Comparative Study of the Effects of Indomethacin and NS-398, a Selective Cyclooxygenase 2 Inhibitor, on Duodenal Bicarbonate Secretion Induced by Luminal Acidification in Rats

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Received May 12, 1997 Accepted July 29, 1997

ABSTRACT—To clarify the mechanisms of duodenal ulcerogenic activity of non-steroidal anti-inflammatory drugs (NSAIDs), the effects of indomethacin (IND) on acid-stimulated duodenal bicarbonate secretion and histamine-induced duodenal ulcerogenic responses were studied in comparison with NS-398, a selective cyclooxygenase (COX)-2 inhibitor, in rats. IND (1 and 5 mg/kg, s.c.) significantly decreased duodenal bicarbonate secretion and potentiated duodenal lesion in a dose-dependent manner. On the other hand, NS-398 had no effect on these parameters. These findings suggest that duodenal ulcerogenicity of IND in the presence of histamine is mainly due to the inhibitory action on acid-stimulated bicarbonate secretion mediated by COX-1, but not by COX-2.

Keywords: Indomethacin, NS-398, Duodenal bicarbonate

It is well-known that non-steroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal damage (1). However, mechanisms of the ulcerogenic activities of NSAIDs are not fully understood. In general, the underlying mechanism of the anti-inflammatory effect of NSAIDs is mainly attributed to the decrease of prostaglandin (PG) production at inflammatory sites, which is due to the inhibition of cyclooxygenase (COX) activity (2); and this inhibition of PG biosynthesis is associated with ulcerogenic activities in the gastroduodenal tract by NSAIDs (1). In addition, PGs have an action to increase bicarbonate secretion from the gastroduodenal tract (3). Bicarbonate secretion maintains the pH within the mucus gel on the mucosal surface of the duodenum at neutrality, even when the intraluminal pH of the duodenum is low (4). A close relationship between duodenal ulcerogenicity and impaired bicarbonate secretion is reported. Indomethacin (IND) inhibited the acid-induced bicarbonate secretion and caused damage in the duodenum under acid hypersecretion (5). This inhibition is due to the decrease of PGE2 biosynthesis accompanied by the inhibition of COX. Therefore, PGs are playing an important role as one of the defensive factors against acid in the gastroduodenal tract.

Recently, it has been shown that COX has two different isozymes, COX-1 and COX-2. COX-1 existing in most tissues is involved in the maintenance of essential physiological functions (6). In vitro, COX-2 is induced by variety of agents (7). Furthermore, in vivo, COX-2 is induced significantly under inflammatory conditions (8). However, it is unclear which isozyme mediates the physiological actions such as acid-induced bicarbonate secretion in the duodenum.

Thus, in the present study, we studied in rats using IND and NS-398 whether COX-1 or COX-2 is involved in generation of PG that plays an important role in duodenal bicarbonate secretion after luminal acidification.

Male Sprague-Dawley rats (6 weeks of age; Charles River Japan, Yokohama) were used in this study. Rats were kept in individual cages and deprived of food overnight with free access to water 2 hr before the experiment. Indomethacin (IND; Sigma Chem. Company, St. Louis, MO, USA) and NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methanesulphonamide; synthesized in our laboratories) were suspended in 0.5% carboxymethylcellulose sodium salt (CMC·Na; Wako Pure Chemical Co., Osaka). Each drug was administered subcutaneously (s.c.) in a volume of 1 ml/200 g body weight. Control animals were administered vehicle alone.

Determination of duodenal bicarbonate secretion was
according to the report by Takeuchi et al. (3) with some modifications. Animals were anesthetized with urethane (1.25 g/kg, i.p.; Sigma Chem. Company). The abdomen of each rat was incised, and a duodenal loop was made between the pyloric ring and the area just proximal to the outlet of the common bile duct (17 mm). This loop was perfused at a flow rate of 0.7 ml/min with 0.9% saline heated at 37°C. The perfusate was collected every 15 min and HCO$_3$^- output was determined by back-titrating each sample with 10 mM HCl to pH 5.0 using a pH meter (F-15; Horiba, Kyoto). After stable basal secretion had been obtained for at least 30 min, test drugs were administered s.c. One hour after administration of test drugs, the duodenum was exposed to 10 mM HCl for 10 min. After exposure, mucosa was rinsed gently with saline, and HCO$_3$^- was measured for 2 hr thereafter. Duodenal lesions were induced as follows: Histamine dihydrochloride (40 mg/kg; Nacalai Tesque, Kyoto) was dissolved in 10% gelatin (Wako Pure Chemical Co.) and administered s.c. in a volume of 1 ml/200 g body weight 3 times every 2.5 hr. Test drugs were administered s.c. 30 min before the first administration of histamine. Animals were sacrificed at 8 hr after administration of test drugs. The duodenum and stomach were dissected out, inflated with 2% formalin (Nacalai Tesque) and then opened along the mesenteric attachment or along the greater curvature. The damage area (in square millimeters) was measured under a dissecting microscope (x 10, SMZ-10; Nikon, Tokyo) with a square grid. Histamine-stimulated gastric acid secretion was determined as follows: The abdomen was incised under ether anesthesia, and the pylorus was ligated. Test drugs were administered s.c. 30 min before ligation. Histamine dihydrochloride (40 mg/kg) dissolved in 10% gelatin in a volume of 1 ml/200 g body weight was given s.c. immediately after ligation. Animals were sacrificed at 3 hr after pylorus ligation. The gastric juice was collected and analyzed for volume (ml/rat) and acidity (mEq/1). Acidity was determined by titration of the gastric juice against 0.1 N NaOH to pH 7.0 using a pH meter (F-15, Horiba). Acid output was expressed as μEq/hr. All results are expressed as the mean ±S.E. The differences in values among various groups were analyzed by one-way analysis followed by Dunnett's t-test. A P value of less than 0.05 was considered statistically significant.

The proximal duodenum secreted HCO$_3$^- at a steady basal rate of 1.4–1.8 μEq/15 min in each group. In the control, bicarbonate secretion was increased about 2 times greater than basal levels in response to acidification of mucosa, and this level was maintained even 90 min later. Total increase of bicarbonate output from basal secretion was 11.8 ± 1.2 μEq/1.5 hr after the duodenal acidification. IND (1 and 5 mg/kg) significantly inhibited duodenal bicarbonate secretion in a dose-dependent manner; total increase of bicarbonate output from basal secretion was 6.5 ± 1.4 μEq/1.5 hr and 4.4 ± 1.7 μEq/1.5 hr, respectively, and the inhibition from the control level was 45.2% and 62.4%, respectively. On the other hand, NS-398 (10 and 50 mg/kg) had no influence on the increase of duodenal bicarbonate secretion in response to acidification. The increase of bicarbonate secretion from basal values was similar to that in the control group. Total increase of bicarbonate output from basal secretion after the duodenal acidification was 12.1 ± 1.5 μEq/1.5 hr and 7.7 ± 1.4 μEq/1.5 hr, respectively (Fig. 1A). In the
Table 1. Effects of indomethacin (IND) and NS-398 on histamine-stimulated gastric secretion in pylorus-ligated rats

| Drug     | Dose (mg/kg) | Volume (ml/rat) | Acidity (mEq/l) | Acid output (µEq/hr) |
|----------|--------------|-----------------|-----------------|----------------------|
| Control  | 9.8±0.5      | 139.2±2.0       | 454.9±22.4      |                      |
| IND      | 1            | 9.0±0.1         | 136.3±2.0       | 407.8±5.9            |
|          | 5            | 9.2±0.4         | 133.3±1.2       | 408.3±18.0           |
| NS-398   | 10           | 8.8±0.6         | 135.8±4.0       | 398.5±31.6           |
|          | 50           | 8.4±0.4         | 138.3±2.0       | 389.2±22.5           |

Histamine was administered s.c. immediately after ligation. Each drug was administered s.c. 30 min before ligation. Animals were sacrificed at 3 hr after ligation. Data represent the mean ± S.E. from 6 rats.

control animals administered histamine 3 times, s.c., a small hemorrhagic lesion was observed in the proximal part of the duodenum with an area of 4.3±0.6 mm². However, IND (1 and 5 mg/kg) remarkably enhanced lesions in the duodenum, the area of lesion being 14.9±2.1 mm² and 18.6±3.7 mm², respectively. NS-398 (10 and 50 mg/kg) had no influence on gastroduodenal lesions. The area of duodenal lesion was 4.4±0.9 mm² and 5.0±1.3 mm², respectively (Fig. 1B). Three hours after bolus administration of histamine, volume of gastric juice, acidity and acid output were 9.8±0.5 ml, 139.2±2.0 mEq/l and 454.9±22.4 µEq/hr, respectively. IND (1 and 5 mg/kg) and NS-398 (10 and 50 mg/kg) had no significant effects on these parameters (Table 1).

Since IND inhibits both COX-1 and COX-2, it is not understood whether COX-1 or COX-2 are concerned with the maintenance of mucosal integrity. If duodenal lesions occurred in response to NSAIDs by inhibiting bicarbonate secretion due to COX-1 inhibition, then selective COX-2 inhibitors may be used safely without inducing duodenal functional alteration and mucosal damage. Futaki et al. (9) reported that IND inhibited COX-1 and COX-2 with comparable potencies and also reported that NS-398 was at least a 25-fold more potent inhibitor of COX-2 than COX-1. Doses of NS-398 (10 and 50 mg/kg) used in the present study were higher than doses that inhibited PGs biosynthesis in inflamed tissues in rat carrageenin-air-pouch inflammation (10). Furthermore, NS-398 inhibited the activity of COX-2 in gastric mucosal lesions induced by acetic acid in mice (11). Thus, it can be estimated that doses of NS-398 in the present study are enough to inhibit COX-2 in the duodenum. NS-398 showed the selective inhibition for COX-2 and has no influence on acid-stimulated duodenal bicarbonate secretion. From these findings, the inhibitory action of IND on acid-stimulated duodenal bicarbonate secretion may be due to COX-1, but not COX-2. Excessive gastric secretion is thought to be a causative factor of duodenal ulceration (12). The experimental ulcer model used in this study may be due to excessive gastric acid secretion stimulated by histamine. Since IND (1 and 5 mg/kg) and NS-398 (10 and 50 mg/kg) had no effect on histamine-stimulated gastric secretion, the mechanisms of the duodenal ulcerogenicity of IND are mainly due to the inhibition of acid-stimulated bicarbonate secretion mediated by COX-1. These results support the hypothesis that inhibition of COX-1 causes unwanted side effects in the gastroduodenal mucosa and that inhibition of COX-2 produces anti-inflammatory effects.

On the other hand, induction of COX-2 is observed in gastric mucosal lesions by acetic acid and healing of this lesion is delayed by NS-398 (11). It is considered that PGs induced by COX-2 play an important role in the repair process of gastric lesion. Although inhibition of COX-2 did not affect generation of duodenal lesion in this study, inhibition of COX-2 may influence the repair process of duodenal lesions as well as the stomach. Recently COX-2 was found to be localized not only in inflammatory sites but also in normal gut or kidney. In the stomach, Iseki (13) reported that COX-2 is distributed in surface mucus cells of both the fundic and pyloric regions. In the duodenum, there is not any information about the expression of COX-2 that would suggest a physiological role in the normal gastrointestinal tract.

In conclusion, the present study suggests that duodenal ulcerogenicity induced by IND is probably due to the inhibitory action on bicarbonate secretion mediated by COX-1, but not by COX-2.

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