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Antisecretory and antiulcer effect of T-330, a novel reversible proton pump inhibitor, in rats

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Abstract

The antisecretory and antiulcer effects of T-330 (2-[(2-dimethylaminobenzyl)sulfinyl]-1-(3-methylpyridine-2-yl)imidazole), a novel reversible proton pump inhibitor, were studied in rats. T-330 suppressed dibutyryl cyclic AMP-stimulated acid formation in isolated rat gastric mucosal cells with the IC_{50} value of 0.57 μM. In chronic fistula rats, intravenous, intraduodenal and oral administration of T-330 inhibited pentagastrin-stimulated gastric acid secretion; the ED_{50} values calculated from the peak inhibition were 0.36, 0.43 and 0.73 mg/kg, respectively. T-330 also reduced dimaprit-stimulated gastric acid secretion following its intraduodenal injection (ED_{50} 0.85 mg/kg). The antisecretory activities of T-330 following its intraduodenal and oral administration were 3–6- and 4–10-times more potent than those of omeprazole and ranitidine, respectively, while the duration of action of T-330 was apparently shorter than that of omeprazole and was almost equal to that of ranitidine. Oral or duodenal administration of T-330 inhibited the development of acid-related damage (water-immersion- and aspirin-induced gastric lesions, cysteamine-induced duodenal ulcers and reflux esophagitis) with equal or higher potency than omeprazole or ranitidine. Furthermore, T-330 prevented ethanol-induced gastric lesions. These findings indicate that T-330 exerts its antiulcer effect mainly via its potent antisecretory action and partly via its gastroprotective action.

Keywords: T-330; H^+/K^+-ATPase; Gastric acid secretion; Omeprazole; Ranitidine

1. Introduction

The presence of acid is a fundamental factor in the pathogenesis of gastric and duodenal ulcers, reflux esophagitis, and nonsteroidal antiinflammatory drug-induced lesions (Hunt et al., 1995). Therefore, it is mandatory to control acid secretion for treatment of these diseases. While acid secretion by parietal cells is regulated through several stimulatory receptors such as histamine H_2 receptor, muscarinic M_3 receptor and gastrin receptor, the final step of acid secretion is mediated by the gastric H^+/K^+-ATPase, the so-called proton pump (Hersey and Sachs, 1995). Thus, effective therapeutic control of acid secretion involves both blockade of these receptors and inhibition of the proton pump.

Inhibition of acid secretion by histamine H_2 receptor antagonists has revolutionized the treatment of gastroduodenal acid-related disease (Bodemar et al., 1977). However, the effectiveness of histamine H_2 receptor antagonists is limited because acid secretion is mediated by several stimuli and histamine H_2 receptor antagonist-resistant ulcers and esophagitis have been reported (Hirschowitz et al., 1995; Hunt et al., 1995). Proton pump inhibitors have been expected to have potential clinical advantages over histamine H_2 receptor antagonists, because acid secretion is inhibited irrespective of the stimulus. The first class of proton pump inhibitors, substituted benzimidazole derivatives, irreversibly suppress gastric H^+/K^+-ATPase, resulting in a long-lasting and potent antisecretory effect (Herling and Weidmann, 1994; Hersey and Sachs, 1995). Although these derivatives exert superior healing of acid-related disorders compared to histamine H_2 receptor antagonists (Lindberg et al., 1990), long-term treatment with omeprazole, one of this class of inhibitor, causes bacterial overgrowth in the upper gut due to profound long-standing acid suppression (Wingate, 1990; Larner and Lendrum, 1992). Sustained acid inhibition due to a long-term treatment with acid inhibitors also induces hypergastrinemia, resulting in the development of gastric

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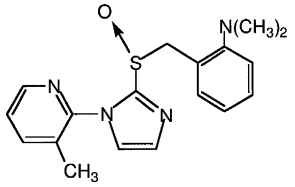

Fig. 1. Chemical structures of T-330.

enterochromaffin-like (ECL) cell carcinoids in rats (Carlsson et al., 1986; Lindberg et al., 1990; Herling and Weidmann, 1994) and a slight hyperplasia of ECL cells in humans (Lamberts et al., 1988, 1993; Freston et al., 1995).

A reversible H^+/K^+-ATPase inhibitor, the second class of proton pump inhibitor, is expected to exert a potent (irrespective of stimuli) yet short-lasting antisecretory action that should provide greater dosing flexibility to reduce both bacterial overgrowth and excessive hypergastrinemia. T-330 (2-[(2-dimethylaminobenzyl)sulfinyl]-1-(3-methylpyridine-2-yl)imidazole; Fig. 1) reversibly inhibits H^+/K^+-ATPase through a mechanism distinct from that of the previous reversible H^+/K^+-ATPase inhibitors, which competitively bind to the high affinity K^+-site on the gastric H^+/K^+-ATPase (Pope and Parsons, 1993; Kinoshita et al., 1996). The present study evaluates the antisecretory and antiulcer effects of T-330 in comparison with omeprazole and ranitidine, a histamine H_2 receptor antagonist.

2. Materials and methods

2.1. Animals and chemicals

Male Sprague-Dawley rats (Charles River Japan, Kanagawa, Japan) weighing 160–230 g were used.

T-330, omeprazole and dimaprit were synthesized, and ranitidine was extracted from Zantac (Glaxo, Toronto, Canada) and purified in the Organic Chemistry Research Laboratory of Tanabe Seiyaku (Saitama, Japan). [^{14}C]aminopyrine (specific activity, 4.4 GBq/mmol) was obtained from Dupont-New England Nuclear (Wilmington, DE, USA). Pentagastrin, aspirin and cysteamine were purchased from Nacalai Tesque (Kyoto, Japan), and dibutyryl cyclic AMP (db-cAMP) from Sigma (St. Louis, MO, USA). T-330, omeprazole and ranitidine were first dissolved in dimethylsulfoxide (DMSO) and diluted with the medium C mentioned below to the final concentration of 0.1% (v/v) DMSO for in vitro experiments. For intravenous injection, test drugs were dissolved in DMSO and the solution was diluted with saline to make the final concentration of 5% (v/v) DMSO. The drugs were suspended in a 0.2% (v/v) polyoxyethylene sorbitan monooleate (Tween 80) solution to prepare the volume for 2 ml/kg and 10 ml/kg for intraduodenal and oral administration, respectively.

2.2. Acid formation in rat gastric mucosal cells

2.2.1. Preparation of gastric mucosal cells

Three kinds of media (pH 7.4) were prepared. Medium A (mM) consists of NaH_2PO_4 0.5, Na_2HPO_4 1.0, $NaHCO_3$ 20.0, NaCl 70.0, KCl 5.0, glucose 11.0, EDTA 2.0, Hepes 50.0 and 2.0% bovine serum albumin. Medium B had the same composition as medium A, except it was without EDTA but with 1.0 mM $CaCl_2$, 1.5 mM $MgCl_2$ and 1.0% bovine serum albumin. The composition of medium C was the same as medium B, except it contained 0.1% instead of 1.0% bovine serum albumin.

Cell isolation was performed according to the method of Lewin et al. (1982). Under ether anesthesia, the stomach was removed, transformed into an everted sac filled with pronase solution (10 mg/ml) and incubated at 37°C in medium A gassed with 95% O_2/5% CO_2 for 90 min. The medium was renewed twice during this period. The sac was then transferred into medium B and the solution was gently stirred for 30 min by a magnetic stirrer. The isolated cells were collected by centrifugation ($600 \times g$, 5 min). The pellet was resuspended in medium C. The viability of the cells, judged by Trypan blue exclusion, was $95 \pm 1.5\%$ and the content of parietal cells was $25.8 \pm 2.2\%$ (mean $\pm$ S.E., $n = 4$), as identified under the light microscope by their unique morphological feature.

2.2.2. Measurement of acid formation

Acid formation in the parietal cells was determined by measuring the cellular uptake of [^{14}C]aminopyrine (Sewing et al., 1983). Gastric mucosal cells (approx. 5×10^6 cells) were incubated with 0.2 μCi of [^{14}C]aminopyrine, 200 μM db-cAMP and the test drugs in 1.0 ml of medium C at 30°C for 45 min. The reaction was stopped by centrifugation (4°C, $9000 \times g$, 1 min, Microcentrifuge himac CR 15D, Hitachi-Koki, Tokyo, Japan). The pellet was washed with 1 ml of ice-cold medium C and subsequently recentrifuged. The pellet was digested with 250 μl of tissue solubilizer (Soluen, New England Nuclear) and then the radioactivity was counted by a scintillation counter (Tri-Carb 460CD, Packard, Meriden, CT, USA).

2.3. Gastric secretion in rats

2.3.1. Chronic fistula rat

Rats were chronically implanted with a plastic fistula by the method previously reported (Larsson et al., 1983). Under pentobarbital anesthesia (35 mg/kg, i.p.), a plastic fistula was placed in the forestomach near the glandular part. In some experiments, a polyethylene tube (PE 50) was inserted into the upper part of the duodenum and fixed for the administration of the test drugs. The tube was subcutaneously guided to the neck. The procedure was carried out under aseptic conditions. One week of recovery was allowed before experiments.

Rats were deprived of food but allowed free access to water for 24 h prior to the experiments. Each animal was placed in a Bollman cage and gastric juice was collected by free drainage through the fistula in 30-min samples. After determination of the volume of each sample, the acid concentration was measured by titration against 0.1 M NaOH to the endpoint of pH 7.0 using an autotitrater (TTT 85; Radiometer Copenhagen, Copenhagen, Denmark), and the acid output was expressed as μEq of H^+ per 30 min.

90 min after intravenous infusion of pentagastrin (0.1 mg/kg per h) or dimaprit (20 mg/kg per h), test drugs were injected intravenously through the tail vein or intraduodenally through the implanted tube. The ED_{50} values were calculated from the ratio of acid secretion at peak inhibition to that just before drug administration.

In the experiments for determination of effects after oral drug administration, pentagastrin (0.1 mg/kg) was subcutaneously injected, and gastric juice was collected by drainage through the fistula. One hour later the content of the stomach was rinsed out through the collecting fistula. Immediately after the fistula was capped, the test drugs were orally administered. 1 h after the administration, the cap was removed and pentagastrin (0.1 mg/kg) was subcutaneously given again. Acid output during the 30-min period after pentagastrin injection was measured as mentioned above. The first pentagastrin-stimulated acid secretion was defined as the control for calculation of the inhibitory activity of the drugs.

2.3.2. Pylorus-ligated rat

Rats were deprived of food but allowed free access to water for 24 h prior to experiments. Following ligation of the pylorus under ether anesthesia, the test drugs were intraduodenally given, and the abdomen was closed by suturing. 5 h later, the rat was killed by an overdose of ether, the stomach was removed and the gastric content was collected. After centrifugation ($900 \times g$, 10 min), the volume of the supernatant was measured and the acid concentration was determined by titration as mentioned above. Pepsin activity was determined by Anson's method using bovine hemoglobin as a substrate (Anson, 1938). Total acid and pepsin output were determined by the juice volume (ml) × acid concentration (μEq/ml) and pepsin activity (μg tyrosine/ml), respectively.

2.4. Acute gastroduodenal ulcers and reflux esophagitis in rats

Rats were deprived of food but allowed free access to water for 24 h prior to experiments.

2.4.1. Water-immersion-induced gastric lesions

The experiment was performed according to the method described by Yamamoto et al. (1984) with a slight modification. Fifteen minutes after the oral administration of test drugs, rats were placed in a stainless-mesh cage and were immersed vertically to the level of the xiphisternum process in a water bath maintained at 24 ± 1°C. 6 h later the rats were killed by an overdose of ether. The stomach was excised and fixed with 1% formalin. The area (mm^2) of lesions in the corpus was measured and used as a lesion index.

2.4.2. Aspirin-induced gastric mucosal lesions

1 h after oral administration of the test drugs, aspirin (200 mg/kg) was orally administered. 4 h later the rats were killed by an overdose of ether, and the stomach was excised and fixed with 1% formalin. The length of each lesion in the stomach was measured and used as a lesion index (mm).

2.4.3. Ethanol-induced gastric mucosal lesions

One hour after oral administration of the test drugs, 99.5% ethanol (1 ml/rat) was orally administered. One hour later the rats were killed by an overdose of ether, and the stomach was excised and fixed with 1% formalin. The length of each lesions in the stomach was measured and used as a lesion index (mm).

2.4.4. Cysteamine-induced duodenal ulcers

Cysteamine (400 mg/kg) was orally given 30 min after the oral administration of the test drugs. 16 h later the rats were killed by an overdose of ether and the duodenum was excised. The severity of each ulcer was scored macroscopically, using an ulcer index according to the method of Groves et al. (1974): normal mucosa scored as 0; reddening, irritation or sloughing of mucosa as 1; superficial erosion as 2; deep ulcer as 3; perforating ulcer as 4.

2.4.5. Reflux esophagitis

Under ether anesthesia, the pylorus and the junction between the forestomach and corpus were ligated according to the method of Inatomi et al. (1991) with a slight modification. The test drugs were intraduodenally administered immediately after the ligation. 4 h later, the rats were killed by an overdose of ether and the gastroesophageal portion of the digestive tract was excised. The lesion in the thoracic esophagus was scored macroscopically, using a lesion index according to the following criteria: no lesion as 0; edema as 1; reddening as 2; the length of hemorrhagic lesion area (< 20 mm) as 3; the length of hemorrhagic area (20–30 mm) as 4; the length of hemorrhagic area (30–40 mm) as 5; the length of hemorrhagic area (> 40 mm) or perforation as 6.

2.5. Statistical analysis

All data are expressed as means ± S.E. and were analyzed by analysis of variance or Kruskal–Wallis rank test followed by the Bonferroni's methods. A P value less than 0.05 was considered to be statistically significant. IC_{50} and ED_{50} values were calculated from the concentra-

tion- or dose-inhibition relationships by the method of least squares.

3. Results

3.1. Effect on acid formation in rat gastric mucosal cells

T-330 (0.001–30 μM) and omeprazole (0.001–30 μM) inhibited db-cAMP-stimulated acid formation in a concentration-dependent manner; the IC_{50} values were 0.57 μM and 0.14 μM, respectively (Table 1). Ranitidine at 100 μM elicited no effects on the db-cAMP-induced acid formation.

3.2. Effect on gastric acid secretion in rats

3.2.1. Effect on pentagastrin-stimulated acid secretion in chronic fistula rats

T-330 (0.31–1.25 mg/kg), omeprazole (0.31–1.25 mg/kg) and ranitidine (0.31–5 mg/kg), when intravenously administered, dose dependently inhibited pentagastrin-stimulated gastric acid secretion. Intraduodenal administration of T-330 (0.31–2.5 mg/kg), omeprazole (1.25–10 mg/kg) and ranitidine (1.25–20 mg/kg) also dose dependently depressed gastric acid secretion caused by pentagastrin. As shown in Figs. 2 and 3, the peak inhibition induced by T-330, omeprazole and ranitidine was attained within 1 h for both intravenous and intraduodenal injections. However, the duration of the action of T-330 was shorter than that of omeprazole and almost the same as that of ranitidine. The potency of intravenous T-330 was comparable to that of omeprazole but was 6-times greater than that of ranitidine (Table 1). The

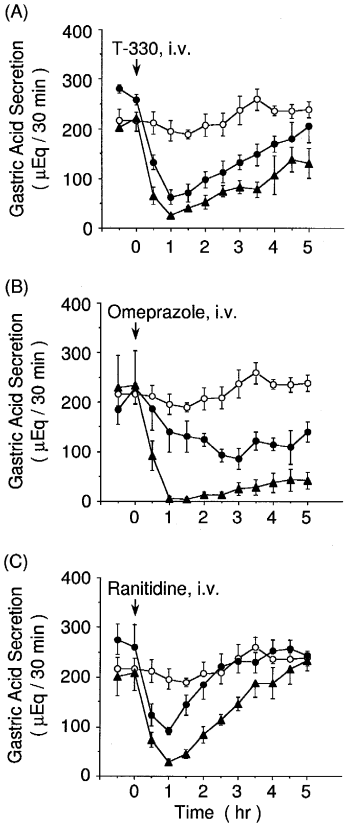

Fig. 2. Time-course of antisecretory effect of intravenously administered T-330, omeprazole and ranitidine on pentagastrin-induced gastric acid secretion in chronic fistula rats. After 90 min of intravenous infusion of pentagastrin (0.1 mg/kg per h), the test drugs were intravenously injected. Control (○). (A) T-330, 0.62 mg/kg (●), 1.25 mg/kg (▲); (B) omeprazole, 0.62 mg/kg (●), 1.25 mg/kg (▲); ranitidine, 1.25 mg/kg (●), 5.0 mg/kg (▲). Data are expressed as the means ± S.E. for 5–6 rats.

Table 1
IC_{50} (μM) and ED_{50} (mg/kg) values of antisecretory and antiulcer effects of T-330, omeprazole and ranitidine in rats

Item	Route	IC_{50}/ED_{50} values		
		T-330	Omeprazole	Ranitidine
Gastric acid secretion				
Aminopyrine uptake				
db-cAMP stim.	in vitro	0.57 (0.37–0.89)	0.14 (0.08–0.25)	> 100
Chronic fistula rats [a]				
Pentagastrin stim.	i.v.	0.36 (0.21–0.40)	0.44 (0.30–0.55)	2.19 (0.73–7.01)
	i.d.	0.43 (0.32–0.60)	2.44 (1.93–3.08)	4.10 (1.89–7.42)
	p.o.	0.73 (0.11–3.95)	1.92 (0.45–14.2)	N.C. [b]
Dimaprit stim.	i.d.	0.85 (0.71–1.01)	3.61 (2.75–4.37)	4.83 (1.11–21.4)
Pylorus-ligated rats [c]	i.d.	1.85 (1.21–2.70)	1.54 (0.80–2.63)	16.8 (3.49–98.2)
Gastric lesions				
Water immersion	p.o.	4.72 (1.55–13.5)	7.36 (2.69–40.9)	27.8 (13.5–47.1)
Aspirin	p.o.	6.60 (3.05–13.0)	15.5 (7.00–42.8)	8.82 (2.69–27.1)
Ethanol	p.o.	9.78 (6.28–15.2)	8.32 (5.00–13.6)	> 100
Duodenal ulcers				
Cysteamine	p.o.	4.92 (1.22–18.8)	25.5 (10.8–98.4)	79.9 (34.8–426)
Reflux esophagitis	p.o.	2.87 (1.94–4.43)	2.31 (1.21–4.10)	93.0 (54.6–903)

Numbers in parentheses indicate 95% confidence limits. [a] The ED_{50} value for the antisecretory effect (i.v. and i.d.) in chronic fistula rats was calculated from the peak inhibition. [b] N.C.: not calculated. [c] The ED_{50} value for antisecretory effect in pylorus-ligated rats was calculated from the total acid output.

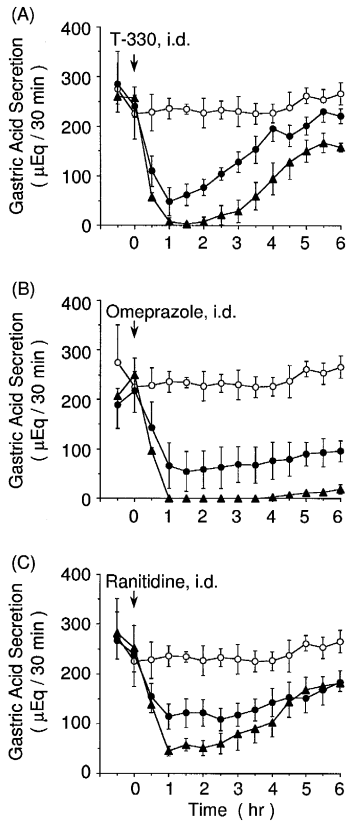

Fig. 3. Time-course of antisecretory effect of intraduodenally administered T-330, omeprazole and ranitidine on pentagastrin-induced gastric acid secretion in chronic fistula rats. After 90 min of intravenous infusion of pentagastrin (0.1 mg/kg per h), the test drugs were intraduodenally injected. Control (○). (A) T-330, 1.25 mg/kg (●), 2.5 mg/kg (▲); (B) omeprazole, 5 mg/kg (●), 10 mg/kg (▲); (C) ranitidine, 5 mg/kg (●), 20 mg/kg (▲). Data are expressed as the means ± S.E. for 6 rats.

antisecretory effect of intraduodenal T-330 was approximately 6- and 10-times more potent than that of omeprazole and ranitidine, respectively (Table 1).

In chronic fistula rats, subcutaneous injection of pentagastrin (0.1 mg/kg) caused transient gastric acid secretion (144.1 ± 10.7 μEq/30 min; mean ± S.E., $n = 7$) which lasted for 1 h. Repeated treatment with s.c. pentagastrin produced a reproducible acid output. When administered orally, T-330 (0.31–2.5 mg/kg) and omeprazole (0.62–5 mg/kg) dose-dependently reduced pentagastrin-induced acid secretion; the ED_{50} values were 0.73 and 1.92 mg/kg, respectively (Table 1). T-330 was about 3 times as potent as omeprazole regarding the antisecretory effect. The inhibitory effect of ranitidine at the dose of 10 mg/kg was approximately the same as that of T-330 at 2.5 mg/kg (data not shown).

3.2.2. Effect on dimaprit-stimulated acid secretion in chronic fistula rats

Intravenous infusion of dimaprit (20 mg/kg per h) caused a sustained acid output for about 6 h; the acid output during steady state secretion was 248.5 ± 9.6 μEq/30 min (mean ± S.E. $n = 6$). T-330 (0.31–2.5 mg/kg, i.d.) inhibited the dimaprit-stimulated gastric acid secretion in a dose-dependent manner. While the inhibition of gastric acid secretion caused by T-330, omeprazole and ranitidine reached the maximum within 1 h of the injection, recovery from the T-330-induced response was faster than that for omeprazole and was comparable to that for ranitidine (data not shown). The antisecretory activity of T-330 was about 4 and 5 times more potent than that of omeprazole and ranitidine, respectively, when judged by the peak inhibition (Table 1).

3.2.3. Effect on gastric secretion in pylorus-ligated rats

Intraduodenal administration (1–10 mg/kg) of T-330 and omeprazole inhibited gastric juice volume and acid concentration over a similar dose range, with a weak inhibitory effect on the pepsin activity in pylorus-ligated rats (Table 2). As a result, the inhibitory activities on both total acid output (ED_{50}: T-330, 1.85 mg/kg; omeprazole,

Table 2
Effects of intraduodenal administration of T-330, omeprazole and ranitidine on gastric secretion in pylorus-ligated rats ($n = 8$)

Drug	Dose (mg/kg)	Volume (ml/5 h)	Acid		Pepsin	
			Conc. (μEq/ml)	Total output (μEq/5 h)	Activity (μg tyr./ml)	Total output (μg tyr./5 h)
Control		6.1 ± 0.6	99.8 ± 3.6	619.5 ± 84.8	15.5 ± 1.3	92.6 ± 10.9
T-330	1	4.9 ± 0.3	83.4 ± 2.3	408.4 ± 30.1 [a]	16.0 ± 1.1	74.5 ± 7.1
	3	3.7 ± 0.3 [a]	61.1 ± 4.8 [b]	222.9 ± 24.4 [b]	16.7 ± 1.1	60.9 ± 5.3 [a]
	10	2.7 ± 0.3 [b]	24.9 ± 3.0 [b]	67.2 ± 12.6 [b]	11.5 ± 1.6	30.6 ± 5.3 [b]
Omeprazole	1	5.3 ± 0.4	75.6 ± 6.1	410.9 ± 54.0 [a]	15.6 ± 0.8	83.1 ± 7.0
	3	3.5 ± 0.3 [a]	41.3 ± 6.3 [b]	142.0 ± 23.7 [b]	16.8 ± 1.3	58.4 ± 5.5 [a]
	10	2.9 ± 0.2 [b]	10.2 ± 2.7 [b]	28.2 ± 7.5 [b]	11.1 ± 1.7	31.5 ± 5.1 [b]
Ranitidine	1	5.5 ± 0.6	102.6 ± 5.7	573.3 ± 84.7	17.2 ± 1.5	88.7 ± 5.8
	10	3.8 ± 0.4 [a]	98.1 ± 5.6	389.6 ± 62.3 [a]	20.1 ± 0.9	76.0 ± 7.3
	100	3.2 ± 0.3 [b]	35.3 ± 6.8 [b]	121.9 ± 32.8 [b]	17.1 ± 1.9	56.4 ± 10.0 [b]

[a] $P < 0.05$ and [b] $P < 0.01$ compared with control, respectively.

1.54 mg/kg) and total pepsin output (ED_{50}: T-330, 4.99 mg/kg; omeprazole, 5.17 mg/kg) were almost the same for the two drugs. Ranitidine reduced gastric juice volume and acid concentration in a dose-dependent manner without affecting pepsin activity (Table 2). Ranitidine was about 9 and 30 times less potent than T-330 in inhibiting the total acid output (ED_{50}: 16.8 mg/kg) and the total pepsin output (ED_{50}: > 100 mg/kg), respectively.

3.3. Effect on gastroduodenal ulcers and esophagitis in rats

3.3.1. Effect on water immersion-induced gastric lesions

Water immersion at 24°C for 6 h caused punctate lesions mainly in the glandular stomach and the mean lesion area in the control group was 5.90 ± 0.80 mm^2 ($n = 10$). T-330 (2.5–10 mg/kg, p.o.), omeprazole (2.5–10 mg/kg, p.o.) and ranitidine (20–80 mg/kg, p.o.) prevented the formation of these lesions in a dose-related manner, and T-330 was about 2 and 5 times more potent than omeprazole and ranitidine, respectively (Table 1).

3.3.2. Effect on aspirin-induced gastric lesions

Aspirin (200 mg/kg, p.o.) produced elongated erosions in the glandular potion of stomach and the lesion index of the control was 44.4 ± 2.9 mm (mean $\pm$ S.E., $n = 8$). T-330, omeprazole and ranitidine (3–30 mg/kg, p.o.) dose dependently inhibited the formation of aspirin-induced lesions. As shown in Table 1, the inhibitory activity of T-330 was about 2 times as high as that of omeprazole and almost the same as that of ranitidine.

3.3.3. Effect on ethanol-induced gastric lesions

Oral administration of absolute ethanol induced band-like hemorrhagic lesions in the glandular portion of stomach. The lesion index in the control group was 76.9 ± 9.3 mm (mean $\pm$ S.E., $n = 12$). Both T-330 (3–30 mg/kg) and omeprazole (3–30 mg/kg), when orally administered, exerted dose-dependent inhibition of the formation of ethanol-induced gastric lesions, and the inhibitory potency of T-330 was comparable to that of omeprazole (Table 1). Ranitidine even at 100 mg/kg was without effect.

3.3.4. Effect on cysteamine-induced duodenal ulcers

Cysteamine (400 mg/kg, p.o.) induced penetrating or severe ulcers in the proximal duodenum and the mean score of ulcer severity in the control was 3.4 ± 0.2 ($n = 9$). Oral administration of T-330 (2.5–10 mg/kg), omeprazole (10–40 mg/kg) and ranitidine (20 and 80 mg/kg) dose dependently reduced the severity of cysteamine-induced duodenal ulcers. T-330 was about 5 and 16 times more potent than omeprazole and ranitidine, respectively (Table 1).

3.3.5. Effect on reflux esophagitis

In the double ligated animals, hemorrhagic lesions were observed in almost the entire area of the thoracic esophagus. Perforation of the esophagus was also observed in two-thirds of the animals and the mean severity of esophagitis of the control was 5.33 ± 0.37 ($n = 9$). T-330 (1.3–5.0 mg/kg, i.d.) dose dependently inhibited the formation of esophagitis, and the potency of the inhibition for T-330 was almost the same as that for omeprazole and 31 times that for ranitidine (Table 1).

4. Discussion

In the present study, T-330 exerted more potent antisecretory and antiulcer effects than either omeprazole or ranitidine following oral and intraduodenal administration. The duration of inhibition of acid secretion induced by T-330 was shorter than that elicited by omeprazole and was comparable to that elicited by ranitidine. These findings reflect the results of a previous study showing that T-330 inhibits gastric H^+/K^+-ATPase reversibly and more potently than omeprazole in vivo (Kinoshita et al., 1996).

T-330 as well as omeprazole inhibited the acid formation in rat parietal cells induced by db-cAMP, an analog of cAMP that is an intracellular mediator of gastric acid secretion, and the inhibitory activity of T-330 was weaker than that of omeprazole. However, T-330 was equally potent to omeprazole by the i.v. route and even more potent than omeprazole by the i.d. and p.o. routes in chronic fistula rats. These findings suggests a higher bioavailability of T-330 compared to omeprazole and its effective absorption from the gastrointestinal tract.

In contrast to the long-lasting inhibitory effect of omeprazole (Larsson et al., 1983; Satoh et al., 1989), the duration of the antisecretory action of T-330 was as short as that of ranitidine, a reversible histamine H_2 receptor antagonist. In the acidic intracellular compartment of the parietal cells omeprazole is transformed into an active intermediate, the sulfenamide, which binds covalently to the sulfhydryl groups of H^+/K^+-ATPase to inhibit the enzyme's activity (Lindberg et al., 1990). Since the tight covalent bond formation between the enzyme and the sulfenamide causes irreversible inhibition, de novo synthesis of the H^+/K^+-ATPase is required for restoration of enzyme activity and acid secretion (Im et al., 1985; Gedda et al., 1995). In the previous report, we have shown that restoration of H^+/K^+-ATPase activity that is depressed by T-330 does not need de novo synthesis of the enzyme, indicating the reversible inhibition of the H^+/K^+-ATPase by T-330 in vivo (Kinoshita et al., 1996). Yamada et al. (1996) have suggested that the sulfinylimidazole derivative, e.g., T-330, is converted into sulfenic acid but not into sulfenamide in the acidic milieu to interact with thiol groups in the H^+/K^+-ATPase, and that the S-S bridge between the enzyme and the sulfenic acid is easily cleaved by the sulfhydryl agent. In fact, a lower concentration of 2-mercaptoethanol is required to restore H^+/K^+-ATPase activity that is inhibited by T-330 than by omeprazole (Kinoshita et al., 1996). Therefore, the short-lasting antise-

cretory action of T-330 is likely to be based on the distinct reversible inhibition of the enzyme's activity.

When intraduodenally administered, T-330 was more potent than and equally effective to omeprazole in chronic fistula rats and pylorus-ligated rats, respectively. This discrepancy is explained by the short duration of inhibitory action of T-330. The acid secretion determined in the pylorus-ligated rats was the total secretion for 5 h. The duration of the action of T-330 might not be sufficient to inhibit acid secretion completely for the 5-h period, in contrast to omeprazole, which exerts a sustained antisecretory effect.

Profound long-standing suppression of acid secretion causes bacterial overgrowth in the upper gut (Wingate, 1990; Larner and Lendrum, 1992) and hypergastrinemia (Carlsson, 1989). Life-long elevation of serum gastrin levels often stimulates the development of gastric ECL cell carcinoids in rodents (Poynter et al., 1985; Carlsson et al., 1986; Betton et al., 1988). In humans, hypergastrinemia induced by antisecretory agents causes hyperplastic change but not dysplasia in endocrine cells of the stomach (Lamberts et al., 1993). Therefore, optimal control of acid secretion is desirable for treatment of acid-related disorders to avoid the possibility of microbial overgrowth and excessive hypergastrinemia. The short-lasting reversible inhibition obtained with T-330 may make acid secretion controllable.

T-330 exhibited potent inhibition of the development of acid-related damage such as water immersion- and aspirin-induced gastric lesions, cysteamine-induced duodenal ulcers, and reflux esophagitis, reflecting its potent antisecretory activity in vivo. Ranitidine was less effective on cysteamine-induced duodenal ulcers and reflux esophagitis than on the other acid-related damage, because the pathogenesis of both of cysteamine-induced ulcer and reflux esophagitis involves vagal pathway-dependent acid secretion (Szabo et al., 1979; Inatomi et al., 1991). Furthermore, T-330 as well as omeprazole exerted a protective effect against ethanol-induced gastric lesions, in contrast to ranitidine. Since ethanol directly causes damage, these agents seem to enhance the gastric mucosal defense. The mechanism of gastroprotection induced by T-330 waits to be determined. Gastroprotective activity may partly contribute to the antiulcer effects of both T-330 and omeprazole in addition to the antisecretory effect. Thus, T-330 looks promising to exert prominent effects against Zollinger-Ellison syndrome and gastroesophageal reflux disease, in which histamine H_2 receptor antagonists are less effective.

Taken together, T-330 produces a potent and short-lasting inhibition of acid secretion in rats due to reversible suppression of H^+/K^+-ATPase. The antiulcer effect of T-330 is due mainly to its potent antisecretory action and partly to its gastroprotective action. These findings indicate that T-330 is a useful therapeutic agent in the treatment of peptic ulcer diseases.

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