Role of Gastric Acid and Bile Acids in the Pathogenesis of Compound 48/80-induced Gastric Lesions in Rats

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Abstract—We studied the effects of various agents, which influence gastric acidity and bile acids, on compound 48/80 (48/80)-induced gastric lesions in rats. 48/80-induced gastric lesions were produced by repeated intraperitoneal administration of 48/80 at 0.75 mg/kg once daily for 4 days. Test agents were given orally twice daily (30 min before and 9 hr after 48/80 administration) for 4 days. Al(OH)₃ and sucralfate at 2000 mg/kg/day, a weak antacid dose, significantly inhibited (about 50-60%) the development of 48/80-induced lesions. Propantheline at 60 mg/kg/day and omeprazole at 60 or 200 mg/kg/day, which reduced gastric secretion for more than 12 hr, also significantly inhibited (about 30-40%) these lesions. Cimetidine at 200 mg/kg/day, which reduced gastric secretion for only 5 hr, had little effect on the lesion formation. Cholestyramine, which is a potent bile acids binding agent, had no effect on 48/80-induced lesions in doses of 600 or 2000 mg/kg/day. These results suggest that gastric acid, but not bile acids, is partly involved in the pathogenesis of 48/80-induced gastric lesions.

We reported a new method for producing gastric lesions by repeated administration of compound 48/80 (48/80) to rats (1). Since these lesions were all but completely inhibited by cyproheptadine or methysergide, the vascular changes caused by released serotonin seemed to be the main causal factor. The present study was done to determine whether or not a gastric acid factor is also involved in the pathogenetic mechanism of 48/80-induced lesions. In addition, refluxed bile acids are postulated to be involved in the pathogenesis of some experimentally-induced gastric lesions (2-6). Thus, the influence of bile acids on the pathogenesis of 48/80-induced lesions was also studied using a bile acids binding agent, cholestyramine.

Materials and Methods

Male Donryu rats (220–240 g) were fed a normal rat chow and given water ad libitum throughout the experimental period, except during the gastric secretory studies.

Lesion induction: 48/80 (Sigma), dissolved in distilled water, was given intraperitoneally at 0.75 mg/kg once daily for 4 days (1). Twenty-four hr after the final administration, the rats were killed and the stomachs were removed and inflated with 10 ml of 2% formalin for 10 min. They were then opened along the greater curvature for examination of lesions in the glandular stomach under a dissecting microscope (×10). The area (mm²) of each lesion was measured, summed per stomach, and arbitrarily divided into 5 degrees according to the following lesion indices:

| Total area (mm²): |
|------------------|
| 0, 1–30, 31–60, 61–90, 91–120, >120 |

| Lesion index: |
|--------------|
| 0 1 2 3 4 5 |

The person measuring the lesions had no knowledge of which treatment an animal had received. The following agents were given orally twice daily (30 min before and 9 hr after the administration of 48/80) for 4 days. Al(OH)₃ (Maruishi) as an antacid and sucralfate (Chugai) as a weak antacid and
potent antipepsin agent were suspended in distilled water, and propantheline bromide (Sigma) and cimetidine (SK&F) as antisecretory agents were dissolved in saline. Omeprazole (Hässle) as an antisecretory agent was suspended in 1% carboxymethylcellulose solution containing 0.2% NaHCO₃ (w/w), and the pH was adjusted to 9.0 with 2 N NaOH. Cholestyramine (Nakarai) as a bile acids binding agent was suspended in distilled water. These agents were given in a volume of 0.5 ml/100 g body weight.

Gastric secretory study: Under ether anesthesia, the abdomen of 24 hr fasted rats was incised and the pylorus ligated. Four hr later, they were killed and the stomachs removed. The gastric contents were analyzed for volume, pH, and acidity (the samples were titrated against 0.1 N NaOH to pH 7.0). Acid output was expressed as μEq/hr. Test agents were given orally 1 or 8 hr before pylorus ligation.

Statistical analysis: Student’s t-test was used to determine the statistical significance of the data, and P<0.05 was regarded as significant.

Results

Effects on 48/80-induced lesions: Four days treatment with 48/80 produced severe and consistent mucosal lesions in the glandular stomach. As shown in Fig. 1, Al(OH)₃, sucralfate and propantheline dose-dependently inhibited the 48/80-induced lesions, the inhibition percentage varying from about 30 to 60%. Omeprazole also significantly inhibited the lesions, but there was no dose-dependency at the doses used.
Cimetidine at 100 and 300×2 mg/kg/day and cholestyramine even at 1000×2 mg/kg/day had no effect on these lesions.

**Effects on gastric secretion:** As shown in Figs. 2 and 3, Al(OH)₃ at 1000 mg/kg and sucralfate at 1000 mg/kg slightly reduced the acid output and increased the pH level up to 3.0 at 5 hr after treatment. In contrast, 30 mg/kg propantheline and 100 mg/kg omeprazole markedly reduced the gastric acid output and increased the pH level up to 6 to 7 for 5 hr. The antisecretory effect of these agents persisted even for 12 hr after administration. Cimetidine at 100 and 300 mg/kg also potently reduced the gastric acid output for 5 hr, but the antisecretory effect was not observed 12 hr later. Cholestyramine at 300 and 1000 mg/kg had no effect on gastric secretion at 5 hr after treatment.

**Discussion**

Since 48/80-induced gastric lesions were significantly inhibited by agents which reduce gastric acidity, gastric acid was suggested to be involved in the pathogenesis of the lesions. Our previous study (7) showed that a single intraduodenal administration of 100 mg/kg of omeprazole inhibited the gastric secretion for more than 14 hr in pylorus-ligated rats. Omeprazole given orally at 100 mg/kg was also found to markedly inhibit the gastric acid secretion for up to 12 hr in the present study. Thus, it is possible...
that two administrations of omeprazole per day may sufficiently reduce the noxious influence of gastric acid during an entire day for 4 days of an experimental period. However, the inhibition of 48/80-induced lesions by omeprazole was limited, i.e., less than 40% at the maximum. Similar results were also observed with propantheline. Why cimetidine had little effect on reduction of the gastric lesions is unknown, but the limited effect on gastric secretion as demonstrated in this study may be one explanation. The effects of Al(OH)₃ or sucralfate on gastric acidity were weak and short, whereas they had more potent inhibitory effects on 48/80-induced lesions than the effects of omeprazole. Therefore, it is possible that the effects of these agents may relate to other mechanisms in addition to antacid activity. Nagashima and Hirano (8) and Steiner et al. (9) have suggested that sucralfate which selectively adheres to ulcerous and eroded areas of the stomach and forms a protective layer may protect the mucosa against further untoward effects of gastric acid. In addition, sucralfate stimulates the biosynthesis of endogenous prostaglandins in the rat stomach (10, 11). In an earlier study, we demonstrated that cholestyramine at the dose given in the present study potently inhibited water-immersion and indomethacin-induced gastric lesions (12). Our data suggested that bile acids refluxed into the stomach play an important role in the pathogenesis of these lesions. Cholestyramine, however, had no effect on the 48/80-induced lesions, thereby indicating that bile acids have no relation to the pathogenetic mechanism of these lesions.

All these results taken together suggest that 48/80-induced gastric lesions might be caused mainly by vascular disturbances in response to released serotonin and partly by gastric acid.

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