Effects of 12-Sulfodehydroabietic Acid Monosodium Salt (TA-2711), a New Anti-Ulcer Agent, on Gastric Secretion and Experimental Ulcers in Rats

Yuichi ONODA, Tetsuo MAGARIBUCHI and Hajime TAMAKI

Biological Research Laboratory, Tanabe Seiyaku Co., Ltd., 2-2-50, Kawagishi, Toda, Saitama 335, Japan

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Abstract—Effects of 12-sulfodehydroabietic acid monosodium salt (TA-2711), a new anti-ulcer agent, on gastric secretion and experimental ulcers were investigated in rats. Oral administration of TA-2711 at doses of 25 to 100 mg/kg immediately after pyloric ligation markedly reduced pepsin activity and slightly lowered acid concentration without affecting the volume of gastric juice. Addition of TA-2711 (0.25–16 mg/ml) directly to gastric juice also reduced pepsin activity in vitro. Oral TA-2711 dose-relatedly inhibited the formation of pylorus-ligated ulcers (50–200 mg/kg), aspirin-induced gastric erosions (25–100 mg/kg) and cysteamine-induced duodenal ulcers (100–800 mg/kg). In addition, this drug prevented both the formation of gastric lesions (6.3–100 mg/kg, p.o.) and the fall in gastric potential difference (100 mg/kg, p.o.) induced by ethanol. The preventive effect against ethanol-induced lesions was suppressed by pretreatment with indomethacin (10 mg/kg, s.c.). Intravenous dosing of TA-2711 (10–100 mg/kg) never produced such effects on ethanol-induced lesions and pepsin activity as observed by oral administration. These results indicate that TA-2711 exerts its anti-ulcer effect by a local action, and it is suggested that both reduction of pepsin activity and a mucosal prostaglandin-mediated process are involved in the anti-ulcer action of TA-2711.

In extensive research aimed at the development of a new anti-ulcer agent, we found that some derivatives of dehydroabietic acid exhibited antisecretory and/or antipepsin activity (1). A preliminary experiment revealed that among these compounds, 12-sulfodehydroabietic acid monosodium salt (TA-2711, Fig. 1) had both potent antipepsin and anti-ulcer activity with low toxicities. Accordingly, we selected it for detailed studies. The present study describes the effects of TA-2711 on gastric secretion and experimental ulcers in rats. In addition, the effects of TA-2711 were compared with those of carbenoxolone, sucralfate and cimetidine.

Materials and Methods

1. Drugs

TA-2711 was synthesized at the Organic Chemistry Research Laboratory, Tanabe Seiyaku. Other drugs used were carbenoxolone disodium (Tokiwa Shokubutsu Kagaku), sucralfate (Ulcerlmine®; Chugai Seiyaku), cimetidine (Farmatis), aspirin (Iwaki Seiyaku), cysteamine hydrochloride (Tokyo Kasei), indomethacin (Sigma), ethanol (Kanto Chemical) and urethane (Wako Pure...
In oral experiments, test drugs were dissolved or suspended in deionized water and given to animals in a volume of 4 or 5 ml/kg. In intravenous experiments, TA-2711 was dissolved in 1 N NaOH and cimetidine in 1 N HCl. The pH of each solution was neutralized, and the solution was diluted with physiological saline solution so as to make the administered volume 1 ml/kg. In in vitro experiments, the test drugs were suspended in 0.01 N HCI. Aspirin was suspended in 0.5% carboxymethylcellulose sodium containing 0.2% Tween 80, and indomethacin was suspended in physiological saline containing 0.2% Tween 80. Cysteamine and urethane were dissolved in deionized water.

2. Methods
1) Gastric secretion
Male SD rats (Charles River Japan, 150–190 g) were fasted for 48 hr. The animals were anesthetized with ether, and the pylorus was ligated according to the method of Shay et al. (2). Test drugs were administered immediately after the ligation. The stomach was removed 3 hr after the intravenous administration and 5 hr after the oral administration. The gastric contents were centrifuged at 900×g for 10 min, and then the volume of gastric juice (supernatant) was measured. The acidity was titrated with 0.1 N NaOH to pH 7.0 using an autoburette (TTT 2, Radiometer) and expressed as mEq/l.

Pepsin activity was determined by Anson’s method using bovine hemoglobin as a substrate (3). The centrifuged gastric juice sample was diluted to 100-fold in 0.01 N HCl. Each dilution, 0.2 ml, was incubated with 1 ml of 10 mg/ml hemoglobin (Sigma, dissolved in 0.06 N HCl) at 30°C. After 10 min, the reaction was stopped by 7% trichloroacetic acid, and the solution was centrifuged. The supernatant was made alkaline by the addition of 0.55 M Na₂CO₃. The Folin-Ciocalteu reagent (Merck) was then added, and the optical density was measured with a spectrophotometer (UV-150-02, Shimadzu) at 660 nm. The pepsin activity was expressed as mg tyrosine/ml.

2) Pepsin activity of gastric juice in vitro
Gastric juice was collected from pylorus-ligated rats (male, SD, Charles River Japan, 150–190 g). A test drug, 0.25 ml, was added to 2.25 ml of gastric juice, and the mixture was incubated at 30°C for 10 min. After centrifugation (24,000×g at 4°C for 10 min), the pepsin activity of the diluted (1/50, in 0.01 N HCl) supernatant was determined in the same way as described above.

3) Experimental ulcers
a) Pylorus-ligated gastric ulcers: Male Wistar rats (Charles River Japan, 200–250 g) were fasted for 48 hr. Under ether anesthesia, the abdomen was incised and the pylorus was ligated (2). Test drugs were given orally immediately after ligation. Sixteen hr later, the stomach was removed and incised along the greater curvature. Degree of ulceration in the forestomach was estimated according to the method of Takagi et al. (4) using the following scoring system: ulcers, 0.5–1 in diameter (mm), scored as 1; 1.1–3 as 3; 3.1–5 as 5 and >5 or perforation as 10. The ulcer index was obtained from the sum of the number of ulcers multiplied by the corresponding score.

b) Aspirin-induced gastric erosions: Male Donryu rats (Shizuoka Laboratory Animal Center, 245–295 g) were fasted for 24 hr. Four hr after the oral administration of 200 mg/kg aspirin, the stomach was removed and fixed by instilling 10 ml of 1% formalin solution. The stomach was incised along the greater curvature and examined for lesions in the glandular portion. The sum of the length (mm) of each erosion was used as an erosion index. Test drugs were administered orally 30 min before the treatment with aspirin.

c) Water immersion stress-induced gastric erosions: Male SD rats (Charles River Japan, 160–240 g) were fasted for 24 hr, and the stress erosion was induced according to the method of Takagi and Okabe (5). The animals were given the test drug orally 10 min before being placed in a stress cage and immersed vertically to the level of the xiphoid process in a water bath maintained at 24±0.5°C. Seven hr later, the stomach was removed and fixed by instilling 10 ml of 1% formalin solution. The stomach was incised along the greater curvature and examined for mucosal lesions. Severity of each erosion was scored as 0.25 for weak hyperemia, 0.5 for severe hyperemia or weak hemorrhage, 1 for hemorrhage, and 2 for severe hemorrhage.
Areas of mucosal damage were measured in mm² using a planimeter, multiplied by the corresponding score, and summed up to give an erosion index.

d) Cysteamine-induced duodenal ulcers: Female SD rats (Charles River Japan, 160–200 g) were fasted for 24 hr. Cysteamine (400 mg/kg) was given orally to the animals 1 hr before the administration of test drugs. Seventeen hr after cysteamine, the duodenum was excised. The severity of each ulceration was scored as an ulcer index according to the method of Groves et al. (6): normal mucosa scored as 0; reddening, irritation or sloughing of mucosa, as 1; superficial ulcer, as 2; deep ulcer, as 3; and perforating ulcer, as 4.

e) Ethanol-induced gastric mucosal lesions: Male SD rats (Charles River Japan, 150–210 g) were fasted for 48 hr and were deprived of water during the last 24 hr of fasting. Ethanol-induced lesions were produced according to the method of Nagashima et al. (7). Three hr after oral administration of 50% ethanol (8 ml/kg), the stomach was removed and fixed by instilling 10 ml of 1% formalin solution. The stomach was opened along the greater curvature and examined for the lesions in the glandular portion. The sum of the length (mm) of each lesion was used as a lesion index. Test drugs were given orally 1 hr before ethanol treatment. Indomethacin was administered subcutaneously 20 min before the dosing of TA-2711.

4) Gastric mucosal potential difference
Male SD rats (Charles River Japan, 300–400 g) were fasted for 24 hr. Test drugs were administered orally 30 min before the oral treatment with 8 ml/kg of 50% ethanol or deionized water. Thirty min after treatment with ethanol or deionized water, the animals were anesthetized with urethane (1.2 g/kg, i.p.) and surgically prepared for the measurement of gastric mucosal potential difference (PD) according to the method described by Tarnawski and Ivey (8).

Upon completion of the surgery, the gastric lumen was rinsed with warmed (37°C) physiological saline solution. After instillation of 2 ml of physiological saline into the stomach, recording of the PD was started, and each PD value was read at 90 min after ethanol or deionized water.

3. Statistical analysis
All the results except those of cysteamine-induced ulcers were expressed as the mean±S.E. Statistical analysis was performed by one-way analysis of variance followed by Bonferroni’s method or by Dunnett’s method. Data on pylorus-ligated ulcers and cysteamine-induced ulcers were analyzed by the Kruskal Wallis test. A ‘P’ value of less than 0.05 was regarded as significant.

Results
1. Effect on gastric secretion
As shown in Fig. 2, oral administration of TA-2711 at doses of 25 to 100 mg/kg reduced pepsin activity of gastric juice in a dose-related manner, while the volume of gastric juice was not changed significantly. Nearly complete loss in pepsin activity was observed at a dose of 100 mg/kg. TA-2711 at 100 mg/kg showed only a slight reduction of acid concentration. Oral carbenoxolone at a dose of 50 mg/kg or more also significantly reduced pepsin activity, but its effect was less potent than that of TA-2711. Carbenoxolone had no significant effect on acid concentration. Sucralfate (100 and 200 mg/kg, p.o.) did not have any significant effect on gastric secretion.

In contrast to the oral administration, intravenous dosing of TA-2711 (30 and 100 mg/kg) and carbenoxolone (30 mg/kg) did not reduce pepsin activity. Intravenous administration of cimetidine at a dose of 30 mg/kg significantly reduced acid concentration, while it produced no appreciable change in pepsin activity (Fig. 3).

2. Effect on pepsin activity of gastric juice in vitro
The addition of TA-2711 at 0.25 to 16 mg/ml to gastric juice concentration-dependently reduced the pepsin activity in the supernatant of the mixture and that of 16 mg/ml reduced it almost completely. Carbenoxolone also reduced pepsin activity at concentrations greater than 0.5 mg/ml. On the other hand, sucralfate caused only a slight reduction of the pepsin activity (Fig. 4).

3. Effect on experimental ulcers
a) Pylorus-ligated gastric ulcers: As shown in Fig. 5, orally administered TA-2711 at 50 to 200 mg/kg inhibited the ulceration: the inhi-
b) Aspirin-induced gastric erosions: Figure 6 shows that TA-2711 at oral doses of 25 to 100 mg/kg inhibited aspirin-induced erosions in a dose-related manner. Carbenoxolone inhibited the erosions at 100 mg/kg or more, but sucralfate at 50 to 200 mg/kg had no significant effects. Cimetidine (100 mg/kg) potently inhibited the erosions.

c) Water immersion stress-induced gastric erosions: TA-2711 as well as sucralfate at doses of 200 and 800 mg/kg, p.o., showed no significant effect on the development of stress-induced erosions (erosion index: control, 25.8±1.9; TA-2711 at 800 mg/kg, 20.3±1.8; sucralfate at 800 mg/kg, 21.5±1.7; n=8).
Fig. 4. Effect of TA-2711, carbenoxolone (CBX) and sucralfate (SCF) on pepsin activity of rat gastric juice in vitro. After incubation of the mixture of the test drug and gastric juice for 10 min at 30°C, the pepsin activity of the supernatant was determined. Each point represents the mean±S.E. (n=3).

Fig. 5. Effect of oral administration of TA-2711, carbenoxolone (CBX) and sucralfate (SCF) on pylorus-ligated gastric ulcers in rats. Test drugs were administered immediately after the ligation, and the stomach was removed 16 hr after the administration. Each column represents the mean±S.E. (n=6–8). *P<0.05.

Carbenoxolone at 200 mg/kg, p.o., showed a tendency to aggravate the erosions (erosion index: 32.4±3.8, n=8). On the other hand, cimetidine (100 mg/kg, p.o.) inhibited the erosions significantly (erosion index: 14.8±0.9, n=8).

d) Cysteamine-induced duodenal ulcers: As shown in Table 1, TA-2711 (100–800 mg/kg, p.o.) dose-relatedly reduced the severity of the ulceration induced by cysteamine, and the 800 mg/kg dose completely protected 7 of the 9 animals. Sucralfate at 400 mg/kg and cimetidine at 100 mg/kg also inhibited the ulcer formation. Carbenoxolone even at the high dose of 800 mg/kg did not show any significant effect.

e) Ethanol-induced gastric mucosal lesions: As shown in Fig. 7, oral administration of TA-2711 at doses of 6.3 to 100 mg/kg dose-dependently prevented the formation of gastric lesions induced by ethanol. Oral carbenoxolone (50–200 mg/kg) and sucralfate (50–200 mg/kg) also inhibited the mucosal lesions. Cimetidine at 100 mg/kg, p.o., caused only a slight inhibition.
Table 1. Effect of oral administration of TA-2711, carbenoxolone (CBX), cimetidine (CIM) and sucralfate (SCF) on cysteamine-induced duodenal ulcers in rats

<table>
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<th>Ulcer index</th>
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Test drugs were administered 1 hr after treatment of cysteamine (400 mg/kg, p.o.). Seventeen hr after cysteamine, the duodenum was removed. a: Number of observations. *P<0.05.

Fig. 7. Effect of oral administration of TA-2711, carbenoxolone (CBX), sucralfate (SCF) and cimetidine (CIM) on gastric lesions induced by ethanol in rats. Test drugs were given 1 hr before ethanol (50% v/v), and the stomach was removed 3 hr after ethanol. Each column represents the mean±S.E. (n=9–20). *P<0.05.

The intravenous administration of TA-2711 (10–100 mg/kg) 10 min prior to ethanol had no protective effect (lesion index: control, 57.8±3.1; TA-2711 at 100 mg/kg, 48.4±7.1; n=8).

Figure 8 shows that the inhibitory effect of TA-2711 at 100 mg/kg on ethanol-induced lesions was significantly suppressed by the pretreatment with indomethacin (10 mg/kg, s.c.). The effect of TA-2711 at 25 mg/kg was nullified by indomethacin. Indomethacin itself hardly affected the ethanol-induced lesions.

4. Effect on ethanol-induced fall in gastric mucosal PD

Oral administration of TA-2711, sucralfate and cimetidine at 100 mg/kg had no influence on gastric PD in normal rats (Fig. 9A), while oral treatment with ethanol lowered gastric PD by about 20 mV (Fig. 9B). The ethanol-induced fall in PD was significantly suppressed by the oral pretreatment of TA-2711 at 100 mg/kg (Fig. 9B). On the other hand, both sucralfate and cimetidine at 100 mg/kg, p.o., had no significant effect (Fig. 9B).

Discussion

The results obtained in the present study show that TA-2711 reduces the pepsin activity of gastric juice and inhibits the development of various types of experimental ulcers in rats.

TA-2711, when administered orally im-
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Fig. 8. Effect of TA-2711 on ethanol-induced gastric lesions in rats in the presence or absence of indomethacin. TA-2711 was given 1 hr before treatment with ethanol (50% v/v). Indomethacin (IND, 10 mg/kg) was given subcutaneously 20 min before oral administration of TA-2711. Three hr after ethanol, the stomach was removed. Each column represents the mean±S.E. (n=8-10). *P<0.05.

Oral TA-2711 exhibited a preventive effect on the development of lesions induced by pyloric ligation, aspirin and cysteamine. It has been suggested that acid and pepsin are important factors for the pathogenesis of these ulcerations (2, 9-11). TA-2711 has a potent reducing effect on gastric pepsin activity, while it causes only a weak inhibition on gastric acidity. Therefore, the anti-ulcer effect elicited by TA-2711 in these ulcer models may be ascribed to the reduction of pepsin activity.

On the other hand, oral TA-2711 prevented both ethanol-induced gastric mucosal lesions and ethanol-induced fall in gastric mucosal PD, which is known to be a good index of the integrity of the mucosal barrier (8, 12); and the effect of TA-2711 against ethanol-
induced lesions was suppressed by the pretreatment with indomethacin, a cyclooxygenase inhibitor. These results indicate that the effect of TA-2711 is mediated through an increase in mucosal prostaglandins which are known to play an important role in maintaining mucosal integrity (13) and to protect gastric mucosa against various damaging agents (14, 15). Thus, it is suggested that both gastric antipepsin activity and a mucosal prostaglandin-mediated process are involved in the anti-ulcer action of TA-2711.

The effect of TA-2711 on ethanol-induced lesions as well as that on the pepsin activity of gastric juice was produced by oral administration and was never produced by intravenous dosing. Therefore, it is likely that TA-2711 exerts its effects mainly by a local action. TA-2711 had no significant inhibitory effect on water-immersion stress-induced gastric erosions. The increase in acid secretion (16) and gastric motility (17) has been postulated to be involved in the pathogenesis of the mucosal damage. In addition to the weak effect of TA-2711 on gastric acidity, the possible prompt elimination of this drug from the stomach to the duodenum caused by the increased gastric motility during stressing (18) may explain the low efficacy of TA-2711 on the stress erosions. Sucralfate and carbenoxolone, both of which are known to act locally and have no substantial effect on acid secretion, also did not show any inhibitory effect on the development of the stress erosions, whereas cimetidine, a histamine H2 receptor antagonist, which inhibits gastric acid secretion, significantly prevented the development of erosions.

The pharmacological properties of TA-2711, which acts locally at the gastric lumen and exerts its anti-ulcer action through mechanisms other than gastric acid inhibition, are obviously different from those of cimetidine. Sucralfate caused no inhibition of gastric pepsin activity and had no preventive effect against aspirin-induced erosions, while TA-2711 did. The result of sucralfate in gastric pepsin activity was consistent with that reported by Okabe et al. (18). On the other hand, Okabe et al. (18) have reported that sucralfate inhibits aspirin-induced gastric erosions in pylorus-ligated rats. The discrepancy in results may be due to the difference in the experimental procedure. The effect of TA-2711 to reduce gastric pepsin activity was similar to that of carbenoxolone, but the anti-ulcer activity of TA-2711 was much more potent than that of carbenoxolone. In addition, TA-2711 markedly prevented the formation of gastric mucosal lesions and the fall in gastric mucosal PD induced by ethanol, while cimetidine did not show such a marked effect against ethanol. The preventive effect of TA-2711 against ethanol was more potent than that of sucralfate and carbenoxolone.

In conclusion, it is indicated that TA-2711, by acting locally, prevents the development of various types of experimental ulcers in rats. It is suggested that both gastric antipepsin activity and a mucosal prostaglandin-mediated process are involved in the anti-ulcer action of TA-2711.

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References

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