Pathogenic Mechanisms Involved in Mepirizole-Induced Duodenal Damage in the Rat

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Accepted July 28, 1986

Abstract—Mepirizole (60 and 200 mg/kg) administered s.c. induced damage in the surface epithelial cells of the rat proximal duodenum as early as 2 hr after the treatment. 16,16-Dimethyl prostaglandin E2 (dmPGE2, 30 μg/kg) administered s.c. significantly protected the duodenal mucosa against mepirizole-induced damage for up to 6 hr. Gastric acid secretion in acute fistula preparations was significantly reduced 1 hr after administration of mepirizole (60 and 200 mg/kg). The secretion reverted to the control level 2 hr later. In the 60 mg/kg-treated group, however, there was a significant increase in the acid output for up to 6 hr. Duodenal HCO3− secretion stimulated with 10 mM HCl was significantly inhibited with mepirizole (60 and 200 mg/kg). Mepirizole (60 and 200 mg/kg) significantly increased the amount of acid in the duodenum for 2 to 6 hr after the treatment. dmPGE2 (30 μg/kg) significantly inhibited gastric acid secretion, stimulated duodenal HCO3− secretion, and reduced the increased amount of acid in the duodenum in response to mepirizole. Endogenous prostaglandin E2 and 6-keto prostaglandin F1α in the duodenal mucosa were significantly reduced by mepirizole (200 mg/kg) 1 to 2 hr later. Mepirizole-induced duodenal damage appears to be caused by the increased amount of acid in the duodenum.

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 8–15 animals per group.

Damage in the duodenum: An early phase of duodenal damage induced by mepirizole was studied using the scanning electron microscope (SEM), according to our method. Briefly, mepirizole (Daiichi, 60 and 200 mg/kg), suspended in 1% carboxymethylcellulose, was given in a volume of 0.5 ml/100 g body wt. Control animals were given the vehicle alone. Two, 4 and 6 hr after the administration of mepirizole, ether was administered, and the duodenum was removed, fixed in phosphate-buffered 5% paraformaldehyde-4% glutaraldehyde for 3 hr at 4° C, and then postfixed into 1% OsO4 for 1 hr. After the fixation and dehydration in a graded series of ethanol solutions, the tissues
were 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:

1. **Index 0**: normal villus
   - an exfoliation of a few epithelial cells on the apical parts of the villus

2. **Index 1**: an exfoliation of a number of epithelial cells on the apical parts of the villus and an exposure of the lamina propria

3. **Index 2**: low and broad villus by an exfoliation of the epithelial cells and by destruction of the lamina propria

4. **Index 3**: a formation of an avillous surface and erosion

The entire duodenum was scanned, and the area with the most severe damage was recorded for indexing. Most of the duodenum examined almost invariably had two damaged portions. Therefore, the index per duodenum could range from 0 to a maximum of 8.

Effects of dmPGE₂ (Ono) on mepirizole-induced duodenal damage were studied. dmPGE₂ (30 μg/kg) was given s.c. 30 min before treatment with mepirizole (200 mg/kg) in a volume of 0.5 ml/100 g body wt. The dose of dmPGE₂ was that which significantly prevented mepirizole-induced duodenal damage in rats (1). Control animals were given the vehicle alone.

**Gastric secretion**: Under ether anesthesia, the abdomen was incised, and the stomach and duodenum were exposed. According to the method described previously (5), a duodenal loop was made between the pyloric ring and the position just proximal to the common bile duct (15 mm), thereby excluding the influence of the bile and pancreatic juice. An acute fistula prepared using a 2 mm-polyethylene tube was placed in the forestomach through which the gastric contents were withdrawn to prevent an accumulation of gastric juice. 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, 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, Comtrite-7) and by adding 5 mM HCl to the reservoir.

To stimulate duodenal HCO₃⁻ secretion using the above system, 10 mM HCl made isotonic with NaCl was perfused for 10 min by replacing the circulating fluid (6). After perfusion, the acid was removed, the duodenum rinsed gently with saline, and the pylorus was also ligated. The cannula was brought out and the abdominal incision was closed. A fine polyethylene tube was introduced into the tail vein for the infusion of saline (1.2 ml/hr) to compensate for the loss of body fluid by gastric secretion. The gastric lumen was washed with saline until the recovered solution was clear. These animals were kept in Bollman cages, and gastric samples were collected hourly for 6 hr by gravity drainage. Measurements of volume and acidity were made. After centrifugation at 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 microequivalents per hour.

Mepirizole (60 or 200 mg/kg) or dmPGE₂ (30 μg/kg) was given s.c. 2 hr after the stomach was washed. In the case of combined administration, mepirizole (200 mg/kg) was given s.c. 1 hr after the s.c. administration of dmPGE₂ (30 μg/kg). Control animals were given the vehicle alone.

**Duodenal HCO₃⁻ secretion**: Under ether anesthesia, the abdomen was incised, and the stomach and duodenum were exposed. According to the method described previously (5), a duodenal loop was made between the pyloric ring and the position just proximal to the common bile duct (15 mm), thereby excluding the influence of the bile and pancreatic juice. An acute fistula prepared using a 2 mm-polyethylene tube was placed in the forestomach through which the gastric contents were withdrawn to prevent an accumulation of gastric juice. 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, 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, Comtrite-7) and by adding 5 mM HCl to the reservoir.

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tissue reperfused with saline. The HCO₃⁻ secretion was measured for 6 hr thereafter. At least 1 hr after the basal HCO₃⁻ secretion had stabilized, mepirizole (60 and 200 mg/kg) or dmPGE₂ (30 μg/kg) was given s.c. Control animals were given the vehicle alone. In cases of acid-stimulated HCO₃⁻ secretion, mepirizole was given 1 hr before exposure of the duodenum to 10 mM HCl. Effect of dmPGE₂ on the basal HCO₃⁻ secretion in rats treated with mepirizole (200 mg/kg) was also examined. At that time, dmPGE₂ (30 μg/kg) was given s.c. 1 hr after mepirizole treatment.

Amount of acid in the duodenum: Under ether anesthesia, the abdomen was incised and the duodenum exposed. An acute fistula prepared using a 2-mm polyethylene tube was placed in the duodenum proximal to the outlet of the common bile duct (15 mm distally to the pylorus), thereby excluding the influence of bile and pancreatic juice. The fistula was withdrawn through the abdominal incision and was held in place by a ligature. After recovery from anesthesia, the animals were kept in Ballman cages, and the duodenal outflow was collected hourly for 6 hr by gravity drainage. Saline was continuously infused at the rate of 1.2 ml/hr into the tail vein to compensate for the loss of body fluid during the collection of duodenal contents. After centrifugation, the samples were measured for volume and titrated for acidity against 0.1 N NaOH to pH 7.0. The amount of acid (volume x acidity per hour) was expressed as microequivalent per hour.

Mepirizole (60 and 200 mg/kg) or dmPGE₂ (30 μg/kg) was given s.c. after a 2 hr basal period. In combined administration, mepirizole (200 mg/kg) was given s.c. 1 hr after the s.c. administration of dmPGE₂ (30 μg/kg). Control animals were given the vehicle alone.

Prostaglandin levels in the duodenal mucosa: Effects of mepirizole on the levels of endogenous prostaglandin E₂ (PGE₂) and 6-keto prostaglandin F₁α (6-keto PGF₁α) in the duodenal mucosa were also studied. Mepirizole (60 and 200 mg/kg) was given s.c., and the control animals were given the vehicle alone. One, 2, 4 and 6 hr later, ether was administered, the duodenum was removed, incised along the antimesenteric border, and the lumen was washed with ice-cold saline. To separate the mucosal layer from the underlying one, the duodenal sample was placed between two glass slides, and quickly placed in hexane frozen with dry ice and acetone (7). The mucosae were collected, weighed and put into 5 ml of 100% methanol (vol./vol.) containing 1 X 10⁻⁴ M sodium meclofenac (Parke-Davis) to prevent new formation of prostaglandins. After homogenization, each sample was processed for extraction and chromatography of prostaglandins, as described (8, 9).

Levels of PGE₂ and 6-keto PGF₁α were determined by radioimmunoassay using rabbit anti-PGE₂ (Institute Pasteur Production) and anti-6-keto PGF₁α serum (Cappel Laboratories). Each assay was performed in duplicate, and the results were expressed in nanograms per gram of wet tissue wt.

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

Results
Effects of mepirizole and mepirizole plus dmPGE₂ on the duodenal mucosa: There was little or no damage to the villi throughout the duodenum of the control animals (Fig. 1, 0 hr). An apparent exfoliation of epithelial cells in the villi of two portions (one in the anterior wall and the other in the posterior wall) was evident in nearly all animals as early as 2 hr after treatment with mepirizole (60 and 200 mg/kg). In most of the villi, the lamina propria was exposed (Fig. 1, 2 hr). Four hours later, the damage progressed to shortened villus by an exfoliation of segments of epithelial cells and destruction of the lamina propria (Fig. 1, 4 hr). The mean indices ±S.E. in the 60 and 200 mg/kg treated groups were 3.6±0.6 and 5.5±0.5, respectively; the latter was significantly higher than the former (Fig. 2). At 6 hr, multiple villi in 2 portions of the duodenum were totally damaged, resulting in the formation of an avillous surface and erosions (Fig. 1, 6 hr). The mean indices ±S.E. in 60 and 200 mg/kg treated groups were 3.6±0.8 and 7.6±0.2, respectively; the latter was significantly higher than the former. Pretreatment with dmPE₂ (30 μg/kg) sig-
Fig. 1. Scanning electron micrographs showing normal (0 hr) and damaged duodenal mucosa of rats 2, 4 and 6 hr after s. c. administration of mepirizole (200 mg/kg, ×100). Note that an exfoliation of the surface epithelial cells was evident 2 hr after administration of mepirizole.

Fig. 2. Development of duodenal damage in rats after s. c. administration of mepirizole and the effects of 16,16-dimethyl prostaglandin E₂ (dmPGE₂) administered s. c. on duodenal damage induced with mepirizole (200 mg/kg). Damage was determined by scanning electron microscopy (SEM). Data represent the mean±one S.E.
nificantly inhibited duodenal lesions induced by mepirizole (200 mg/kg) in the 2, 4 and 6 hr experiments; the inhibition being 73.4%, 58.7% and 60.8%, respectively. The damage was reduced by pretreatment with dmPGE₂, but the epithelial cells at the top of each villus were exfoliated (Fig. 3).

**Effects of mepirizole and dmPGE₂ on gastric secretion:** The mean volume of gastric juice and acid output in the control group ranged from 1.2 to 1.7 ml/hr and 120 to 150 μEq/hr for 9 hr, respectively (Fig. 4). Mepirizole (60 mg/kg) significantly reduced the volume and acid output 1 hr later compared to the control levels. However, there was a significant increase in the volume and acid output 4 and 3 hr later, respectively. The increased volume and acid output remained significantly high up to 6 hr. At 200 mg/kg, the volume and acid output were also significantly reduced 1 hr later, but the reduction reverted to the control levels thereafter. dmPGE₂ (30 μg/kg) had little or no effect on the volume for 7 hr, but significantly reduced gastric acid output for the initial 3 hr (Fig. 5). The reduced acid output reverted to the control levels thereafter. The administration of mepirizole (200 mg/kg) 1 hr after dmPGE₂ (30 μg/kg) had no effect on the volume, but did significantly reduce the acid output for 3 hr after treatment at the same degree observed with dmPGE₂ alone.

**Effects of mepirizole and dmPGE₂ on duodenal HCO₃⁻ secretion:** The proximal duodenum consistently secreted HCO₃⁻ at a rate of 4 to 6 μEq/15 min for 7 hr as the basal secretion (Fig. 6A). Mepirizole (60 and 200 mg/kg) had no influence on this basal HCO₃⁻ secretion throughout the experimental periods. Exposing the duodenal mucosa to 10 mM HCl for 10 min stimulated HCO₃⁻ secretion 15 min later, and this stimulation reached the maximum 45 min later (Fig. 6B). Levels of HCO₃⁻ secretion were approximately double the basal level 45 min after exposure. Mepirizole (60 mg/kg) significantly inhibited HCO₃⁻ secretion in response to 10 mM HCl for 75 min. At 200 mg/kg, the increase in HCO₃⁻ secretion by HCl was totally blocked for more than 2 hr. The administration of dmPGE₂ (30 μg/kg) significantly increased the basal HCO₃⁻ secretion for 2 hr (Fig. 7A). The level of HCO₃⁻ secretion stimulated with 30 μg/kg was approximately double the basal level seen 1 hr later. This stimulatory effect of dmPGE₂ on the basal HCO₃⁻ secretion was not affected by pretreatment with mepirizole (200 mg/kg, Fig. 7B).

**Effects of mepirizole and dmPGE₂ on the amount of acid in the duodenum:** The mean volume and amount of acid in duodenal drainage in the control group ranged from 1.0 to 1.5 ml/hr and 10 to 30 μEq/hr for 6 hr, respectively (Fig. 8). When mepirizole (60 mg/kg) was given, the volume of duodenal drainage was significantly increased 3 and 5 hr later compared to the control levels. At 200 mg/kg, there was a slight increase in the volume. It should be noted that the amount of acid was significantly increased 2 hr after the administration of mepirizole (60 and 200 mg/kg). The increase in amount of acid reached the maximum 3 hr later.

**Fig. 3.** Scanning electron micrographs showing duodenal damage induced 6 hr after administration of mepirizole (200 mg/kg, A) and the effect of dmPGE₂ (30 μg/kg) on the damage (B). Although the surface epithelial cells on the top of the villi were exfoliated, other structures of the villi appear to be intact with dmPGE₂ treatment (×200).
Fig. 4. Effects of mepirizole administered s.c. on gastric secretion in acute fistula rats. Gastric contents were collected hourly and acidity was measured by titrating the samples to pH 7.0. Data represent the mean±one S.E.

(about 120–130 μEq/hr), and these levels were maintained for up to 6 hr. There was no difference in the increase of amount of acid between the 60 and 200 mg/kg treated groups. dmPGE2 (30 μg/kg) significantly increased the volume of duodenal drainage for 3 hr after administration, but significantly reduced the amount of acid for 4 hr after administration (Fig. 9). Mepirizole (200 mg/kg) administered 1 hr after dmPGE2 (30 μg/kg) produced no change in the volume for 1 hr, but reduced the volume to the control levels 2, 3 and 4 hr later. Five and 6 hr later, the volume was significantly reduced as compared to the control levels. At that time, the amount of acid was significantly reduced for the initial hour, but reverted to the control levels thereafter.

Effects of mepirizole on PGE2 and 6-keto PGF1α levels in the duodenal mucosa: Levels of PGE2 and 6-keto PGF1α in the duodenum ranged from 260 to 300 ng/g tissue (Fig. 10). The administration of mepirizole (60 mg/kg) had little or no effect on the levels of both PGE2 and 6-keto PGF1α up to 6 hr. However, the administration of the agent (200 mg/kg) significantly reduced the levels of both PGE2 and 6-keto PGF1α for 2 hr. These prostaglandin levels reverted to the control levels 4 and 6 hr later.
Discussion

These studies indicate that mepirizole induced microscopic damage on the surface epithelial cells of the proximal duodenum as early as 2 hr after treatment. This epithelial cell damage, most evident in the case of 200 mg/kg, progressed to destruction of numerous villi with time up to 6 hr. These morphological changes resembled those induced by other duodenal ulcerogens such as cysteamine and propionitrile (10–12). Several investigators (13–16) who used these ulcerogens provided evidence that such agents interfere with the balance between aggressive and defensive factors in the duodenum within several hours after treatment followed by damage formation. Our objective was to determine whether or not the mechanism by which mepirizole induces damage in the duodenum also involves alteration in the above two factors.

With regard to the aggressive factors, Tabata et al. (17) reported that mepirizole (200 mg/kg) administered s.c. significantly reduced gastric acid output in fistula rats 1 hr later, but the reduction reverted to the control levels 2 hr later. We confirmed these findings. It may be noted that mepirizole (60 mg/kg) first reduced the volume and acid output, but conversely increased both volume and acid output 3 to 4 hr later. This increase in gastric acid secretion, however, does not play a meaningful role in the pathogenesis of mepirizole-induced damage for the following reasons: (A) the duodenal damage observed...
Fig. 6. Effects of mepirizole on basal (A) and acid-stimulated (B) duodenal HCO$_3^-$ secretion in rats. Duodenal HCO$_3^-$ secretion was determined by perfusing a duodenal loop (made proximal to the common bile duct) with saline and titrating the perfusate to pH 7.4 using the pH-stat method. Mepirizole significantly inhibited the duodenal HCO$_3^-$ secretion in response to 10 mM HCl. Data represent the mean ± one S.E.

with 60 mg/kg was significantly less severe than that observed with 200 mg/kg. (B) The duodenal acid output did not increase in parallel to the increase in gastric acid output, probably because of the weak inhibition of duodenal HCO$_3^-$ secretion with 60 mg/kg. These results suggest that an increase in aggressive factors such as gastric acid is not a causal factor related to the initial damage of duodenal mucosal cells in response to mepirizole, regardless of the dose. Nevertheless, the mepirizole-induced duodenal damage was significantly inhibited by pretreatment with antacids and antisecretory agents (1). Therefore, the presence of a certain amount of gastric juice appears to be required for damage to ensue.

Recent studies showed that the duodenal mucosa of various experimental animals or amphibians secretes a significant amount of HCO$_3^-$, as one defensive factor against intraluminal acid (18, 19). Tabata et al. (17) reported that mepirizole, administered at an ulcerogenic dose, reduced this basal duodenal HCO$_3^-$ secretion, maximally by 45% in rats. In our study, however, the basal HCO$_3^-$ secretion remained unchanged for 6 hr after treatment when the same dose of
Fig. 7. Effects of dmPGE$_2$ alone (A) or dmPGE$_2$ plus mepirizole (B) on basal duodenal HCO$_3^-$ secretion in rats. dmPGE$_2$ was administered s.c. 1 hr after mepirizole (200 mg/kg) when they were combined. Pretreatment with mepirizole did not affect the stimulating activity of dmPGE$_2$ on duodenal HCO$_3^-$ secretion. Data represent the mean±one S.E.

Mepirizole was administered. This apparent difference may be partly explained by different methods used for measurement of HCO$_3^-$ secretion. They measured HCO$_3^-$ secretion indirectly, whereas we measured HCO$_3^-$ secretion by a direct titration using the pH-stat method. Alternatively, it is possible that the endogenous prostaglandin levels observed by Flemström et al. (6) is different between the animals used by these two groups. The duodenal mucosa of the rats they used may contain higher levels of endogenous prostaglandins.

The presence of acid in the lumen of the duodenum is a potent stimulant for duodenal HCO$_3^-$ secretion, possibly via the stimulation of endogenous prostaglandins (6, 20, 21). We also confirmed that the instillation of 10 mM HCl in the duodenal loop for 10 min markedly stimulated duodenal HCO$_3^-$ secretion. In contrast to the case of basal HCO$_3^-$ secretion, mepirizole reduced this acid-stimulated HCO$_3^-$ secretion in the duodenum in a dose-related manner. Therefore, it is possible that gastric acid emptied by the stomach will not be sufficiently neutralized in the duodenum, thereby resulting in an increased amount of acid. Indeed, a gradual
Fig. 8. Effects of mepirizole on the volume and amount of acid in the rat duodenum. Duodenal contents (without bile and pancreatic juice) were hourly collected through an acute fistula prepared in the proximal duodenum. Note that the amount of acid was gradually increased after s.c. administration of mepirizole (60 and 200 mg/kg). Data represent the mean±one S.E.

Increase in the amount of acid, which is nearly equal to gastric acid output, was observed beginning from 2 hr after the administration of mepirizole (60 and 200 mg/kg). As described above, duodenal mucosal damage was observed as early as 2 hr after treatment with mepirizole. A causal relationship between these two effects is thus likely; the duodenal damage is caused by an increase in the amount of acid in the duodenum. The initial damage of the duodenal mucosa induced 2 hr after treatment with mepirizole (60 mg/kg) was not aggravated until 6 hr, although there was an increased amount of acid in the duodenum. In contrast, the initial damage caused by the agent (200 mg/kg) became severe 4 and 6 hr later. The degree of increase in the amount of acid was nearly the same after treatment with mepirizole (60 and 200 mg/kg). Therefore, an impairment of other defensive factors such as mucosal blood flow or mucus secretion in
the duodenum may also be involved in the progress of the damage induced by 200 mg/kg of the agent.

As expected from our previous work (1), pretreatment with dmPGE₂ (30 µg/kg) did protect the duodenal mucosa against the damage induced by mepirizole in the 2, 4 and 6 hr experiments. dmPGE₂ alone, at the dose which inhibited duodenal damage, significantly inhibited gastric acid output and stimulated duodenal HCO₃⁻ secretion, thereby resulting in a reduced amount of acid in the duodenum. The hourly duodenal outflow after dmPGE₂ treatment exceeded the hourly volume of gastric juice, thereby suggesting the stimulation of duodenal secretion by the agent. The increase in the amount of acid in the duodenum in response to mepirizole was prevented by pretreatment with dmPGE₂. This is most likely the mechanism of action of dmPGE₂ in preventing mepirizole-induced duodenal damage. These findings also support the idea that impairment in defense factors is mainly responsible for the progress of mepirizole-induced damage.

Various antiinflammatory agents interfere with the biosynthesis of endogenous prostaeglandins by inhibiting cyclooxygenase
activity (22). In addition, mepirizole reduced duodenal $\text{HCO}_3^-$ secretion in response to HCl which is possibly mediated by endogenous prostaglandins. Therefore, it is assumed that mepirizole reduces endogenous prostaglandins in the duodenum. Indeed, mepirizole (200 mg/kg) reduced the levels of endogenous $\text{PGE}_2$ and 6-keto-$\text{PGF}_{1\alpha}$, albeit transiently. Whether this reduction in prostaglandins is related to the inhibition of cyclooxygenase activity, to an enhancement of degradation of prostaglandins, or to another mechanism is unknown. Since the reduction of duodenal $\text{HCO}_3^-$ secretion in response to HCl by mepirizole persisted up to 6 hr, it is unlikely that the reduced $\text{HCO}_3^-$ secretion is due only to a reduction in prostaglandin levels. Indomethacin and aspirin reduce endogenous prostaglandins in the gastrointestinal mucosa more extensively and persistently than does mepirizole. However, these agents seldom, if ever, produce duodenal damage within such a short period after treatment. Mepirizole (60 mg/kg) had...
little or no effect on endogenous prostaglandin levels, but did induce duodenal mucosal damage. Thus, it is questionable that changes in endogenous prostaglandins are involved to any great extent in the pathogenic mechanisms of mepirizole-induced damage.

All these results taken together suggest that mepirizole induces microscopical damage in the proximal duodenum because of a sustained increase in the amount of acid in the duodenum, caused not by an increased gastric acid secretion, but rather by an impairment in duodenal \( \text{HCO}_3^- \) secretion.

Acknowledgments: We are grateful to Drs. K. Kobayashi and Y. Arakawa for their kind advice concerning the elaborate techniques used for determination of the prostaglandins. We also thank A. Nishida, A. Toibana and H. Wada for technical assistance and thank Y. Okada and H. Wada for secretarial services.

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