COX-2 inhibitors negated the protective activity of the stomach caused by a mild irritant (20% ethanol) in rats. Wallace et al. (2000) found that
cicloprofen, but not SC-560, accelerated the adhesion of leukocytes to intestinal microvessels in rats, suggesting that leukocyte adhesion induced by COX-2 inhibition contributes to NSAID-induced gastric lesions. This is consistent with the present finding that COX-2 IR, but not COX-1 IR, was observed in endothelial cells of capillaries and venules in the duodenum. Together, these findings suggest that inhibition of COX-2 decreases the protective and healing activity of the mucosa, where COX-2 is adaptively or protectively induced by various stimuli, and thereby causes or aggravates lesions in the GI tract under some pathophysiological conditions.

We found that both COX isoforms were present in the duodenum, and that the expressions were up-regulated by feeding, COX-1 and COX-2 positive responses were observed in different cells, and inhibition of either COX-1 or COX-2 alone caused ulcers in the duodenum, suggesting that COX-1 and COX-2 protect the duodenal mucosa in cats differently. Additional studies to elucidate the role of COX isoforms in the protection of GI mucosa under various pathophysiological conditions could provide useful information regarding NSAID safety.

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Authorship Contributions

Participated in research design: Satoh, Takeuchi.
Conducted experiments: Satoh, Amagase, Ebara.
Performed data analysis: Satoh, Amagase, Ebara.
Wrote or contributed to the writing of the manuscript: Satoh, Akiba, Takeuchi.

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