Sulpiride Specifically Attenuates Psychological Stress-Induced Gastric Lesions in Rodents

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ABSTRACT—Gastric lesions were developed in the communication box paradigm (CB) in mice as well as in the activity-stress paradigm (AS) in rats. Treatment with sulpiride (10–320 mg/kg, p.o.) attenuated these psychological stress-induced gastric lesions in a dose-dependent manner, while it failed to suppress those induced by physical stress such as restraint water-immersion (WI) and indomethacin treatment (IND). In contrast, treatment with famotidine (0.32–10 mg/kg, p.o.) dose-dependently attenuated the gastric lesions induced by physical stress but not those by psychological stress. Pylorus-ligation study revealed that famotidine strongly reduced gastric acid secretion, whereas sulpiride minimally affected that. It was also demonstrated that physical stress (WI) enhanced acid secretion while psychological stress (CB and AS) rather depressed that. These results suggest that the mechanisms of gastric lesion formation are clearly different between physical and psychological stress and that sulpiride specifically attenuates psychological stress lesions possibly through a central mechanism.

Keywords: Sulpiride, Psychological stress, Gastric lesion, Acid secretion, Rodent

Since Selye's pioneering work indicating a link between stress and gastric ulcer (1), stress has been considered to be one of the important ulcerogenic factors. Although gastric ulcers are no doubt caused from an imbalance between aggressive factors, such as acid and pepsin, and defensive factors, such as blood flow and mucus secretion, it is still a matter of debate whether gastric secretion is enhanced or depressed by stress that precipitates fear and anxiety in humans. While Mahl (2) suggested that chronic fear increased gastric acidity, Cannon (3) took the position that gastric functions are depressed during exposure to fear.

In rats, the relationship among stress, ulcer and gastric secretion has been extensively studied using a variety of stress lesion models, such as restraint immobilization (4), restraint combined with water immersion (WI) (5) or cold exposure (6), where animals are subjected to some kinds of physical stimuli. These and subsequent studies have suggested that enhanced gastric secretion (7) and/or motility (8, 9) are important in the etiology of stress ulcer, giving a theoretical basis for the development of anti-ulcer drugs that suppress gastric secretion.

Recently, two animal models for psychological stress lesion have been introduced: activity-stress (AS) ulcer in rats (10) and communication box (CB) lesion in mice (11, 12). In both models, gastric lesions are developed in animals that are not subjected to any physical stimuli but are only put under emotionally stressful conditions. Histological observations revealed that AS ulcer penetrates the muscularis mucosa (13), which resembles human stress ulcer (14). Interestingly, anti-secretory agents such as atropine or cimetidine failed to suppress the formation of AS ulcer (15, 16) and CB lesion (17), whereas sulpiride, which is used as a neuroleptic as well as an antidepressant (18) and also has a curative effect on human stress ulcer (19, 20), suppressed AS ulcer formation (18).

The present study was conducted to investigate the profile of the anti-ulcer activity of sulpiride, which may lead to the finding of a new type of anti-ulcer agent. Anti-ulcer activities of sulpiride were studied using physical as well as psychological stress ulceration models, and the results were compared with those of famotidine.

MATERIALS AND METHODS

Animals

Male, 5- to 6-week-old Sprague-Dawley rats (Clea Japan, Tokyo) were used for the ulceration and secretion study. Only in the communication box paradigm, male, 6-week-old ICR mice (Clea Japan) were employed. Prior
to the experiments, the animals were housed in standard laboratory cages with food and water available ad lib. They were habituated to housing conditions for several days before the experiments. The room temperature was maintained at 22 ± 1°C under a 12:12 light/dark cycle with lights on at 8:00 A.M. All experimental procedures were performed under the guidelines of the Animal Experiment Committee of the Fujisawa Pharmaceutical Co., Ltd.

Materials

Sulpiride was synthesized at our company. Indomethacin and famotidine were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). All drugs were suspended in 0.5% methylcellulose solution.

Pylorus-ligation

After 24 hr fasting, rats or mice were lightly anesthetized of ether inhalation. Then, pylorus-ligation was performed according to the method of Shay et al. (21), with modifications. The operation was carried out during the period of 10:30–12:30 A.M. Compounds tested or vehicle were administered to rats intraduodenally immediately after pylorus-ligation. Four hours later, the animals were killed by CO2 inhalation. The stomach was removed, and gastric juice was collected from each animal. Then the gastric volume (ml/100 g body weight) and acidity (μEq/ml) were measured. The acidity of the samples was determined by titration with 0.1 N NaOH. The total acid output (μEq/100 g body weight) was calculated by multiplying the gastric volume by acidity.

AS ulcers

The experimental procedure was performed according to the method of Paré and Houser (10) with modifications. Briefly, each rat was adapted for 6 days to an AS cage (Okazaki Sangyo Co., Ltd., Tokyo) that consisted of an individual cage (31 × 13 × 17 cm) with a running wheel (37 cm in diameter, 10 cm in width). Rats were randomized by both body weight and running activity during the preceding day. Then they were deprived of food at 09:00 and subjected to a 1-hr feeding schedule (21:00–22:00) by using an automatic feeding system (Okazaki Sangyo Co., Ltd.). During the experimental period, rats were allowed free access to water. Compounds tested or vehicle were administered orally in a volume of 5 ml/kg at 09:00 daily from the start day of AS for 4 days. Throughout the experiments, the running activity [m/hr] of each rat was recorded by a computerized system. At 09:00 A.M. on the fifth day from the onset of AS, the stress session was terminated. Rats were then killed by anesthesia with an overdose injection of pentobarbital sodium, and the stomachs were removed and inspected for gastric lesions.

In the pylorus-ligation study, rats were activity-stressed according to the above-described methods without drug treatment. The control rats were fed for 1 hr per day without access to running wheels. On the third day of AS, we performed pylorus-ligation.

CB lesions

In this paradigm, we employed mice instead of rats, because this paradigm singly does not induce gastric lesions in rats (22). The experimental procedure was performed according to the method described before (17). Briefly, mice were randomized by body weight and fasted for 24 hr. Then, the tested compounds or vehicle were administered orally in a volume of 10 ml/kg at 15:30. One hour later, the mice were subjected to the stress session for 16 hr.

The experiments were performed in CBs that consisted of 64 (8 × 8) horizontally arranged clear polycarbonate small compartments (10 × 10 × 43 cm). The animals of one group, called the sender mice, were put individually in the compartments with an electric grid floor. The animals of the other group, called the responder mice, were put individually in the compartments that were insulated from an electric grid floor. The boxes were arranged so that the responder mice were surrounded by the sender mice. During the stress session, the senders were given intermittent electrical shocks (1.6–2.4 mA, 10 sec/2 min) delivered from an electrical shock generator (Nihon Kohden Co., Tokyo) through the grid floor. In addition, the non-stressed control mice were put individually in the compartments of the control box (10 × 10 × 43 cm) without an electric grid floor and without exposure to the senders for the same hours as the stressed mice. When the stress session was terminated, mice were killed by inhalation of CO2 gas, and the stomachs of psychologically stressed (responder) mice were removed and inspected for gastric lesions.

In the pylorus-ligation study, mice were stressed psychologically in the CB immediately after the operation for 4 hr. The control mice were put individually in the control box for 4 hr.

WI lesions

The experimental procedure was performed according to the method of Takagi et al. (5), with slight modifications. Rats were randomized by body weight and fasted for 24 hr. Then the tested compounds or vehicle were administered orally in a volume of 5 ml/kg. One hour later, rats were immersed to the level of xyphoid for 6 hr. When the stress session was terminated, the rats were killed by inhalation of CO2 gas, and the stomachs were removed and inspected for gastric lesions.
In the pylorus-ligation study, rats were immersed immediately after the operation for 4 hr.

**Indomethacin-induced (IND) lesions**

The experimental procedure was performed as described before (23). Briefly, rats were randomized by body weight and fasted for 24 hr. The fasted rats received 32 mg/kg of indomethacin subcutaneously simultaneously with an oral dose of tested drugs or vehicle. Six hours after indomethacin treatment, the rats were injected with 1 ml of 1% Evans blue in saline via the tail vein, killed by inhalation of CO₂ gas, and then the stomachs were removed and inspected for gastric lesions.

In the pylorus-ligation study, the animals were treated with indomethacin immediately after the operation.

**Lesion index**

Dissected stomachs of rats and mice were inflated to the same size by 12 ml and 2 ml of saline and immersed in saline containing 1% formaldehyde, respectively. The lightly fixed stomachs were opened along the greater curvature, washed lightly in water and inspected for gastric lesions by an observer unaware of the protocol employed. The method of measuring the area of each gastric lesion was described in our previous report (17). The total area (mm²) of lesions was expressed as the lesion index.

**Statistics**

The statistical significance of the effect of a drug was evaluated by linear trend analysis. In the case of a comparison between two groups, Student's t-test was used for statistical evaluation. A P-value less than 0.05 was considered to be significant. The ID₅₀ values represent the doses that reduce the lesion index by 50% to the control levels, and they were obtained by a computerized program.

**RESULTS**

**Effects of sulpiride and famotidine on gastric lesion formation in physical stress models**

As shown in Fig. 1, treatment with famotidine dose-

![Graphs showing the effects of sulpiride and famotidine on gastric lesion formation](Graph.png)

**Fig. 1.** Effects of treatment with sulpiride and famotidine on gastric lesion formation in restraint water-immersion stress and indomethacin-induced lesion models. Each bar represents the mean±S.E. NS: not significant. ***P < 0.001: Significant suppressive effects were seen in the dosed groups (by linear trend analysis).
Effects of sulpiride and famotidine on gastric lesion formation in psychological stress models

Rats and mice subjected to AS and CB developed gastric lesions in the corpus area of the stomach that were classified as real ulcer and mucosal erosion, respectively (data not shown). As shown in Fig. 2, treatment with sulpiride dose-dependently attenuated the lesion indices with statistical significance in AS \( F(3,36)=13.1, P<0.001 \) and CB \( F(3,79)=5.04, P<0.05 \). In contrast, famotidine failed to suppress the gastric lesion formation in AS \( F(4,35)=0.0118, P=0.914 \) and CB \( F(3,44)=0.387, P=0.537 \). The ID\(_{50}\) values of sulpiride in AS and CB were calculated to be 53 and 75 mg/kg, respectively.

Effects of sulpiride and famotidine on acid secretion in pylorus-ligated rats

Table 1 shows the effects of sulpiride and famotidine on gastric volume (ml/100 g body weight), acidity (\( \mu \)Eq/ml) and total acid output (\( \mu \)Eq/100 g body weight) in 4 hr pylorus-ligated rats. Treatment with famotidine dose-dependently suppressed gastric volume, acidity and total acid output. The changes were statistically significant in gastric volume \( F(4,35)=21.2, P<0.0001 \), acidity \( F=60.5, P<0.0001 \) and total acid output \( F=49.0, P<0.0001 \). The ID\(_{50}\) value of famotidine in the total acid output was calculated to be 3.2 mg/kg. In contrast, treatment with sulpiride hardly affected gastric volume \( F(3,36)=0.0191, P=0.891 \), acidity \( F=1.66, P=0.206 \) and CB [3.79]=5.04, P<0.05]. In contrast, famotidine failed to suppress the gastric lesion formation in AS [F(3,45)=0.0118, P=0.914] and CB [F(3,44)=0.387, P=0.537]. The ID\(_{50}\) values of sulpiride in AS and CB were calculated to be 53 and 75 mg/kg, respectively.

Effects of sulpiride and famotidine on gastric lesion formation in activity-stress and communication box paradigm. Each bar represents the mean ± S.E. C: control box. NS: not significant. *P<0.05, **P<0.01, ***P<0.001: Significant suppressive effects were seen in the dosed groups (by linear trend analysis). ††P<0.01: Significantly different from control box group (by Student's t-test).
and total acid output [F=0.374, P=0.545].

**Acid secretion in physical and psychological stress models**

Table 2 shows the acid secretion in WI, AS and CB models. Acid secretion of stressed rats in the physical stress model (WI) was enhanced compared with that of the control rats. The changes were statistically significant in acidity (P<0.001) and total acid output (P<0.05). In contrast, acid secretion in AS rats was markedly depressed compared with the control values. The changes were statistically significant in acidity and total acid output (P<0.01 and P<0.05, respectively). In CB, acid secretion decreased in the stressed mice by almost 60% of the control, although the change was not statistically significant (P=0.059 in acidity).

**DISCUSSION**

The most important finding of the present study is that sulpiride specifically attenuated psychological stress-induced gastric lesions (AS and CB), but hardly affected physical stress-induced gastric lesions (WI and IND) or gastric acidity. In contrast, famotidine selectively suppressed physical stress-induced gastric lesions at the doses that inhibit acid secretion. These results show that anti-ulcer mechanisms are clearly different between sulpiride and famotidine.

It has long been considered that human gastric ulcers are caused from an imbalance between aggressive factors (acid and pepsin) and defensive factors (blood flow and mucus). However, the etiological studies on experimental ulceration seem to have placed more emphasis on aggressive factors. This is presumably due to the adoption of
physical stimuli as stressors. In fact, the present study demonstrated that a physical stress (WI) caused enhanced acid secretion, which is in line with the previous study on restraint stress (7). In addition, Ueki et al. (24) suggested that IND lesions require the presence of acid in the lumen. Although these evidence has given a theoretical bases for the development of anti-ulcer drugs such as atropine, famotidine or omeprazole, it is also true that H₂-blocker-resistant gastric ulcers are clinically detected.

In the present study, the gastric secretion studied by the pylorus-ligation method is rather depressed in the animals subjected to the psychological stresses (AS and CB). Our result in AS coincides with the previous report by Paré (25), whereas in CB is a novel finding. Interestingly, Beaumont (26) observed that fear and depression which are most likely to cause psychogenic ulceration (2, 27) decreased gastric secretion. Later, Lamers (28) reported that gastric secretion decreased in patients with gastric ulcer. We thus speculate that AS and CB lesions share a common etiological feature with human stress ulcer and that aggressive factors may not play a major role in the pathogenesis of the psychological stress lesions. The latter part of the speculation agrees with the present result that famotidine hardly attenuated the gastric secretion. Psychological stress lesion appears to involve diminished defensive factors, which may differ from physical stress lesion. Further studies on acid secretion under stress or on other factors such as gastric motility should be done before reaching a final conclusion. Also, the difference in animal species should be further studied.

The above discussions taken together suggest that sulpiride, which has been used for patients with gastric ulcer (19, 20), may enhance a defensive factor(s). Hara and Ogawa (18) suggest that the psychomotor stimulating property of sulpiride, which is used as a neuroleptic as well as an antidepressant, is closely related to its anti-ulcer effect in the AS paradigm. Thus, it is probable that the drug exerts its anti-ulcer activity through the action on the CNS, although the mechanism of action of sulpiride was not elucidated from the present study. In this respect, it is noteworthy that psychological stress induces neurochemical changes that are different from those under physical stress (29, 30).

In conclusion, we postulate in the present study that the mechanisms of gastric lesion formation are clearly different between physical and psychological stress and that sulpiride specifically attenuates psychological stress lesion possibly through a central mechanism. Further studies on the pharmacological profile of sulpiride may lead to the finding of a new type of anti-ulcer agent.

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