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Abstract

The anti-ulcer action of clotiazepam (a thienodiazepine derivative) was studied in mice subjected to non-physical and physical stimuli in a communication box. There were two groups of mice: the "sender" mice that received electric shocks on the feet and responded by squealing and jumping, and the "responder" mice that were affected by the senders' responses without receiving shocks on the feet. Gastric ulcers resulted in both groups. The effect of clotiazepam was compared with that of diazepam. The incidence of gastric ulcers was suppressed by clotiazepam at a dose of 3 mg/kg, per os, in "responder" and "sender" mice, and by diazepam at a dose of 1 mg/kg, per os, in "responder" mice. These results suggest that clotiazepam has a suppressive action against gastric ulcers produced by non-physical or physical stimuli, although its potency is slightly weaker than that of diazepam.

KEYWORDS: gastric ulcer, clotiazepam, non-physical and physical stimuli, mice

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Effect of Clotiazepam on Gastric Mucosal Lesions Produced by Conditioned Emotional Stimuli in Mice

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The anti-ulcer action of clotiazepam (a thienodiazepine derivative) was studied in mice subjected to non-physical and physical stimuli in a communication box. There were two groups of mice: the "sender" mice that received electric shocks on the feet and responded by squealing and jumping, and the "responder" mice that were affected by the senders' responses without receiving shocks on the feet. Gastric ulcers resulted in both groups. The effect of clotiazepam was compared with that of diazepam. The incidence of gastric ulcers was suppressed by clotiazepam at a dose of 3 mg/kg, per os, in "responder" and "sender" mice, and by diazepam at a dose of 1 mg/kg, per os, in "responder" mice. These results suggest that clotiazepam has a suppressive action against gastric ulcers produced by non-physical or physical stimuli, although its potency is slightly weaker than that of diazepam.

Key words : gastric ulcer, clotiazepam, non-physical and physical stimuli, mice

Clotiazepam is a member of the thienodiazepine group and its mode of action is similar to that of the benzodiazepines. However, the anti-pentylenetetrazol and anti-conflict potencies are three times higher than those of diazepam(1), suggesting that clotiazepam has potent anti-convulsive and anti-anxiety activities. Clotiazepam is used for psychosomatic disease in combination therapy(2), and has a suppressive action on ulcers induced by restraint and water immersion(3). Benzodiazepines suppress various stress ulcers in experimental animals(4, 5) and have been used for peptic ulcers(6). We have reported that benzodiazepines exhibit an inhibitory action on gastric ulcers produced by psychological stress, *i. e.*, non-physical stimuli conveyed by the communication box method

(7, 8). In the present experiment, the effects of clotiazepam on gastric ulcers produced in mice by non-physical stimuli conveyed in a communication box were examined.

Materials and Methods

Animals. Male ddY mice weighing 20-25 g (Kyudo Co. Ltd., Kumamoto, Japan) were used. Animals were housed in a plastic cage (30×28×18 cm) with free access to food and water at the temperature of 20-24°C and humidity of about 60%.

Apparatus and Procedure. A communication box apparatus(9) was used to expose the animals to the conditioned emotional stimuli (CES). The apparatus consisted of a box of 60×80×37 cm with stainless steel grid (5 mm in diameter) on

the floor. The inside of the box was divided into 48 small compartments (10 × 10 × 37 cm) with transparent plastic boards. An electric shock (0–10 mA) was delivered to half of the compartments, with a shock generator (Shinwa Electric Co., Fukuoka, Japan). A plastic plate was placed on the floor of the other half of the compartments to avoid foot-shock. The animals placed in the former compartments responded to shocks on the feet with squeals, jumps and piloerection, and were designated as “senders”. The animals in the latter compartments, which were influenced by the “senders”, were called “responders”. The “responder” mice received no physical stimuli such as shocks.

The CES consisted of a monotone (2,000 Hz, conditioned stimulus) and shock given to the feet (1.5–2.0 mA, unconditioned stimulus) in “sender” mice. The conditioned stimulus (10 sec) was applied before the unconditioned stimulus (10 sec). A lamp was lighted while the unconditioned stimulus was applied. This stimulus schedule was repeated at intervals of 120 sec. Our previous studies (7, 10) have shown that gastric ulcers in “senders” were induced with a high incidence by 12 h of CES, and that a 4-hour session of CES in “senders” was equivalent to a 12-hour session in “responders” in terms of the incidence of ulcers. Therefore, for the evaluation of anti-ulcer effects of drugs, “responders” were subjected to CES for 12 h in the dark (21:00–9:00), and “senders” were subjected to CES for 4 h (21:00–1:00).

Drugs. Clotiazepam and diazepam were suspended in 0.5% sodium carboxymethylcellulose (CMC-Na) solution. The dose was adjusted to 0.1 ml per 10 g of body weight. Drugs were admin-

istered orally, and a CMC-Na solution was given as the control. The drugs were given 1 h before starting CES to both “sender” and “responder” groups. In addition, the drug was administered a second time 6 h after CES in the “responder” group.

Examination for Gastric Ulcers. Immediately after finishing CES, mice were decapitated and the stomach was isolated, immersed in 10% formalin for about 10 sec., and opened along the greater curvature. The presence or absence of gastric ulcers was determined macroscopically.

Statistical Analysis. The inter-group comparisons of drug effects were made by the two-tailed χ^2 -test (11).

Results

Effects of the two administrations (at an interval of 7 h, hereafter abbreviated as ×2) of clotiazepam and diazepam on gastric ulcers in “responders” are shown in Table 1. Clotiazepam slightly suppressed the development of ulcers at 1 mg/kg ×2 and significantly suppressed it at 3 mg/kg ×2 ($\chi^2 = 6.42$, $p < 0.05$). Diazepam significantly suppressed the development of ulcers at 1 mg/kg ×2 ($\chi^2 = 6.42$, $p < 0.05$). Effects of clotiazepam and diazepam on ulcers in “senders” are shown in Table 2. While no remarkable changes were found in the incidence of ulcers at 1 mg/kg, clotiazepam significantly suppressed the incidence at 3 mg/kg ($\chi^2 = 6.98$, $p <$

Table 1 Protective effects of drugs against gastric ulcers produced by conditioned emotional stimuli in “responder” mice

Group	Drug	Dose ^a (mg/kg)	Incidence ^b (%)	χ^2 -value ^c
1	Vehicle (10 ml/kg)	0	82.6% (19/23)	
2	Clotiazepam	1	50.0% (6/12)	2.666
3	Clotiazepam	3	33.3% (4/12)	6.423*
4	Diazepam	1	33.3% (4/12)	6.423*

a: Drugs dissolved in the vehicle (0.5% sodium carboxymethylcellulose) were administered orally twice at an interval of 7 h.

b: The denominator denotes the number of mice used, and the numerator denotes the number of mice in which a gastric ulcer was induced.

c: Significant differences from the vehicle group are indicated by *($p < 0.05$).

Table 2 Protective effects of drugs against gastric ulcers produced by conditioned emotional stimuli in "sender" mice^a

Group	Drug	Dose (mg/kg)	Incidence (%)	χ^2 -value
1	Vehicle(10 ml/kg)	0	83.3%(20/24)	
2	Clotiazepam	1	66.7%(8/12)	0.502
3	Clotiazepam	3	25.0%(3/12)	6.980**
4	Diazepam	1	50.0%(6/12)	2.925 ⁺

a: Drugs were administered once and evaluated as described in Table 1. Significant differences from the vehicle group are indicated by **($p < 0.01$) and +($p < 0.1$).

0.01). Diazepam at a dose of 1 mg/kg tended to suppress the incidence of ulcers ($\chi^2 = 2.93$, $p < 0.1$).

Discussion

Clotiazepam has potent anti-conflict and anti-pentylentetrazol actions, but it has weaker hypnotic and sedative actions than diazepam(1). Clotiazepam suppresses ulcers experimentally induced by physical stress such as restraint, water immersion and foot shock(3). Clotiazepam is particularly useful for treating psychosomatic diseases and ulcers(2). We have reported that ulcers can be experimentally induced by psychological stress, such as CES produced in a communication box(12). Gastric ulcers induced by this method were suppressed by not only anti-ulcer agents like cetraxate, gefarnate or cimetidine(10), but also diazepam, oxazolam and tricyclic antidepressants(7, 8). In the present study, the incidence of gastric ulcers in "responder" mice, which were affected by "senders", was significantly suppressed by two administrations of 3 mg/kg of clotiazepam. Significant suppression was also observed by administering 1 mg/kg of diazepam twice. Gastric ulcers of the "sender" group induced by physical stimuli, *i. e.*, shocks on the feet, were also suppressed by clotiazepam at a dose of 3 mg/kg. These findings suggest that clotiazepam can be clinically useful as an anti-ulcer drug, although the potency of

clotiazepam is slightly weaker than that of diazepam.

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