Antiulcer Activity of Clotiazepam in Rats*

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Abstract—Effect of the anti-anxiety drug clotiazepam on the experimental gastric ulceration induced by restraint and water-immersion stress or aspirin was studied in rats. Clotiazepam prevented the development of each gastric ulcer. From the effect of clotiazepam on aspirin-induced ulceration, we presumed that clotiazepam should have some other antiulcer mechanism in addition to its action on the central nervous system. There was an appreciable correlation between the decrease in the hexosamine level of gastric tissue and associated ulceration. After treatment with aspirin, the hexosamine level was abruptly reduced and was maintained at a low level for several hours. In the clotiazepam-pretreated group, the hexosamine level reduced by aspirin was progressively restored to the intact level. By histological examination with periodic acid-Schiff (PAS)/alcian-blue (AB) staining, clotiazepam increased the amount of gastric mucopolysaccharides decreased by aspirin. Clotiazepam did not affect gastric secretion in pylorus-ligated rats. Atropine and cimetidine inhibited ulceration induced by stress or aspirin and gastric secretion, but did not affect the hexosamine level reduced by aspirin. These results indicate that the antiulcer efficacy of clotiazepam may be attributed to its action not only on the central nervous system, but also on the mucus in gastric mucosa.

Therapeutic evidence has shown that the anti-anxiety drug clotiazepam is beneficial to patients with gastrointestinal diseases (1). Okuse et al. (2) have recently reported that clotiazepam used in combination therapy was effective not only in the improvement of subjective symptoms, but also in the healing of ulcers. Protection by anti-anxiety drugs against experimental ulceration induced by stress has been reported (3–5). On the other hand, little has been reported about the effect of these drugs on other kinds of experimental ulceration. Clinical observations have suggested that even in the absence of overt anxiety some peptic ulcer patients may benefit from combination therapy which includes anti-anxiety drugs (6). We speculate that clotiazepam has other antiulcer mechanisms in addition to its action on the central nervous system.

The present experiments were undertaken to investigate the effect of clotiazepam on ulceration induced by water-immersion stress or aspirin, which have been proposed to develop from reduction of the gastric mucus content (7–9).

Materials and Methods

Wistar rats of both sexes, weighing about 180 g, were used. The animals were deprived of food but allowed free access to water for 20 hr prior to experiments.

Drugs used: Clotiazepam and cimetidine were synthesized in the Yoshitomi Research Laboratories, and atropine sulfate was obtained from the Tokyo Kasei Kogyo Co., Ltd. These drugs were suspended or dissolved in 0.5% methylcellulose solution.

Stress-induced ulceration: Rats were placed in a stress cage and immersed to the level of the xiphoid process in a water bath at 23°C for 6 hr. The examined drugs were given orally 30 min before water-immersion.

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The animals were then stunned and decapitated. The stomach of each rat was excised and inflated by 8 ml of 2% formalin solution, and then it was immersed in 2% formalin solution for 120 min. The stomach was incised along the greater curvature, and the lengths (mm) of the ulcer were measured and summed. The logarithm of the sum was used as an ulcer index.

Aspirin-induced ulceration: Aspirin suspended in 0.5% methylcellulose solution was given orally at 100 mg/kg to rats. Each drug was given orally 30 min before aspirin administration. The animals were stunned and decapitated 5 hr after the aspirin treatment. Each stomach was then examined for ulceration as described above.

Gastric secretion: A modification of the pylorus-ligated stomach method (10) was used to study gastric secretion. Under ether anesthesia, the abdomen was incised and the pylorus was ligated. Each drug was given subcutaneously immediately after the operative procedure. The animals were killed 4 hr after the administration. The gastric contents were centrifuged, and the volume, acidity and pepsin activity of the gastric juice were examined. The acidity of the gastric juice was determined by automatic titration against 0.1 N NaOH to pH 7.0 (ATR-107 Kyoto Densi), and the pepsin activity was determined by Anson's method (11).

Determination of hexosamine: The stomach was quickly removed and incised along the greater curvature. Each stomach was washed with cold saline solution. The gastric tissues were divided into the corpus and antrum region and then dehydrated and defatted. The dried tissues were hydrolyzed in 4 N HCl in sealed ampoules at 100°C for 16 hr. Hexosamine in the hydrolyzate was determined colorimetrically according to Boas (12). Hexosamine was estimated as glucosamine hydrochloride and expressed in μg/mg dry tissue weight.

Histological examination: The gastric tissues were fixed with Carnoy's fixing solution. After fixation, the tissues were infiltrated with paraffin and sectioned in 4 μm slices. The tissue slices were stained with periodic acid-Schiff (PAS)/alcian-blue (AB, pH 2.5).

Statistical analysis: The significance of differences between values was examined by Student's t-test.

Results

Stress-induced ulceration: The restraint and water-immersion stress produced long and narrow lesions in the glandular stomach. These lesions were erosions classified histologically by Murakami as UL-1 (13). However, many authors (4, 5, 14, 15) call these lesions “experimental ulcers,” so we followed this

![Fig. 1](image-url)

**Fig. 1.** Effect of clotiazepam, atropine and cimetidine on the gastric ulceration induced by restraint and water-immersion stress in rats. Each drug was administered orally 30 min before water-immersion. The animals were killed 6 hr after the procedure. Each column represents the mean (N=9–10). *P<0.05 and **P<0.01 when compared with the control.
nomenclature. Figure 1 shows that clotiazepam (10–100 mg/kg) dose-dependently inhibited the ulceration. Atropine (1, 3 mg/kg) and cimetidine (30–300 mg/kg) also significantly inhibited it.

**Aspirin-induced ulceration:** Linear lesions similar to that described above of the glandular stomach were observed 5 hr after aspirin administration. However, no ulceration could be detected within 3 hr. As shown in Fig. 2, clotiazepam at oral doses of 30 and 100 mg/kg significantly inhibited the ulceration. Atropine and cimetidine also significantly inhibited it.

**Gastric secretion:** In pylorus-ligated rats, clotiazepam (100 mg/kg) did not affect the volume of gastric juice, acid and pepsin output. On the other hand, atropine and cimetidine significantly reduced the value of measured variables (Table 1).

**Hexosamine content:** Figure 3 shows that hexosamine level in the corpus tissue was lower than that in the antrum tissue. Hexosamine level in both tissues of the corpus and antrum was significantly reduced 4 hr after aspirin administration (100, 300 mg/kg). Moreover, these doses produced definite ulceration. Figure 4 shows that the rapid decrease of hexosamine level in both tissues was observed 1 hr after aspirin administration, which was maintained for 8 hr in the corpus and 4 hr in the antrum. Clotiazepam (10, 30 mg/kg, p.o., 10 mg/kg, i.p.) significantly prevented the hexosamine decrease in both tissues (Tables 2 and 3). Atropine and cimetidine did not significantly affect the hexosamine level in the antrum. As shown in Fig. 4, in the clotiazepam pretreated group, the hexosamine level reduced by aspirin tended to increase toward the intact level 1 to 2 hr after aspirin administration and approximately attained it.

![Fig. 2. Effect of clotiazepam, atropine and cimetidine on the gastric ulceration induced by aspirin in rats. Each drug was administered orally 30 min before the aspirin treatment (100 mg/kg, p.o.). The animals were killed 5 hr after the aspirin treatment. Each column represents the mean (N=10). **P<0.01 when compared with the control.](image)

**Table 1. Effect of clotiazepam, atropine and cimetidine on gastric secretion in pylorus-ligated rats**

| Treatment      | mg/kg s.c. | Volume ml/100 g | Acid output μEq/100 g | Pepsin output μg/100 g |
|----------------|------------|-----------------|-----------------------|------------------------|
| Control        | 4.5±0.3    | 475±24          | 1402±91               |
| Clotiazepam    | 4.6±0.2    | 546±35          | 1497±65               |
| Control        | 4.7±0.2    | 585±35          | 1765±77               |
| Atropine       | 0.1        | 1.6±0.4**       | 205±48**              | 759±146**              |
| Clotiazepam    | 2.9±0.2**  | 294±27**        | 1331±72**             |

Drugs were administered immediately after pylorus-ligation. The animals were killed 4 hr after pylorus-ligation. Values are means±S.E. (N=9–10). **P<0.01 when compared with the control.
after 4 to 8 hr in the corpus and antrum.

**Histological examination:** The intact gastric mucus consisted of a superficial layer which was stained well by PAS and a profound layer which was stained well by AB. Aspirin clearly decreased the PAS- and AB-stained substances in the corpus and antrum mucosa. Pretreatment of clotiazepam, 30 min before aspirin administration, significantly reduced the diminution in both stained substances in the gastric mucosa (Fig. 5).

**Discussion**

Our results confirm that clotiazepam is effective against gastric ulcer formation induced by stress or aspirin and enhances the biosynthesis and/or the accumulation of the mucus in the gastric mucosa.

It is now generally accepted that the antiulcer effect of anti-anxiety drugs on stress-induced ulceration results from their action on the central nervous system (4, 5). Nakanishi et al. have reported that clotiazepam has more potent anti-pentylene-tetrazole activity and conflict attenuation activity than does diazepam (16). It is certain that the anti-anxiety effect of clotiazepam contributes to its preventive effect against stress-induced ulceration.

It has been proposed that aspirin induces gastric ulceration by reducing the gastric mucus which protects the gastric mucosa from acid and pepsin (7–9) and by weakening the gastric mucosal barrier (17). We determined the hexosamine, one of the major components of mucus, in the tissues of the corpus and antrum.

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**Fig. 3.** Effect of aspirin on gastric hexosamine level and ulceration in rats. The animals were killed 4 hr after the aspirin treatment (100 mg/kg, p.o.). Each column represents the mean±S.E. (N=10–12). **P<0.01 when compared with the intact group.

**Fig. 4.** Effect of clotiazepam on gastric hexosamine level in rats. Clotiazepam (30 mg/kg) was administered orally 30 min before the aspirin treatment (100 mg/kg, p.o.). Each column represents the mean±S.E. *P<0.05, **P<0.01 when compared with the intact or aspirin-treated group.
Table 2. Effect of clotiazepam on the hexosamine level of the gastric corpus in aspirin-treated rats

| Treatment     | mg/kg | No. of rats | Hexosamine μg/mg | % Change |
|---------------|-------|-------------|------------------|----------|
| p.o. Control  | 3     | 10          | 8.17±0.28        | 4        |
| Clotiazepam   | 10    | 15          | 8.49±0.58        | 4        |
| Control       | 30    | 20          | 7.61±0.24        | 15       |
| Clotiazepam   | 10    | 20          | 8.75±0.36**      | 15       |
| Control       | 10    | 15          | 8.12±0.28        | 10       |
| Clotiazepam   | 30    | 20          | 8.89±0.24*       | 10       |

Clotiazepam was administered 30 min before the aspirin treatment (100 mg/kg, p.o.). The animals were killed 4 hr after the administration of aspirin. Values are means±S.E. *P<0.05, **P<0.01 when compared with the control.

Table 3. Effect of clotiazepam, atropine and cimetidine on the hexosamine level of gastric antrum in aspirin-treated rats

| Treatment     | mg/kg | No. of rats | Hexosamine μg/mg | % Change |
|---------------|-------|-------------|------------------|----------|
| p.o. Control  | 3     | 14          | 11.53±0.43       | 2        |
| Clotiazepam   | 10    | 12          | 11.77±0.71       | 2        |
| Control       | 3     | 14          | 11.75±0.45       | 10       |
| Clotiazepam   | 10    | 13          | 13.82±0.90*      | 18       |
| Control       | 30    | 18          | 12.52±0.55       | 18       |
| Clotiazepam   | 30    | 18          | 15.08±0.51**     | 20       |
| p.o. Atropine | 1     | 12          | 10.82±0.34       | 10       |
| Control       | 100   | 20          | 11.89±0.74       | 10       |
| Cimetidine    | 100   | 20          | 10.78±1.05       | 10       |
| Control       | 10    | 18          | 11.76±0.75       | 15       |
| Clotiazepam   | 10    | 18          | 12.04±0.34**     | 15       |

Drugs were administered 30 min before the aspirin treatment (100 mg/kg, p.o.). The animals were killed 4 hr after the administration of aspirin. Values are means±S.E. *P<0.05, **P<0.01 when compared with the control.

Our results agree with other reports (7–9) that aspirin-induced ulceration correlates with the reduction in the hexosamine level of gastric tissue. Although the hexosamine level fell to a low level abruptly after aspirin administration, ulceration did not develop until several hours after the treatment. This observation suggests that the low hexosamine level is a cause of ulceration and not a result, that the visual ulcer is exerted by acid and/or pepsin after the gastric mucus has been reduced by aspirin. The reduced hexosamine
level in the tissues of both the corpus and antrum was restored to the intact level by pretreatment with clotiazepam.

Moreover, we examined histologically the effect of clotiazepam on the gastric mucopolysaccharides which contain hexosamine and constitute the gastric mucus with binding proteins. Additionally, in a biochemical study, aspirin decreased the neutral and acid mucopolysaccharides in the corpus and antrum tissues, and clotiazepam was effective against the decrease of them. It is possible that the mucus-restoring effect of clotiazepam may contribute to its preventive effect against aspirin-induced ulceration.

We have observed that in pylorus-ligated rats, in contrast with cimetidine and atropine, clotiazepam did not inhibit gastric secretion at the antiulcer dose (100 mg/kg, s.c.). The preventive effects of cimetidine and atropine against ulceration induced by aspirin correlate poorly with the hexosamine content. They probably prevent experimental ulceration in animals through a gastric acid-dependent mechanism. Compared with the close relationship of the antiulcer effect to the antisecretory activity for cimetidine and atropine, clotiazepam seems to protect the gastric mucosa through some acid-independent mechanism.

Accordingly, our results suggest that the effect of clotiazepam on mucus may contribute to the antiulcer efficacy in concert with the anti-anxiety property and that clotiazepam may have therapeutic applications for peptic ulcers which are different from those of H2-blockers and anticholinergics.

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