Effects of a New Histamine H2-Receptor Antagonist, Z-300, on Gastric Secretion and Gastro-Duodenal Lesions in Rats: Comparison with Roxatidine

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ABSTRACT — We examined the effects of a new compound, N-[3-[3-(piperidinomethyl)phenoxy]propyl]-2-(2-hydroxyethyl-l-thio)acetamido 2-(4-hydroxy benzoyl)benzoate (Z-300), on the histamine H2-receptor, gastric secretion in rats and dogs, and acute gastro-duodenal lesions or chronic gastric ulcers in rats. Roxatidine acetate hydrochloride (roxatidine), a known histamine H2-receptor antagonist, was used as a reference compound. The pA2 values for Z-300 and roxatidine for the isolated guinea pig atrium were 6.8 and 7.0, respectively. These agents at < 10⁻⁵ M did not affect the contraction of guinea pig ileum in response to carbachol. Z-300, administered either orally or parenterally, significantly inhibited the basal and histamine-stimulated gastric acid secretion in rats. Gastric acid secretion stimulated by histamine, pentagastrin or carbachol in Heidenhain pouch dogs was also significantly inhibited by the compound. The effect persisted for >7 hr in the case of histamine-stimulation. Oral Z-300 significantly protected the gastric mucosa from water-immersion stress-, indomethacin-, aspirin- and HCl·ethanol-induced lesions and protected the duodenal mucosa against mepirizole- and cysteamine-induced ulcers. These effects on gastric secretion and lesion formation were, as a whole, stronger than those observed with roxatidine. Z-300, but not roxatidine, significantly accelerated the spontaneous healing of acetic acid ulcers induced in rats and prevented the delay in ulcer healing caused by indomethacin. The mechanism of action of Z-300 on acute lesions and chronic ulcers appears to be mostly related to its potent antisecretory and mucosal-protective activities.

Keywords: Z-300, Histamine H2-receptor antagonist, Gastric secretion, Gastro-duodenal lesions, Ulcer healing

Since cimetidine was developed as a potential histamine H2-receptor antagonist (H2-antagonist) (1), several other H2-antagonists with different structures have been designed, and established to be useful drugs for the treatment of peptic ulcers or acid-related diseases (2). For example, famotidine, ranitidine hydrochloride, roxatidine acetate hydrochloride (roxatidine) and nizatidine are each now widely used as a potential gastric antisecretory and anti-ulcer drug. Recently, a new compound, N-[3-[3-(piperidinomethyl)phenoxy]propyl]-2-(2-hydroxyethyl-l-thio)acetamido 2-(4-hydroxy benzoyl)benzoate (Z-300, Fig. 1), was synthesized by modifying the structure of roxatidine. The prototype of this compound, the hydrochloride salt (ZP), had already been synthesized and reported by Ueda et al. (3). However, ZP was an oil and unstable so that this salt was altered to 2-(4-hydroxy benzoyl)benzoate, a compound that could be crystallized, thereby greatly increasing its stability. The present study was performed firstly to identify Z-300 as a H2-antagonist by using the isolated atria and ilea of guinea pigs. Secondly, the effects of Z-300 on gastric secretion in rats and dogs and the gastric and duodenal lesions (including penetrating ulcers) induced in rats were determined. Roxatidine was used as a reference compound because of its structural resemblance to Z-300 and its well-elucidated pharmacological properties (4, 5).
MATERIALS AND METHODS

Histamine $H_2$- and cholinergic receptor antagonistic activity

Male Hartley guinea pigs, weighing 380 to 500 g, were killed by a blow on the head and then their hearts or ilea were dissected out in warmed incubation medium. The right atrium was isolated from each heart and suspended in a 10-ml Magnus chamber filled with a Krebs-Henseleit solution of the following composition (mM): 118.2 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM MgSO$_4$, 24.8 mM NaHCO$_3$, and 10.0 mM dextrose. The incubation medium was bubbled with a 95% O$_2$-5% CO$_2$ gas mixture and warmed at 37°C. The preparations were loaded with 0.5 g resting tension and allowed to equilibrate for about 60 min. The amplitude of contraction was measured with an isometric transducer (TB651T, Nihon Kohden), and the beating rate was determined by a cardiotachometer (AT601G, Nihon Kohden) triggered by contractile wave signals. On the other hand, each ileum was suspended in a 10-ml Magnus chamber with 1 g resting tension and the contraction measured by the same method as described above. The temperature of the incubation medium was 32°C. The composition of the medium and gas bubbling were the same as those for the atrial preparations. Through the cumulative application of histamine ($10^{-8}$–$10^{-4}$ M) for the atria and carbachol ($10^{-8}$–$3 \times 10^{-6}$ M) for the ilea into the chamber, concentration-response curves were obtained as a control. Subsequently, test compounds were administered for 5 min prior to determination of the next concentration-response curve. The curves were recorded with a thermal pen recorder (Recti Horitz 8K, Nihondenki Sanei). The compounds were dissolved in incubation medium to approximately $10^{-2}$ M and diluted before use. The pA$_2$ values were calculated by means of Schild plots using Tallarida’s method (6).

Gastric secretory studies in rats

Male Sprague-Dawley rats (Nihon Charles-River), weighing 220–250 g, were deprived of food for 24 hr before the experiments. Tap water was given freely during fasting, but withheld during the last 2 hr. The rats were kept in raised mesh-bottom cages to prevent coprophagy. Each study was carried out with 8–16 animals per group.

Basal secretion: Under ether anesthesia, the abdomen of each rat was incised and the pylorus ligated. The animals were killed 4 hr after the pylorus ligation, and the gastric contents collected and analyzed as to the volume and acidity. The acidity was determined by titration of the gastric contents against 0.1 M NaOH to pH 7.0 with an autoburette (Comtite B, Hiranuma). Acid output (volume \times acidity) was expressed as $\mu$Eq/hr. A test compound or the vehicle alone was administered p.o. at 0.5, 8 or 20 hr before the ligation, or i.d. (intraduodenally), i.p., s.c., and i.v. immediately after the ligation.

Stimulated secretion: Under ether anesthesia, the abdomen was incised, and the stomach and duodenum exposed. A polyethylene cannula (7 mm, ID) was introduced into the stomach through the forestomach and secured tightly with two purse-string sutures. The cannula had two small polyethylene tubes (1.5 mm, ID) attached; one was inserted into the distal part of the esophagus to exclude saliva from the gastric samples, and the other was inserted into the proximal duodenum through the pylorus to inject test compounds (7). The pylorus was then ligated together with the tube. The
cannula was brought out and the incision closed. After recovery from anesthesia, the animals were placed in Bollman cages and gastric juice was collected. The first 0.5-hr sample was discarded and the following 2-hr sample used for the basal secretion measurement. Histamine·2HCl (Nacalai Tesque), dissolved in saline, was injected s.c. at 20 mg/kg (as the salt) twice with a 2-hr interval. Gastric samples were collected 4 hr later; and the volume and acid output were expressed as ml/hr and μEq/hr, respectively. A test compound or the vehicle alone was administered once i.d. 0.5 hr prior to the first administration of histamine.

**Gastric secretion in dogs**

Four beagles of both sexes, weighing 12–15 kg, with Heidenhain pouches were used. The pouches were prepared at least 5 months before the experiments. The animals were deprived of food with free access to water for 18 hr before the secretory test. A fine polyethylene tube was inserted into the foreleg vein to continuously infuse the gastric secretagogues. Gastric juice was drained through the implanted cannula into collection tubes every 15 min for 8 hr after beginning secretagogue infusion. Histamine·2HCl (Nacalai Tesque, 160 μg/kg/hr as the base), pentagastrin (ICI, 8 μg/kg/hr) or carbachol chloride (Sigma, 8 μg/kg/hr) was infused at the rate of 10 ml/hr, these doses being the ones that induced nearly maximal acid secretion. The gastric samples were analyzed as to volume and acidity as described above, and acid output was expressed as mEq/15 min or mEq/30 min. Secretory studies were performed once a week throughout the experimental periods. Each test compound was administered p.o. 1 hr after the administration of each secretagogue.

**Induction of acute gastric and duodenal lesions**

Male Sprague-Dawley rats, weighing 220–250 g, were fasted for 24 hr before the experiments. The conditions for fasting were the same as described for the gastric secretory study. Each study was carried out with 7–16 animals per group.

**Stress-induced gastric lesions:** The animals were placed in a Tohdai-Yakusaku type stress cage (Natsume) and then immersed to the level of the xiphoid process in a water bath (23°C) for 8 hr (8). The animals were then killed, and the stomach of each rat was removed, inflated by injecting 8 ml of 2% formalin and then immersed in 2% formalin for 10 min. This formalin treatment for light fixing of the gastric or duodenal wall was performed in all the following experiments. In the cases of HCl·ethanol- and aspirin-induced lesions, the gastric contents were withdrawn through the duodenum by gentle pushing of the gastric wall before the formalin treatment. The stomach was then incised along the greater curvature and examined for mucosal lesions in the glandular portion. A test compound or the vehicle alone was administered p.o. 0.5 hr before the water immersion.

**Indomethacin-induced gastric lesions:** Indomethacin (Merck), suspended in saline with a minimal amount of Tween 80, was administered s.c. at 25 mg/kg. The animals were killed 7 hr later and then their stomachs examined for lesions (9). A test compound or the vehicle alone was administered p.o. 0.5 hr before the indomethacin treatment.

**Aspirin-induced gastric lesions:** Aspirin (Nacalai Tesque), suspended in a 0.5% carboxymethylcellulose (CMC) solution, was administered p.o. at 150 mg/kg to rats 5 min after pylorus ligation at a volume ratio of 1 ml/200 g of body weight (10). The animals were killed 7 hr after the aspirin dosing, and then their stomachs examined for lesions. A test compound or the vehicle alone was administered p.o. 0.5 hr before the pylorus ligation.

**HCl·ethanol-induced gastric lesions:** Gastric lesions were produced by giving p.o. 1 ml/200 g body weight of 60% ethanol (v/v) in 150 mM HCl (HCl·ethanol) (11). The animals were killed 1 hr later. After formalin treatment, each stomach was examined for lesions. A test compound or the vehicle alone was administered p.o. 0.5 hr before the administration of HCl·ethanol.

**Mepirizole-induced duodenal ulcers:** Mepirizole (Daichi), suspended in 0.5% CMC, was administered s.c. at 200 mg/kg (12). The animals were killed 24 hr later and the duodenum examined for ulcers. A test compound or the vehicle alone was administered p.o. once 0.5 hr before the mepirizole treatment.

**Cysteamine-induced duodenal ulcers:** Cysteamine·HCl (Nacalai Tesque), dissolved in saline, was administered s.c. at 190 mg/kg twice (9 hr apart) (13). The animals were killed 24 hr after the first injection of cysteamine and examined for ulcers in the duodenum. Each test compound was administered p.o. twice 0.5 hr before each administration of cysteamine.

**Induction of acetic acid-induced gastric ulcers**

Male Donryu rats, weighing 240–260 g, at the time of ulcer induction, were used. Each study was carried out with 22–27 animals per group. Under ether anesthesia, the abdomen was incised and the anterior portion of the stomach exposed. Then, 0.03 ml of 20% acetic acid (v/v) was injected into the submucosal layer at the junction of the fundus and antrum, using a microsyringe (Terumo, 0.25 ml) (14, 15). Postoperatively, the animals were maintained on rat chow and water ad libitum. The following two kinds of experiments were
performed. To study the effects of the test compounds on spontaneous healing of ulcers, a test compound or the vehicle alone was given twice a day for 2 weeks after ulceration. Indomethacin significantly delays the healing of acetic acid-induced ulcers (16). Thus, the effects of the test compounds on this delayed healing of ulcers were studied. Indomethacin (1 mg/kg, Sigma), suspended in saline with a minimal amount of Tween 80, was administered s.c. once daily (9:00 AM) for 2 weeks after ulceration. A test compound or the vehicle alone was administered p.o. twice (9:30 AM and 6:00 PM) for 2 weeks after ulceration. The animals were killed 24 hr after the final administration of a test compound, and then their stomachs were examined for ulcers.

**Determination of lesions and ulcers**

The length (mm) of each gastric lesion or the area (mm²) of each ulcer was determined under a dissecting microscope with a square grid (Olympus, ×10) and summed per stomach. The person (S.O.) measuring the lesions did not know the treatment given to the animals.

**Test compounds**

Both Z-300 and roxatidine were provided by Zeria Pharmaceutical Co., Ltd. (Tokyo). These compounds were suspended in 0.5% CMC and administered at a volume ratio of 1 ml/200 g body weight.

**Analysis of data**

A two-tailed Dunnett's multiple comparison test was employed to determine the statistical significance of the data obtained in this study at the level of P < 0.05. The ED₅₀ values (the doses which inhibit gastric acid output, and gastric and duodenal lesions by 50%, expressed as mg/kg or μM/kg) and 95% confidence limits were calculated by the Litchfield-Wilcoxon method. Since the molecular weight of Z-300 and roxatidine is very different (Z-300: 608.8 Da, roxatidine: 384.9 Da), the potency of Z-300 to roxatidine was compared on a molar basis. In the gastric secretory study on dogs, the ED₅₀ values and 95% confidence limits of test compounds were calculated from the inhibitory responses observed 1.5 hr after the administration.

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**Fig. 2.** Effects of Z-300 and roxatidine on histamine-induced increase of beating rate in isolated guinea pig atria and carbachol-induced contraction of guinea pig ilea. All values are means ± S.E.
RESULTS

Effects on H2-receptors and cholinergic receptors

Histamine at $10^{-8}$ to $10^{-4}$ M increased the beating rate of the guinea pig right atrium in a concentration-dependent manner. The basal beating rate was about 180 beats/min, and the maximum induced by $10^{-4}$ M histamine was about 380 beats/min. Z-300 at $10^{-7}$ to $10^{-6}$ M antagonized the histamine-induced increase in the beating rate and caused a parallel shift of the concentration-response curve to the right (Fig. 2). Nearly the same results were obtained with roxatidine. The pA2 values for Z-300 and roxatidine were 6.80 ± 0.08 and 7.01 ± 0.07, respectively. The slopes of the Schild plots for Z-300 and roxatidine were 1.17 and 1.28, respectively. The ileum preparations contracted with carbachol at concentrations of $10^{-8}$ to $3 \times 10^{-6}$ M concentration-dependently. Pretreatment with Z-300 and roxatidine acetate at $10^{-6}$ to $10^{-5}$ M did not influence the concentration-response curves with carbachol. However, the curves shifted to the right when these drugs were administered at $10^{-4}$ M (Fig. 2).

Effects on gastric secretion in rats

Basal secretion: Four hours after pylorus ligation, the volume of the gastric contents, acidity and acid output in the controls were about 6 ml/rat, 100 mEq/l and 100 - 230 μEq/hr, respectively. When Z-300 was administered p.o. at >10 mg/kg, the volume and acid output significantly decreased, almost in a dose-dependent manner (Table 1). Roxatidine also significantly decreased these parameters, but the effective dose was >30 mg/kg. The ED50 values for Z-300 and roxatidine were 18.9 and 27.8 mg/kg, respectively. When administered p.o. 8 hr before the ligation, Z-300 still significantly decreased the acid output in a dose-dependent manner. At 200 mg/kg, the rates of inhibition of the volume and acid output were 58.3% and 81.4%, respectively. The ED50 value was 37.9 mg/kg. In contrast, roxatidine, even at 200 mg/kg, had an insignificant effect on gastric secretion. When administered 20 hr before the ligation, Z-300 showed a tendency to in-

Table 1. Effects of orally administered Z-300 and roxatidine on basal gastric secretion in pylorus-ligated rats

| Treatment | Dose (mg/kg) | No. of rats | Volume (ml/rat) | Inhibition (%) | Acid output (μEq/hr) | Inhibition (%) | ED50 mg/kg (95% confidence limits) |
|-----------|-------------|-------------|----------------|----------------|---------------------|----------------|----------------------------------|
| A         | Control     | 16          | 6.0 ± 0.6      | 172.3 ± 21.6   | 151.9 ± 21.5       | 11.8           |                                   |
| Z-300     | 1           | 8           | 5.5 ± 0.6      | 8.3            | 132.9 ± 30.0       | 22.9           |                                   |
|           | 3           | 8           | 5.4 ± 0.9      | 10.0           | 99.3 ± 13.8        | 42.4           |                                   |
|           | 10          | 8           | 5.1 ± 0.7      | 15.0           | 72.1 ± 25.1        | 58.2           |                                   |
|           | 30          | 8           | 4.3 ± 0.7      | 28.3           | 41.3 ± 5.4         | 76.0           |                                   |
|           | 100         | 8           | 3.1 ± 0.4      | 48.3           | 40.2 ± 7.3         | 76.7           |                                   |
|           | 200         | 8           | 3.2 ± 0.3      | 46.7           | 40.2 ± 7.3         | 76.7           |                                   |
| B         | Control     | 8           | 7.7 ± 0.6      | 234.0 ± 23.5   | 176.7 ± 23.8       | 24.5           |                                   |
| Roxatidine| 3           | 8           | 6.1 ± 0.7      | 20.8           | 164.7 ± 22.8       | 29.6           |                                   |
|           | 10          | 8           | 6.3 ± 0.7      | 18.2           | 83.3 ± 15.8        | 64.4           |                                   |
|           | 30          | 8           | 4.5 ± 0.6      | 41.6           | 90.9 ± 11.1        | 61.2           |                                   |
|           | 100         | 8           | 4.9 ± 0.5      | 36.4           | 67.3 ± 11.6        | 71.2           |                                   |
|           | 200         | 8           | 4.2 ± 0.4      | 45.5           | 67.3 ± 11.6        | 71.2           |                                   |
| Z-300     | Control     | 8           | 6.0 ± 0.6      | 155.5 ± 22.0   | 117.4 ± 20.3       | 24.5           |                                   |
|           | 10          | 8           | 5.0 ± 0.6      | 16.7           | 77.3 ± 12.2        | 50.3           |                                   |
|           | 30          | 8           | 3.9 ± 0.5      | 35.0           | 58.0 ± 20.6        | 62.7           |                                   |
|           | 100         | 8           | 3.6 ± 0.9      | 40.0           | 28.9 ± 8.0         | 81.4           |                                   |
|           | 200         | 8           | 2.5 ± 0.5      | 58.3           | 28.9 ± 8.0         | 81.4           |                                   |
| C         | Roxatidine  | 8           | 5.9 ± 0.7      | 1.7            | 158.1 ± 28.0       | 1.7            |                                   |
|           | 100         | 8           | 5.2 ± 0.7      | 13.3           | 144.5 ± 23.9       | 7.1            |                                   |
|           | 200         | 8           | 4.5 ± 0.6      | 25.0           | 112.2 ± 22.5       | 27.8           |                                   |
| Z-300     | Control     | 8           | 5.1 ± 0.6      | 136.3 ± 19.4   | 133.8 ± 25.7       | 1.8            |                                   |
|           | 30          | 8           | 5.0 ± 0.9      | 2.0            | 100.9 ± 18.3       | 26.0           |                                   |
|           | 100         | 8           | 3.9 ± 0.7      | 23.5           | 88.5 ± 13.6        | 35.1           |                                   |

Each agent was given p.o. at either 0.5 hr (A), 8 hr (B) or 20 hr (C) before the ligation. Control animals were given the vehicle alone. Animals were killed 4 hr after the ligation. All values are means ± S.E. *P < 0.05.
### Table 2. Effects of intraduodenally or intraperitoneally administered Z-300 and roxatidine on basal gastric secretion in pylorus-ligated rats

| Treatment | Dose (mg/kg) | Route | No. of rats | Volume (ml/rat) | Inhibition (%) | Acid output (μEq/hr) | Inhibition (%) | ED$_{50}$ mg/kg (95% confidence limits) |
|-----------|--------------|-------|-------------|----------------|---------------|---------------------|---------------|----------------------------------------|
| Z-300     |              | i.d.  | 8           | 5.7 ± 0.9      | 21.1          | 94.3 ± 25.6         | 37.8          |                                         |
|           | 3            | i.d.  | 8           | 4.5 ± 0.9      | 38.6          | 60.1 ± 6.8*        | 60.3          | 6.0                                     |
|           | 10           | i.d.  | 8           | 3.5 ± 0.2*     | 49.1          | 42.3 ± 10.7          | 72.1          | (0.7 – 13.8)                            |
|           | 30           | i.d.  | 8           | 2.9 ± 0.5*     | 56.1          | 30.5 ± 7.0*         | 79.9          |                                         |
| Control   |              | i.d.  | 8           | 4.1 ± 0.4      | 26.8          | 131.7 ± 27.9        | 24.8          |                                         |
| Roxatidine|              | i.d.  | 8           | 3.5 ± 0.5      | 32.0          | 58.8 ± 12.1*        | 44.3          | 23.8                                    |
|           | 10           | i.d.  | 8           | 3.2 ± 0.5      | 42.9          | 65.8 ± 11.6*        | 59.2          | (13.0 – 51.2)                          |
|           | 30           | i.d.  | 8           | 2.6 ± 0.5      | 41.3          | 54.9 ± 7.1*         | 68.5          |                                         |
|           | 100          | i.d.  | 8           | 2.3 ± 0.2*     | 36.4          | 45.3 ± 15.1*        | 69.9          |                                         |

Each agent was given either intraduodenally (i.d.) or i.p. immediately after the ligation. Control animals were given the vehicle alone. Animals were killed 4 hr after the ligation. All values are means ± S.E. *P < 0.05.

### Table 3. Effects of subcutaneously or intravenously administered Z-300 and roxatidine on basal gastric secretion in pylorus-ligated rats

| Treatment | Dose (mg/kg) | Route | No. of rats | Volume (ml/rat) | Inhibition (%) | Acid output (μEq/hr) | Inhibition (%) | ED$_{50}$ mg/kg (95% confidence limits) |
|-----------|--------------|-------|-------------|----------------|---------------|---------------------|---------------|----------------------------------------|
| Z-300     |              | s.c.  | 16          | 6.1 ± 0.5      | 202.5 ± 20.9  | 211.9 ± 25.4       | -4.6          |                                         |
|           | 1            | s.c.  | 8           | 6.1 ± 0.6      | 49.2          | 76.1 ± 22.4*       | 62.4          |                                         |
|           | 3            | s.c.  | 8           | 3.1 ± 0.7*     | 37.0 ± 7.8*   | 42.8 ± 13.2*       | 78.9          | (2.5 – 9.4)                            |
|           | 10           | s.c.  | 8           | 2.0 ± 0.2*     | 59.1          | 59.5 ± 11.0*       | 74.1          | (0.73 – 4.82)                         |
|           | 30           | s.c.  | 8           | 2.2 ± 0.6*     | 56.1          | 54.6 ± 12.7*       | 76.3          |                                         |
| Control   |              | s.c.  | 16          | 6.6 ± 0.5      | 230.0 ± 19.1  | 173.3 ± 23.1       | 24.7          |                                         |
| Roxatidine|              | s.c.  | 8           | 5.2 ± 0.5      | 39.4          | 118.5 ± 16.5*      | 48.5          |                                         |
|           | 1            | s.c.  | 8           | 4.0 ± 0.5*     | 40.9          | 105.9 ± 20.4*      | 54.0          | 2.2                                    |
|           | 3            | s.c.  | 8           | 3.9 ± 0.7*     | 59.1          | 59.5 ± 11.0*       | 74.1          | (0.73 – 4.82)                         |
|           | 10           | s.c.  | 8           | 2.7 ± 0.6*     | 56.1          | 54.6 ± 12.7*       | 76.3          |                                         |
|           | 30           | s.c.  | 8           | 2.9 ± 0.6*     | 36.4          | 72.1 ± 12.5        | 68.7          |                                         |

Each agent was given either s.c. or i.v. immediately after the ligation. Control animals were given the vehicle alone. Animals were killed 4 hr after the ligation. All values are means ± S.E. *P < 0.05.
hibit the gastric secretion, but the inhibition was not significant. Even with the parenteral route, Z-300 showed marked inhibition of gastric secretion in a dose-dependent manner (Tables 2 and 3). The ED50 values for Z-300 after i.d.-, i.p.-, s.c. and i.v.-administration were 6.0, 2.8, 5.3 and 12.5 mg/kg, respectively. The corresponding values for roxatidine were 23.8, 7.9, 2.2 and 11.5 mg/kg, respectively.

Histamine stimulation: The basal secretion as to volume and acid output was about 0.3-0.4 ml/hr and 25 μEq/hr. Histamine administered s.c. at 20 mg/kg markedly stimulated gastric secretion. In the controls, the mean volume and acid output were about 1.7 ± 0.2 ml/hr and 237.7 ± 33.0 μEq/hr, respectively (Table 4). Pretreatment with Z-300 at 3 and 10 mg/kg significantly inhibited the stimulated secretion (both in volume and acid output) for 4 hr. The ED50 value for acid output was 3.0 mg/kg. Roxatidine also significantly inhibited the stimulated gastric secretion. The ED50 value for the acid output was 10.4 mg/kg.

Table 4. Effects of Z-300 and roxatidine on histamine-stimulated gastric acid secretion in rats

| Treatment | Dose (mg/kg) | No. of rats | Basal secretion | Stimulated secretion | ED50 mg/kg |
|-----------|-------------|-------------|-----------------|----------------------|------------|
|           |             |             | Volume (ml/hr)  | Acid output (μEq/hr) |            |
|           |             |             |                 |                      |            |
| Control   | 8           | 0.3 ± 0.1   | 25.4 ± 7.2      | 1.7 ± 0.2            | 237.7 ± 33.0 |            |
| Z-300     | 1           | 0.4 ± 0.1   | 23.3 ± 6.6      | 1.4 ± 0.2            | 192.2 ± 32.9 | 19.1 (3.0) |
|           | 3           | 0.4 ± 0.04  | 16.1 ± 3.4      | 1.0 ± 0.1*           | 128.9 ± 20.3* | 45.8 (2.0-45) |
|           | 10          | 0.4 ± 0.1   | 17.3 ± 8.2      | 0.4 ± 0.1*           | 27.2 ± 9.2*  | 88.6       |
| Roxatidine| 8           | 0.4 ± 0.1   | 23.9 ± 9.3      | 1.2 ± 0.2            | 145.6 ± 25.1 |            |
|           | 3           | 0.4 ± 0.1   | 13.5 ± 8.7      | 0.9 ± 0.2            | 108.7 ± 26.4 | 25.3 (10.4) |
|           | 10          | 0.4 ± 0.1   | 35.0 ± 11.8     | 0.9 ± 0.2            | 95.3 ± 31.9  | 34.5 (2.9-50.3) |
|           | 30          | 0.3 ± 0.1   | 16.5 ± 7.2      | 0.3 ± 0.1*           | 22.3 ± 5.5*  | 84.7       |

The first 2-hr samples were used for the basal secretion measurement. Histamine·HCl (20 mg/kg, dissolved in saline) was given s.c., twice, with a 2-hr interval after collecting the basal secretion. The agents were given i.d. at 0.5 hr before the first administration of histamine. All values are means ± S.E. *P < 0.05.

Effects on gastric acid secretion in dogs

Histamine stimulation: Continuous infusion of histamine gradually stimulated gastric acid secretion, which reached a near maximum level (1.6 ± 0.2 mEq/30 min) at 1.5 hr. Oral administration of Z-300 at 0.3 mg/kg 1 hr after beginning histamine infusion had little or no effect on the gastric secretion (Fig. 3A). At 1 mg/kg, the inhibition of gastric secretion was evident for about 3 hr after injection, but was not statistically significant. However, the gastric secretion was potentially and significantly inhibited after injection of 3 mg/kg of Z-300 for >7 hr. The onset of inhibition was fast, and the maximal inhibition was observed 45 min later. At 1.5 hr after the administration, the inhibition rates were 3.7, 36.6 and 75.8% at 0.3, 1 and 3 mg/kg, respectively.

The ED50 value was 1.2 (0.7-2.9) mg/kg. Roxatidine, administered p.o. at 0.3 and 1 mg/kg, also tended to inhibit gastric acid secretion for about 3-4 hr (Fig. 3B). At 3 mg/kg, however, it significantly inhibited the gastric acid secretion for 4.5 hr after the injection. The inhibited secretion then returned to the control level thereafter. The inhibition observed 1.5 hr after the administration of 0.3, 1 and 3 mg/kg were 19.6, 48.9 and 96.5%, respectively. The ED50 value was 0.8 (0.4-1.6) mg/kg.

Pentagastrin stimulation: Pentagastrin also stimulated the gastric acid secretion, although the degree of stimulation was considerably lower compared with that of histamine. At 1 hr, the acid output reached the maximum levels (0.34 ± 0.12 mEq/15 min). Z-300, administered p.o., significantly decreased the acid output in a dose-dependent manner (Fig. 4A). The onset of the inhibition was slow compared with that for histamine stimulation, the maximal inhibition being observed 75 min later. The antisecretory activity persisted for >3 hr after administration. At 1.5 hr, the inhibition rates were 40.6, 58.2 and 87.5% at 0.3, 1 and 3 mg/kg, respectively. The ED50 value was 0.5 (0.2-0.9) mg/kg. Nearly the same inhibition was observed with roxatidine (Fig. 4B), the ED50 value being 0.5 (0.1-0.8) mg/kg.

Carbachol stimulation: Infusion with carbachol also apparently stimulated gastric acid secretion. The degree of stimulation was much higher than that of pentagastrin, but weaker than that of histamine. Near maximal acid output (0.54 ± 0.83 mEq/15 min) was observed 60 min after infusion. There was a significant and persistent (>3 hr) dose-dependent decrease in gastric acid output after p.o.-administration of Z-300 (Fig. 5A). The onset of inhibition was fast, i.e., the near maximal inhibition at 3 mg/kg was observed 45 min later. At 1.5 hr, the inhibition rates were 34.4, 60.7 and 73.5% at
0.3, 1 and 3 mg/kg, respectively. The ED$_{50}$ value was 0.7 (0.02–1.8) mg/kg. Roxatidine also significantly and persistently (>3 hr) inhibited the gastric acid secretion with a fast onset (Fig. 5B). The ED$_{50}$ value was 0.4 (0.2–0.5) mg/kg.

**Effects on acute gastric lesions**

**Water-immersion stress-induced lesions:** Water-immersion stress for 8 hr induced multiple mucosal lesions, mainly in the corpus area, with a 100% incidence. Z-300 significantly prevented the stress-induced lesions in a dose-dependent manner (Fig. 6A). The inhibition rate was 75.0% when it was administered at 100 mg/kg. Roxatidine also significantly prevented the development of these lesions. The ED$_{50}$ values for Z-300 and roxatidine were 10.8 (6.1–19.8) and 6.4 (1.4–13.0) mg/kg,

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**Fig. 3.** Effects of Z-300 (A) and roxatidine (B) on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. Each drug was administered p.o. 1 hr after the histamine infusion. Note that Z-300 significantly and persistently (>7 hr) inhibited the gastric secretion. *Significantly different from the controls at P < 0.05.
Fig. 4. Effects of Z-300 (A) and roxatidine (B) on pentagastrin-stimulated gastric acid secretion in Heidenhain pouch dogs. Each drug was administered p.o. 1 hr after the pentagastrin infusion. Both drugs significantly inhibited the gastric secretion for >3 hr. *Significantly different from the controls at P < 0.05.

respectively.

Indomethacin-induced lesions: Indomethacin induced multiple mucosal lesions resembling those induced by water-immersion stress. Development of the lesions was significantly prevented by treatment with Z-300 in a dose-dependent manner (Fig. 6A). The inhibition rate was 82.6% at the dose of 60 mg/kg. Roxatidine also significantly protected the gastric mucosa against these lesions. The ED₅₀ values for Z-300 and roxatidine were 22.8 (10.3 – 109.6) and 24.3 mg/kg, respectively.

Aspirin-induced lesions: Aspirin administered to pylorus-ligated rats induced multiple linear or dotted mucosal lesions 7 hr later. These lesions were significantly prevented by treatment with Z-300 in a dose-dependent manner (Fig. 6B). The inhibition rate was 95.0% at 30 mg/kg of the compound. Roxatidine also
significantly prevented aspirin-induced lesions, the inhibition rate being 73.6% at 60 mg/kg. The ED₅₀ values for Z-300 and roxatidine were 2.1 (1.0–3.4) and 35.7 (6.1–173.8) mg/kg, respectively.

HCl-ethanol-induced lesions: Oral administration of HCl-ethanol induced severe and extensive mucosal lesions in the corpus and antrum 1 hr later. Pretreatment with Z-300 significantly protected the gastric mucosa against HCl-ethanol-induced lesions in a dose-dependent manner (Fig. 6B). The inhibition rate was 85.7% at 100 mg/kg of the compound. Roxatidine also significantly protected the gastric mucosa against the lesions. The ED₅₀ values for Z-300 and roxatidine were 11.1 (3.0–49.4) and 89.5 (45.8–888.2) mg/kg, respectively.

Fig. 5. Effects of Z-300 (A) and roxatidine (B) on carbachol-stimulated gastric acid secretion in Heidenhain pouch dogs. Each drug was administered p.o. 1 hr after the carbachol infusion. Both drugs significantly inhibited the gastric secretion for >3 hr. *Significantly different from the controls at P < 0.05.
Effects on acute duodenal ulcers

Mepirizole-induced ulcers: Mepirizole induced one or two penetrating ulcers in the proximal part of the duodenum 24 hr after administration. The development of these ulcers was significantly prevented by pretreatment with Z-300 in a dose-dependent manner (Fig. 7). The inhibition rate was 96.5% with 10 mg/kg of the compound. Roxatidine also significantly prevented the development of the ulcers. The ED₅₀ values for Z-300 and roxatidine were 1.3 (0.6–2.2) and 12.0 (4.3–25.4) mg/kg, respectively.

Cysteamine-induced ulcers: Similar to the case of mepirizole-induced ulcers, cysteamine also induced severe ulcers in the proximal duodenum 24 hr after the
initial injection. Both Z-300 and roxatidine significantly protected the duodenal mucosa against the cysteamine-induced ulcers in a dose-dependent manner (Fig. 7). The ED50 values for Z-300 and roxatidine were 2.0 (0.7–3.4) and 5.3 (1.5–14.5) mg/kg, respectively.

Effects on acetic acid-induced ulcers

Five days after acetic acid injection, there was the consistent development of a deep ulcer in the area corresponding to the injection. The average area of these ulcers was about 35–40 mm². Two weeks later, the area of the ulcers was 4.1 ± 0.5 mm² (n = 25). Daily administration of Z-300 at 200 mg/kg/day for 2 weeks tended to accelerate the spontaneous healing of ulcers (29.3%) (Fig. 8). At 400 mg/kg/day, however, it significantly accelerated the ulcer healing, the rate being 56.1%. Roxatidine, administered at 200 and 400 mg/kg/day had no effect on ulcer healing. Indomethacin apparently delayed the ulcer healing, the area in the controls being 10.1 ± 1.3 mm² (n = 24). Z-300, when administered at 200 mg/kg/day, did not prevent the delay in ulcer healing caused by indomethacin. However, it significantly prevented the delayed healing by 34.7%, when given at 400 mg/kg/day. Roxatidine, administered at 200 and 400 mg/kg/day, had no preventive effect on the delayed healing of ulcers.

Comparison with ED50 values

The comparative potencies of Z-300 to roxatidine on gastric secretion and acute gastro-duodenal lesions were calculated based on ED50 values (Table 5). As to the gastric secretion in rats and dogs, the ED50 ratio of Z-300 to roxatidine varied from 0.7 to 6.2 according to the route of administration and 0.8 to 1.1 depending on the gastric secretagogue employed. As to the gastric or duodenal lesions, the ratio largely varied with the lesion model, from 0.9 to 26.5.

DISCUSSION

The present study showed that Z-300 is a highly selective and competitive H₂-antagonist which exhibits potent antisecretory activity in rats and dogs. It markedly protected the gastric and duodenal mucosa from various acute lesions and accelerated the healing of acetic acid ulcers.

First, it was clearly indicated that Z-300 at 10⁻⁷ to 10⁻⁶ M competitively antagonized the histamine-induced response of the isolated guinea pig atrium. The potency of the compound was almost the same as that of roxatidine. Similar to roxatidine, Z-300 competitively antagonized cholinergic receptors when used at the concentration of 10⁻⁴ M. Such a phenomenon was also observed with ranitidine (competitively) or tiotidine.
Fig. 8. Effects of Z-300 and roxatidine on the spontaneous and delayed healing (by indomethacin) of acetic acid-induced gastric ulcers in rats. Each drug was administered p.o. twice daily for 2 weeks after ulceration. The numbers in parentheses are the numbers of animals used. *Significantly different from the controls at P < 0.05.

Table 5. Comparison of antisecretory and antilesion activities of Z-300 and roxatidine, based on ED50 values (μM/kg), in rats and dogs (noncompetitively) at concentrations >10^{-4} M (17). These results indicate that Z-300 is a highly selective H2-antagonist, as other established antagonists are.

Concerning gastric secretion, Z-300 significantly and persistently inhibited both the basal and stimulated secretion in rats and dogs. The overall pharmacological potency of the compound was nearly the same or 6 times that of roxatidine, when determined from the ED50 ratio (molar basis). However, it should be noted that Z-300 persistently inhibited the gastric acid secretion by >80% in both rats (>12 hr) and dogs (>7 hr). This long and potential activity would be a beneficial property when this compound is used for the treatment of peptic ulcers or acid-related diseases, since it can reduce the times of administration.

Various gastric lesions and duodenal ulcers were markedly prevented by pretreatment with Z-300 in a dose-dependent manner. It is generally known that the pathogenesis of water-immersion stress, indomethacin or aspirin-induced gastric lesions, or mepirizole- or cysteamine-induced duodenal ulcers is causally related to gastric secretion (9, 10, 18, 19). Indeed, most antisecretory agents, such as anticholinergic agents, H2-antagonists or gastric proton pump inhibitors, protect the gastric and duodenal mucosa against these lesions or ulcers (20, 21). Therefore, it is most likely that the mechanism of action of Z-300 on these lesions may in-
volve its potential and persistent antisecretry activity.

This compound also markedly protected the gastric mucosa against HCl-ethanol-induced gastric lesions. This lesion model is induced in the presence of HCl in the stomach. Therefore, the underlying mechanism is not related to the antisecretry activity of Z-300, but rather to its so-called cytoprotective activity (22). Roxatidine is also known to have a cytoprotective action against 100% ethanol-, 0.6 N HCl- and 0.2 N NaOH-induced lesions (23). In the present study, we also confirmed the cytoprotective activity of roxatidine against HCl-ethanol-induced lesions. However, the activity was about 7 times weaker than the antisecretry activity, based on the ED50 value. The cytoprotective effect of Z-300 was 12.7 times more potent than that of roxatidine, based on the ED50 ratio. The cytoprotective mechanism of Z-300 and roxatidine remains unknown.

One of the most important pharmacological properties of Z-300 appears to be its ability to enhance the healing of chronic gastric ulcers in rats. It is most likely that the mechanism of action of Z-300 in ulcer healing is related to its antisecretry activity, particularly to the property that this activity is long-lasting. Since the antisecretry activity of the compound persists for >12 hr, two administrations appear to be sufficient to extensively suppress the basal and food-stimulated gastric secretion during a day. We do not know whether or not the cytoprotective activity of Z-300 is involved in its mechanism of action for enhancing ulcer healing. The reason why roxatidine failed to show any significant effect on ulcer healing seems to be due to its short-lasting acid suppressive activity.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and indomethacin, are well-known to delay the healing of peptic ulcers or to induce the relapse of healed ulcers (24). A drug that is able to prevent such an adverse effect will be useful for the treatment of patients with peptic ulcers who are receiving NSAID therapy. Z-300 was found to significantly prevent the delay in ulcer healing caused by indomethacin. Therefore, this compound seems to be effective in reducing the hazardous effect of NSAIDs as well as in enhancing ulcer healing.

These results taken together suggest that the new H2-antagonist Z-300, with potential antisecretry, cytoprotective and ulcer healing activities, will be a beneficial drug for the treatment of human peptic ulcers and acid-related diseases.

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