Augmentation of Cysteamine and Mepirizole-Induced Lesions in the Rat Duodenum and Stomach by Histamine or Indomethacin

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Abstract—Repeated administration of histamine·2HCl (40 mg/kg×4) significantly augmented mepirizole (200 mg/kg) or cysteamine (300 mg/kg)-induced duodenal and gastric lesions in rats within 6 hr. Most of the duodenal lesions were penetrating ulcers and were located over the entire duodenum. Gastric lesions were mainly located in the antrum adjacent to the duodenum. Indomethacin pretreatment did not significantly augment the duodenal lesions induced by mepirizole or cysteamine, but did augment the gastric lesions induced by these compounds.

Both duodenal ulcerogens cysteamine and mepirizole can induce microscopic damage to surface epithelial cells of the proximal duodenum of rats within 2-4 hr of administration (1-3). The initial damage usually takes more than 12 hr to progress to visible penetrating ulcers. In addition, ulcerogenic doses (400 mg/kg) of cysteamine frequently lead to high mortality, by its general toxicity. We induced severe ulcers at a much earlier time, thereby facilitating studies on the pathogenesis and also for screening antiulcer agents. Either histamine as a gastric secretagogue or indomethacin which inhibits HCO₃⁻ secretion in the duodenal mucosal cells (4-7) was given before and after or before the above ulcerogens, respectively. Male Sprague-Dawley rats (240-270 g) were deprived of food (but not water) for 24 hr before the experiments. In the first study, the effects of histamine, mepirizole, or cysteamine, either alone or in combination, on the duodenal and gastric mucosa were studied. Histamine·2HCl (Nakarai, 40 mg/kg), dissolved in 10% gelatin, was given subcutaneously in a volume of 0.5 ml/100 g body weight four times, i.e., at 1, 2, 2, 2 hr intervals. Either mepirizole (Daiichi, 200 mg/kg) or cysteamine (Sigma, 300 mg/kg), suspended in 1% carboxymethylcellulose (CMC), was given once in a volume of 0.5 ml/100 g body weight. At that time, 10% gelatin was also given four times, i.e., 1 hr before, concomitantly, 2 and 4 hr after each ulcerogen. In the combined study, histamine, instead of 10% gelatin, was given four times to the ulcerogen treated rats. Animals were killed at 7 hr after the initial administration of histamine or at 6 hr after mepirizole or cysteamine administration. The duodenum and stomach were removed as a single unit and inflated by injecting 12 ml of 2% formalin to fix the inner and outer layers of each organ. Subsequently, the duodenum was incised along the site opposite the mesenteric attachment and the stomach was incised along the greater curvature. The area (mm²) of each lesion in the duodenum and stomach was measured under a dissecting microscope with a square grid (×10), summed in each portion and used as a lesion index. The person measuring the lesions had no knowledge of which treatment an animal had received. Tissues were placed into 10% formalin and stained with hematoxylin and eosin for histological examination. In the second study, the combined effects of indomethacin and ulcerogens were studied. Indomethacin (Sigma, 5 mg/kg), suspended in saline with a trace of Tween 80, was given once subcutaneously in a volume of 0.5 ml/100 g body weight. Mepirizole (200 mg/kg) or cysteamine (300 mg/kg) was also given once subcutaneously. The vehicle used for in-
Domethacin was given 1 hr before ulcerogen administration. In the combined study, each ulcerogen was given 1 hr after indomethacin administration. Animals were killed 6 hr after administration of the ulcerogens. The duodenum and stomach were examined macroscopically for the presence of lesions as described above and examined microscopically to determine the severity of the damage. Student's t-test was used to determine the statistical significance of the data and a P<0.05 value was regarded as significant.

Repeated administration of histamine (40

Fig. 1. Influence of combined administration of histamine (A) or indomethacin (B) together with mepirizole or cysteamine on the duodenal and gastric mucosa in rats. Note that histamine given 4 times significantly augmented both the duodenal and gastric lesions induced by mepirizole and cysteamine at 7 hr after the initial administration of histamine. Indomethacin pretreatment also significantly augmented the gastric lesions induced by these agents, but not the duodenal lesions.
mg/kg×4) alone induced visible but superficial lesions throughout the duodenum 7 hr after the first injection (Fig. 1A). A single administration of either mepirizole (200 mg/kg) or cysteamine (300 mg/kg), given together with 10% gelatin four times, also induced duodenal lesions in 7 of 10 and 2 of 10 rats, respectively. The degree of lesion was less severe than that observed with histamine alone. In the stomach, histamine, mepirizole, or cysteamine given alone produced few or no lesions. Combined administration of histamine plus mepirizole or histamine plus cysteamine significantly augmented the development of duodenal lesions. The lesions developed in the duodenum were observed through the serosal site (Fig. 2). The lesion index in the stomach was also significantly higher than those seen in rats treated with histamine alone or each ulcerogen alone. In the histamine plus mepirizole treated group, a triangular lesion was almost invariably observed in the antrum immediately adjacent to the duodenum. Histologically, most of the lesions induced in the duodenum by histamine plus each ulcerogen penetrated the submucosal layers. Animals given cysteamine alone or together with histamine were all alive during the 6 hr period.

A single administration of indomethacin (5 mg/kg) alone or cysteamine (300 mg/kg) alone induced few or no lesions either in the duodenum or the stomach 6 hr later (Fig. 1B). Mepirizole (200 mg/kg) induced one or two small superficial lesions in the proximal duodenum in 7 of 10 rats. The administration of indomethacin plus mepirizole significantly augmented the gastric lesions but not the duodenal lesions as compared with mepirizole alone. The administration of indomethacin plus cysteamine produced less severe duodenal lesions but significantly augmented the gastric lesions.

These results indicate that persistent increase in the gastric secretion caused by repeated injection of histamine alone can induce visible damage in the rat duodenum within 7 hr. Our previous study showed that three administrations of histamine in the same dose used in this study induced no damage in the duodenum 8 hr later (7). This difference may be caused by an increase in the number of injections and shortening of the injection interval from 2.5 hr in an earlier study to 1 or 2 hr in this study. As expected, the combined administration of histamine plus duodenal ulcerogens significantly augmented the development of duodenal lesions, most being penetrating ulcers. The damaged area of the histamine-cysteamine-induced duodenal lesions was about double that reported with indomethacin-histamine-induced lesions (18 vs. 9.8 mm²) (7). Both mepirizole and cysteamine reduced duodenal HCO₃⁻ secretion in rats in response to acid instillation, thereby resulting in a lowered acid neutralization capacity (3, 8, 9). Therefore, the mechanism by which the combined administration augments duodenal lesions appears to be an increase in aggressive factors in the stomach and a decrease in defensive factors both in the duodenum and stomach. Duodenal lesions induced by mepirizole or cysteamine alone at 24 hr after administration were round, predominantly located in the proximal duodenum and did not extended to the distal duodenum. In contrast, the duodenal lesions induced by a combination with histamine plus ulcerogens were extended throughout the duodenum, although the most severe lesions were present in the proximal area. This finding suggests that the pathogenesis of duodenal lesions induced by the ulcerogen itself and the ulcerogen plus histamine is apparently different. Duodenal lesions induced by the combination of ulcerogens plus histamine appear to be much more dependent on the rapid flow of an excessive amount of acidic juice emptied from the stomach. In contrast, the duodenal lesions induced by ulcerogens may be gradually formed and due to an alteration of several factors, including the gastric juice and duodenal HCO₃⁻ secretion.

In contrast to the case of histamine, indomethacin pretreatment did not significantly augment the duodenal lesions induced by either mepirizole or cysteamine, but did augment the gastric lesions. It is possible that the duodenal HCO₃⁻ secretion in the duodenum was lowered maximally with the ulcerogen itself and was not potentiated with indomethacin treatment. Whether or not indomethacin as well as
cysteamine or mepirizole exerts any influence on the pancreatic and/or biliary $\text{HCO}_3^-$ secretion will be studied and reported in detail elsewhere. The mechanism by which histamine or indomethacin pretreatment significantly augmented the gastric lesions, particularly in the distal antrum, induced by mepirizole or cysteamine was not clear. Indomethacin at the dose used in this study potently and persistently reduces endogenous prostaglandins in the rat stomach (10). Therefore, the reduction of endogenous prostaglandins in the stomach by indomethacin may participate in this augmentation.

All these findings suggest that histamine-mepirizole- or histamine-cysteamine-induced lesions are useful models because of the severity, high incidence, shortness of the experimental periods, and total survival.

Fig. 2. External (top) and internal (bottom) view of the duodenal lesions induced in rats by combined administration of histamine (40 mg/kg × 4) and cysteamine (300 mg/kg) at 7 hr after the initial administration of histamine. Duodenal lesions can be seen from the serosal site.
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