Effects of Indomethacin on the Duodenal Mucosa of Rats: Comparative Study with Cysteamine

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Abstract—Effects of indomethacin and cysteamine on the duodenal mucosa of rats were studied microscopically (using scanning electron microscopy) and also functionally. Indomethacin (5 mg/kg, s.c.) induced no microscopic damage to the duodenal epithelium for up to 6 hr after administration. Indomethacin had no effects on gastric H+ output and the amount of H+ in the duodenum, but did reduce the duodenal HCO3− secretion (both basal and 10 mM-HCl stimulated). PGE2 contents in the duodenal mucosa were markedly reduced by indomethacin for 6 hr. These results suggest that reductions of duodenal HCO3− secretion and endogenous prostaglandins per se do not impair the H+ disposal system of the duodenum and so do not damage the epithelial cells. In contrast, cysteamine (100 mg/kg, s.c.) produced microscopic damage to the duodenal epithelium as early as 2 hr later. Cysteamine significantly increased gastric H+ output and reduced duodenal HCO3− secretion, resulting in an increased amount of H+ in the duodenum 3 hr later. Cysteamine had no effect on PGE2 contents in the duodenum. The time lag between damage formation and functional changes suggests that the earliest damage caused by cysteamine occurs by mechanisms other than erosive action of H+ emptied by the stomach. The increased amount of H+ may contribute to an enhancement of the initial damage.

Mepirizole, a basic antiinflammatory agent, induces microscopic damage in the proximal duodenum of rats as early as 2 hr after administration, and the damage progresses to well-defined deep ulcers with time (1-4). We found that while mepirizole (200 mg/kg) did not increase gastric H+ output, it did significantly reduce HCl-stimulated duodenal HCO3− secretion and increase the amount of H+ in the duodenum within 2 hr (4). Therefore, we postulated that this increased amount of H+ by an impairment of the H+ disposal system may contribute to the pathogenesis of duodenal ulceration by mepirizole. Flemström et al. (5) and we (6) reported that indomethacin significantly reduced HCl-stimulated duodenal HCO3− secretion in anesthetized rats. In contrast to mepirizole, however, indomethacin in the same dose induced no macroscopic damage in the duodenum up to 8 hr (6, 7). It was of interest to re-examine the effects of indomethacin on the duodenal mucosa both microscopically and functionally, including determination of the amount of H+ in the duodenum and prostaglandin contents in the duodenal mucosa. Cysteamine was used as a reference drug, because this agent, as mepirizole, reduces duodenal HCO3− secretion and H+ disposal capacity and induces duodenal damage in rats (8-12).

Materials and Methods

Male Sprague-Dawley rats (weighing 230-250 g) were deprived of food but allowed free access to water for 24 hr before the experiments. They were kept in raised mesh-bottom cages to prevent coprophagy. Each study was carried out using 8 to 10 animals per group.
Damage in the duodenum: The effects of indomethacin and cysteamine on the duodenal epithelium were microscopically examined, using a scanning electron microscope (SEM) by our method (4). Briefly, indomethacin (5 mg/kg, Sigma) suspended in saline with a trace of Tween 80, cysteamine hydrochloride (100 mg/kg, Sigma) dissolved in saline, or the vehicle alone was administered s.c. in a volume of 0.5 ml/100 g body wt. The doses of these drugs were ones which were demonstrated to reduce HCl-stimulated duodenal HCO₃⁻ secretion in rats (5, 6). Two, 4 and 6 hr later, the duodenum was removed under ether anesthesia, fixed in 5% paraformaldehyde-4% glutaraldehyde for 3 hr at 4°C, and then postfixed into 1% OsO₄ for 1 hr. After fixation and dehydration with a graded series of ethanol and isoamyl acetate, the tissues were then critical point dried with CO₂ (Hitachi, HLP-2), mounted on aluminum stubs, and vacuum coated with a palladium-platinum ion sputter (Eicho, IB-3). Thereafter, the samples were examined for damage using a scanning electron microscope (Hitachi, S-510). The severity of damage was divided into five degrees as follows:

Index 0: normal villus
1: an exfoliation of a few epithelial cells on the apical parts of villus
2: an exfoliation of a number of epithelial cells on the apical parts of villus and an exposure of the lamina propria
3: low villus occurred by destruction of the lamina propria
4: a formation of an avillous surface and erosion

The entire duodenum was scanned, and the area with the severest damage was recorded for indexing. The person examining the duodenal samples was unaware of which treatment the animal had received.

Gastric H⁺ secretion: Under ether anesthesia, a polyethylene cannula was placed into the stomach through the forestomach and secured tightly around the inserted part (6). After fixation and dehydration with a graded series of ethanol and isoamyl acetate, the tissues were then critical point dried with CO₂ (Hitachi, HLP-2), mounted on aluminum stubs, and vacuum coated with a palladium-platinum ion sputter (Eicho, IB-3). Thereafter, the samples were examined for damage using a scanning electron microscope (Hitachi, S-510). The severity of damage was divided into five degrees as follows:

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Gastric H⁺ secretion: Under ether anesthesia, a polyethylene cannula was placed into the stomach through the forestomach and secured tightly around the inserted part (6). The pylorus was also ligated, and the cannula was brought out. After recovery from anesthesia, these animals were kept in Bollman cages and gastric samples were collected hourly for 7 hr by gravity drainage. Hartman solution was continuously infused at the rate of 1.2 ml/hr into the tail vein to compensate for the loss of body fluid by gastric drainage. Measurements were made for volume and acidity. After centrifugation with 3,000 rpm for 15 min, acidity was titrated with 0.1 N NaOH to pH 7.0 using an autoburette (Radiometer); acid output was expressed in μEq/hr. Indomethacin (5 mg/kg), cysteamine (100 mg/kg), or the vehicle alone was administered s.c. 2 hr after the stomach was washed.

Duodenal HCO₃⁻ secretion: Under ether anesthesia, a duodenal loop was made between the pyloric ring and the position just proximal to the common bile duct (15 mm), excluding the influence of bile and pancreatic juice (6). Gastric contents were withdrawn by an acute fistula placed in the forestomach to prevent an accumulation of gastric juice in the stomach. After recovery from anesthesia, these animals were kept in Bollman cages. This loop was perfused at a flow rate of 1 ml/min with saline which was adjusted with NaOH to pH 7.4, gassed with 100% O₂, heated to 37°C and kept in a reservoir. The titration was performed at luminal pH 7.4, using the pH-stat method (Hiranuma, Comtite-7) and by adding 5 mM HCl to the reservoir. In another experiment, 10 mM HCl in isotonic NaCl was perfused for 10 min by replacing the circulating fluid to stimulate duodenal HCO₃⁻ secretion. After perfusion, the acid was removed, the duodenum rinsed gently with saline, and the tissue reperfused with saline. The HCO₃⁻ secretion was measured for 6 hr thereafter. At least 1 hr after the basal HCO₃⁻ secretion had stabilized, indomethacin (5 mg/kg), cysteamine (100 mg/kg), or the vehicle alone was administered s.c. In the case of acid-stimulated HCO₃⁻ secretion, these drugs were given 1 hr before exposure of the duodenum to 10 mM HCl.

Amount of H⁺ in the duodenum: To examine H⁺ disposal capacity in the proximal duodenum, the amount of H⁺ in the duodenum was determined as follows. Under ether anesthesia, an acute fistula was placed in the duodenum proximal to the outlet of the common bile duct (15 mm distally to the pylorus) (Fig. 1) (6). After recovery from
anesthesia, the animals were kept in Bollman cages, and the gastric and duodenal juice was collected hourly for 6 hr by gravity drainage. Hartman solution was continuously infused at the rate of 1.2 ml/hr into the tail vein. After centrifugation, the samples were measured for volume and acidity as described for gastric H+ secretion. The amount of H+ (volume x acidity per hr) was expressed as μEq/hr. Indomethacin (5 mg/kg), cysteamine (100 mg/kg), or the vehicle alone was given s.c. after a 2 hr basal period.

Prostaglandin contents in the duodenal mucosa: Effects of indomethacin and cysteamine on prostaglandin E2 (PGE2) contents in the duodenal mucosa were studied. Indomethacin (5 mg/kg), cysteamine (100 mg/kg), or the vehicle alone was given s.c. One, 2, 4 and 6 hr later, the duodenum was removed under ether anesthesia, and incised along the anti-mesenteric border. After washing the lumen with ice-cold saline, the duodenal sample was placed between two glass slides and quickly placed in hexane frozen with dry ice and acetone (13-15). The mucosae were collected, weighed and placed in 5 ml of 100% methanol (v/v) containing 1×10^-4 M sodium meclofenac (Parke-Davis). After homogenization, PGE2 contents were determined by radioimmunoassay using rabbit anti-PGE2 (Institute Pasteur Production). Each assay was performed in duplicate.

Statistics: Data are expressed as the mean±one S.E.M. The mean values were compared using the unpaired Student's t-test, and P<0.05 was regarded as significant.

Results

Effects of indomethacin and cysteamine on the duodenal mucosa: The administration of indomethacin (5 mg/kg) induced no damage to the duodenal mucosa in all animals 2, 4, and 6 hr later (Fig. 2). Each villus remained intact and exfoliation of the epithelial cells was nil for up to 6 hr. In contrast, cysteamine (100 mg/kg) induced an apparent exfoliation of epithelial cells as early as 2 hr after administration (Fig. 3). At this period, an exposure of the lamina propria was already evident in many villi. The damage progressed to a marked exfoliation of epithelial cells with time, thereby resulting in a broad exposure of the lamina propria (Fig. 4). At 6 hr, there were two avillous areas in the proximal duodenum in most of the animals.

Effects of indomethacin and cysteamine on gastric H+ secretion: In the control animals,
the volume of gastric juice ranged from 1.0 to 1.3 ml/hr, and the H⁺ output ranged from 100 to 150 μEq/hr (Fig. 5). The administration of indomethacin (5 mg/kg) had little or no effect on gastric secretion (both volume and H⁺ output) for up to 6 hr, except for a significant reduction in H⁺ output at the initial hr. In contrast, cysteamine (100 mg/kg) significantly increased both volume and H⁺ output during 2 to 4 hr after administration; the volume and H⁺ output were approximately 2.0 ml/hr and 280 μEq/hr, respectively.

Effects of indomethacin and cysteamine on duodenal HCO₃⁻ secretion: The duodenal mucosa perfused with saline consistently secreted HCO₃⁻ at the rate of 5 to 7 μEq/15 min (i.e., 20–28 μEq/hr) (Fig. 6A). Indomethacin (5 mg/kg) significantly inhibited basal duodenal HCO₃⁻ secretion beginning from 1.5 hr after administration for about 2 hr. Cysteamine (100 mg/kg) had no effect on the basal duodenal HCO₃⁻ secretion up to 6 hr. The instillation of 10 mM HCl into the duodenal loop for 10 min markedly stimulated the HCO₃⁻ secretion for about 2 hr (Fig. 6B). The increased HCO₃⁻ (about 9 μEq/15 min, i.e., 36 μEq/hr) approximately doubled the basal value (about 5 μEq/15 min, i.e., 20 μEq/hr). Both indomethacin and cysteamine significantly prevented this increase in duodenal HCO₃⁻ secretion in response to 10 mM HCl to the same degree.

Effects of indomethacin and cysteamine on the amount of H⁺ in the duodenum: In the control animals, the volume of duodenal drainage ranged from 1.6 to 1.8 ml/hr, and the amount of H⁺ ranged from 30 to 60 μEq/hr (Fig. 7). The administration of indomethacin (5 mg/kg) had little or no effect on the volume and amount of H⁺ for 6 hr. In contrast, the administration of cysteamine (100 mg/kg) significantly reduced the volume to 0.8 ml/hr and the amount of H⁺ to nearly zero in the initial hr. However, the volume and amount of H⁺ were conversely increased during 3 to 5 hr after administration, i.e., the
Fig. 3. Microscopic appearances of duodenal villi of rats at 0, 2, 4 and 6 hr after cysteamine administration (200 mg/kg, s.c.). It was evident that these villi were destroyed by cysteamine administration, and the damage became more severe with time. At 6 hr, an avillous surface was present in the proximal duodenum.

Fig. 4. Effects of indomethacin and cysteamine on the duodenal mucosa of rats. Indomethacin (5 mg/kg) or cysteamine (100 mg/kg) was given s.c., and the animals were killed at 0, 2, 4 and 6 hr later. Degree of damage in the duodenal mucosa was quantitatively examined, using a scanning electron microscope (SEM). Indomethacin induced no damage to the duodenal mucosa, but cysteamine did induce damage with time. Data represent the mean±one S.E.M.
Fig. 5. Effects of indomethacin and cysteamine on gastric secretion in acute fistula rats. Indomethacin (5 mg/kg), cysteamine (100 mg/kg), or the vehicle alone was given s.c., and the gastric juice was collected hourly for 6 hr after administration. Indomethacin had little or no influence on the volume and acid output, but cysteamine significantly increased both the volume and acid output 2, 3 and 4 hr later. Data represent the mean±one S.E.M. *Significantly different from control values.

Fig. 6. Effects of indomethacin and cysteamine on basal (A) and HCl-stimulated (B) duodenal \( \text{HCO}_3^- \) secretion in rats. Indomethacin (5 mg/kg), cysteamine (100 mg/kg), or the vehicle alone was given s.c., and \( \text{HCO}_3^- \) was titrated every 15 min using a pH-stat. Indomethacin significantly inhibited both basal and HCl-stimulated duodenal \( \text{HCO}_3^- \) secretion. Cysteamine significantly inhibited HCl-stimulated duodenal \( \text{HCO}_3^- \) secretion alone. Data represent the mean±one S.E.M. *Significantly different from control values.

volume and amount of \( \text{H}^+ \) were approximately 2.75 ml/hr and 180 \( \mu \text{Eq/hr} \), respectively.

Effects of indomethacin and cysteamine on PGE\(_2\) contents: The mean duodenal content of PGE\(_2\) in the control animals was 169±38 ng/g tissue. The administration of indomethacin (5 mg/kg) potently and persistently reduced PGE\(_2\) contents for up to 6 hr (Fig. 8). Reduction in the prostaglandin contents exceeded 80% for the initial 6 hr. However, the administration of cysteamine (100 mg/kg) had no effect on PGE\(_2\) contents for 6 hr.

Discussion

The present studies confirmed microscopically our previous observations that indomethacin (5 mg/kg) induced no macroscopical damage to the duodenal mucosa (6, 7). In addition, we also confirmed in conscious rats the findings that indomethacin (5 mg/kg) had no effect on gastric \( \text{H}^+ \) secretion, but reduced HCl-stimulated duodenal \( \text{HCO}_3^- \) secretion in anesthetized rats (6). It is interesting that indomethacin also significantly reduced basal (unstimulated) duodenal \( \text{HCO}_3^- \) secretion.
Fig. 7. Effects of indomethacin and cysteamine on the amount of H+ in the duodenum of rats. Indomethacin (5 mg/kg), cysteamine (100 mg/kg), or the vehicle alone was given s.c., and the gastric and duodenal juice were collected hourly from the duodenum. Cysteamine, but not indomethacin, significantly increased both the volume and amount of H+ 3, 4 and 5 hr later. Data represent the mean ± one S.E.M. *Significantly different from control values.

during 2 to 4 hr after administration. Flemström et al. (5) and Konturek et al. (16) suggested that the inhibitory effect of indomethacin on duodenal HCO3− secretion in response to HCl may be related to its inhibition of prostaglandins biosynthesis in the duodenal mucosa. Actually, we also demonstrated that indomethacin in a dose which inhibited duodenal HCO3− secretion potently reduced PGE2 contents in the duodenal mucosa within 1 hr after administration.

It is generally considered that endogenous prostaglandins play an important role in maintaining mucosal integrity of the gastro-intestinal tract. This hypothesis is based on the observations that various agents which inhibit prostaglandin synthesis induce gastric or intestinal damage, and exogenous prostaglandins inhibit the development of such damage. However, our present data indicate that the reduction of prostaglandin contents at least for 6 hr did not lead to any appreciable damage to the duodenal epithelium. Therefore, it is questionable that endogenous prostaglandins are necessary factors for normal maintenance of the mucosal integrity of the duodenum.

As described above, indomethacin did not influence the gastric H+ output, but did reduce the duodenal HCO3− secretion. Based on these results, it was expected that the amount of H+ in the duodenum might be increased to a considerable extent by indomethacin. Unexpectedly, the amount of H+ was nearly equal to that seen in the control animals up to 6 hr. This result suggests that the amount of HCO3− secreted from the duodenal mucosa is not enough to influence the H+-disposal system of the duodenal mucosa.

It is well known that the H+-disposal system in the duodenum mainly consists of neutralization of H+ with HCO3− secreted by the duodenal mucosa (including Brunner's glands), the pancreas and biliary system, and H+ absorption in the duodenal mucosa (17–20). In our study, the mean gastric H+ output in the control animals was about 125 μEq/hr. When the gastric contents were collected through the duodenum, the mean volume of H+ was about 45 μEq/hr. This result suggests that about 80 μEq/hr of gastric H+ was disposed of through the duodenum. The duodenal HCO3− secretion in response to 10 mM HCl was about 40 μEq/hr at maximum. Isenberg et al. (21) also reported that luminal instillation of even 100 mM HCl for 5 min stimulated duodenal HCO3− secretion up to 30 μEq/cm/hr (i.e., about 45 μEq/1.5 cm/hr) in conscious rats. If such a response really occurs in the duodenum as a physiological function, 50% of the gastric H+ emptied in the duodenum may be disposed of through neutralization and the other 50% removed by H+ absorption in our model system.

Therefore, the results obtained with
indomethacin may be explained as follows: (a) the amount of \( \text{HCO}_3^- \) secreted from the duodenal mucosa in response to 125 \( \mu \text{Eq/hr} \) of \( \text{H}^+ \) emptied by the stomach is minute and (b) indomethacin may stimulate \( \text{H}^+ \) absorption by duodenal tissues. In any event, the amount of \( \text{H}^+ \) in the duodenum of rats treated with indomethacin was within the normal range throughout the entire experimental period. This probably explains why the duodenal epithelium remained intact after exposure to indomethacin.

In contrast to indomethacin, cysteamine (100 mg/kg, a subulcerogenic dose, determined macroscopically) induced microscopic damage as early as 2 hr after administration. In addition, the epithelial cell damage worsened with time, i.e., an avillous surface in the proximal duodenum was evident 6 hr later. This finding is consistent with the observations by other investigators (9) who reported the development of microscopic damage in rat duodenal epithelium 4 to 8 hr after administration of cysteamine (280 mg/kg, p.o.). From various laboratories (22-27) it was reported that cysteamine at the ulcerogenic dose significantly increased gastric \( \text{H}^+ \) secretion within 1-4 hr in acute or chronic fistula rats, thereby suggesting the close link of increased gastric secretion in the pathogenesis. We also found that cysteamine (100 mg/kg) increased gastric \( \text{H}^+ \) output by about 2-fold of the control values beginning from 2 hr after administration for the following 2 hr. Briden et al. (10) reported that cysteamine (100 mg/kg) significantly reduced duodenal \( \text{HCO}_3^- \) secretion in response to 5 mM HCl. We also confirmed the effect of cysteamine on \( \text{HCO}_3^- \) secretion in our model. The agent had no effect on basal \( \text{HCO}_3^- \) secretion, but potently inhibited it in response to 10 mM HCl. Our data, however, slightly differed from their findings in that cysteamine significantly increased duodenal \( \text{HCO}_3^- \) secretion 1 hr later, but decreased it 5 hr later. This apparent difference appears to relate to the different methods used by their group and ours, i.e., they used duodenal preparations which excluded the proximal part of the duodenum (including...
Brunner’s glands) and determined HCO₃⁻ secretion in anesthetized animals.

As expected from the results on gastric H⁺ and duodenal HCO₃⁻ secretion, the amount of H⁺ in the duodenum of rats treated with cysteamine was markedly increased 3 hr later, i.e., 1 hr after the increase in gastric H⁺ secretion. Gallaghar and Szabo (28) also reported that cysteamine (150 mg/kg, s.c.) showed a tendency to increase the amount of H⁺ in the duodenum 3 to 4 hr later when determined in chronic duodenal fistula rats. However, it should be noted that the amount of H⁺ remained unchanged for the initial 2 hr after cysteamine administration as compared to control values. By that time, the duodenal epithelium had already been damaged. Therefore, the earliest change observed in the duodenal epithelium may be unrelated to the increased amount of H⁺, but rather due to unspecified mechanisms. In this regard, the pathogenesis of cysteamine-induced damage appears to be different from that postulated for the mepirizole-induced duodenal damage as described in an earlier section. Gastric H⁺ output and the amount of H⁺ in the duodenum observed 3 hr later were about 280 and 180 μEq/hr, respectively. Thus, about 100 μEq/hr of H⁺ was disposed of by the duodenum, probably by H⁺ absorption. This implies that the H⁺ disposal system in the duodenum has limitations in capacity or is impaired by the preceding mucosal cell damage. The increased amount of H⁺ in the duodenum is most likely to contribute to the gradual development of the initial damage finally leading to the development of penetrating ulcers.

In contrast to indomethacin, cysteamine had no influence on PGE₂ contents in the duodenum during the entire experimental period. Therefore, the participation of endogenous prostaglandins will be ruled out from the pathogenesis of cysteamine-induced duodenal damage in rats. In addition, these data indicate that the mechanism by which cysteamine inhibits the HCl-stimulated duodenal HCO₃⁻ secretion is at least unrelated to endogenous prostaglandins.

We conclude that (a) indomethacin did not induce even microscopic damage to the rat duodenal epithelium, (b) indomethacin had little or no influence on the H⁺ disposal system despite an apparent reduction in duodenal HCO₃⁻ secretion and prostaglandin contents, and (c) cysteamine damaged the duodenal epithelium and impaired the H⁺ disposal system.

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