Effects of 12-Sulfodehydroabietic Acid Monosodium Salt (TA-2711), a New Anti-Ulcer Agent, on Gastric Mucosal Lesions Induced by Necrotizing Agents and Gastric Mucosal Defensive Factors in Rats

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Abstract—Effects of TA-2711 on gastric mucosal lesions induced by various necrotizing agents and several defensive factors of gastric mucosa were investigated in rats. Oral administration of TA-2711 at 12.5 to 200 mg/kg prevented the formation of gastric mucosal lesions induced by 99.5% ethanol, 0.6 N HCl, 0.2 N NaOH and boiling water with ED50 values of 24, 58, 16 and 101 mg/kg, respectively. Oral TA-2711 at 100 mg/kg increased the gastric mucosal prostaglandin E2 (PGE2) level without any change in transmucosal potential difference. A sustained decrease in gastric mucosal blood flow produced by intragastric administration of 99.5% ethanol was inhibited by oral TA-2711 (50, 100 mg/kg) and 16,16-dimethyl PGE2 (10 μg/kg). The effect of TA-2711 on ethanol-induced decrease in blood flow was suppressed by indomethacin (10 mg/kg, s.c.). Oral TA-2711 (25–100 mg/kg) dose-dependently increased the amount of mucus adherent to the gastric mucosa. In addition, gastric HCO3− secretion was increased by intragastric TA-2711 at 2.5 and 5.0 mg/ml. These results suggest that TA-2711 enhances gastric mucosal resistance by increasing mucus and HCO3− secretion and by maintaining mucosal blood flow, and protects the gastric mucosa against various irritants. The effects of TA-2711 appear to be mediated by mucosal prostaglandins such as PGE2.

A previous study has shown that TA-2711, 12-sulfodehydroabietic acid monosodium salt, acting locally at the upper gastrointestinal tract, has a potent gastric antipepsin activity and prevents the development of various types of experimental ulcers in rats (1). In addition, a mucosal prostaglandin-mediated process has been suggested to be involved in the anti-ulcer action of TA-2711 (1). Several prostaglandins are known to play an important role in the mucosal defense by enhancing such factors as mucus, alkaline secretion and mucosal blood flow and to protect the gastric mucosa against various irritants (2). The present study describes the effects of TA-2711 on the formation of gastric mucosal lesions induced by various necrotizing agents, the mucosal prostaglandin E2 (PGE2) level, and the gastric mucosal defensive factors such as mucus, alkaline secretion and blood flow in rats.

Materials and Methods

1. Animals
Male rats (Crj:CD (SD)) were used.

2. Drugs and reagents
TA-2711 and cetraxate were synthesized for this study at the Organic Chemistry Research Laboratory, Tanabe Seiyaku. Other drugs and chemicals used were sucralfate (Ulcerlmin®, Chugai Seiyaku), 16,16-dimethyl PGE2 (Funakoshi), indomethacin (Sigma), sodium lauryl sulfate (Tokyo Kasei), thiopental sodium (Ravonal®, Tanabe Seiyaku), urethane (Wako Pure Chemical) and alcian blue (Nacalai Tesque). An 125I-PGE2
radioimmunoassay (RIA) kit and \(^{3}\text{H}\)-PGE\(_2\) (New England Nuclear) were used for PGE\(_2\) determination.

In the oral experiments, test drugs were dissolved or suspended in deionized water or physiological saline solution, and given to animals in a volume of 2.5 or 5 ml/kg. In the intravenous experiments, TA-2711 was dissolved in 1 N NaOH, and the pH of the solution was adjusted to neutral. Then, the solution was diluted in physiological saline solution and administered in a volume of 1 ml/kg. Indomethacin was suspended in physiological saline containing 0.2% Tween 80 and administered subcutaneously in a volume of 2 ml/kg.

3. Methods

1) Gastric mucosal lesions induced by necrotizing agents: Rats weighing between 150 and 300 g were fasted for 48 hr and were deprived of water during the last 24 hr of fasting. Gastric mucosal lesions by 99.5% ethanol, 0.6 N HCl, 0.2 N NaOH and boiling water were produced according to the method of Robert et al. (3). Before the treatment of boiling water, the animals were anesthetized with thiopental (30 mg/kg, i.p.). One hour after the oral administration of 1 ml of each irritant, the stomach was removed. The stomach was fixed by instilling 10 ml of 1% formalin solution, opened along the greater curvature, and examined for the lesions in the glandular portion. The sum of the length (mm) of each lesion induced by ethanol, HCl and NaOH, and the sum of the area (mm\(^2\)) of each lesion induced by boiling water were used as a lesion index. Test drugs and the vehicle were given orally 30 min before the administration of the irritants. To obtain a dose-response (% protection against gastric mucosal lesions) relationship, 2 to 4 doses of a test drug were used. In the case of ethanol lesions, TA-2711 and the vehicle were given orally at various intervals before the administration of ethanol.

2) Measurement of gastric mucosal PGE\(_2\): Rats weighing between 160 and 215 g were fasted for 24 hr. The animals were sacrificed 1 hr after oral administration of the test drug. Immediately after the sacrifice, the stomach was removed, placed in ice-cold physiological saline solution, and cut open along the greater curvature. The gastric corpus wall was then placed between two glass slides and frozen by immersion in hexane cooled in a dry ice-ethanol bath. The gastric mucosa was separated from its underlying muscle layer by pulling the two glass slides apart. Extraction and separation of mucosal PGE\(_2\) were performed according to the method of Arakawa et al. (4). The frozen mucosa was homogenized in 2.5 ml of methanol containing 10\(^{-4}\) M indomethacin. The homogenate was filtered, and the filtrate was evaporated at 37°C. Then, 3 ml of CHCl\(_3\) and 5 ml of phosphate buffer (pH 8.0) was added to the residue. After mixing, the two phases were separated by centrifugation (3,000\(\times\)g for 10 min at 4°C). The aqueous layer was acidified to pH 3.5 with HCl, and PGE\(_2\) was extracted from the buffer with ethylacetate. PGE\(_2\) separated by thin-layer chromatography was measured by the \(^{125}\text{I}\)-PGE\(_2\) RIA kit.

3) Measurement of gastric mucosal potential difference (PD): Rats weighing between 370 and 435 g were fasted for 24 hr. The animals were anesthetized with urethane (1.2 g/kg, i.p.) and surgically prepared for the measurement of gastric PD according to the method described by Tarnawski and Ivey (5) using saturated KCl in 5% agar electrodes. Upon completion of the surgery, the gastric lumen was rinsed with warmed (37°C) physiological saline solution. PD measurements were first carried out in the group of animals after intragastric instillation of physiological saline solution to obtain baseline values and then after intragastric instillation of the test drug in physiological saline. Each PD value was read when the PD reached to the steady state level after the instillation.

4) Measurement of gastric mucosal blood flow: Rats weighing between 260 and 410 g were fasted for 24 hr and anesthetized with urethane (1.2 g/kg, i.p.). The measurement of gastric mucosal blood flow was made according to the method of Saita et al. (6) using a laser Doppler blood flowmeter (Laserflo, BPM403; TSI). The optical flow probe (endoscope type) was introduced into the stomach through a forestomach incision to be placed on the gastric mucosa along the greater curvature, and secured by ligation around the incision, and the probe was
mounted in a commercially available balancer (Physio-Tech). The output signal from the flowmeter was continuously recorded on a linearcorder (WR3701, Graphtech). The test drug or the vehicle (physiological saline solution) was administered intragastrically through an esophageal tube in a volume of 2.5 ml/kg. Ethanol (99.5%) in a volume of 5 ml/kg was instilled into the stomach 30 min after the test drug or the vehicle. Indomethacin was administered subcutaneously 30 min before the administration of TA-2711.

5) Determination of gastric mucus: Mucus adhering to the gastric mucosa was estimated according to the method of Corne and Woods. (7) Rats weighing between 160 and 250 g were fasted for 24 hr. The animals were sacrificed under ether anesthesia 1 hr after oral administration of the test drug. The stomach was removed, opened along the greater curvature, and rinsed in ice-cold 0.25 M sucrose.

After being weighed, the stomach was incubated in 10 ml of 0.1% alcian blue solution containing 0.15 M sucrose and 0.05 M sodium acetate (pH 5.8) for 1.5 hr at room temperature. After two successive washes in 0.25 M sucrose, alcian blue bound to mucus was eluted by immersion in 15 ml of 0.5 M MgCl₂ solution for 2 hr. The solution was shaken vigorously with 10 ml of ether, and the optical density of the aqueous phase was measured with a spectrophotometer (UV-150-02, Shimadzu) at 605 nm. Results were expressed as μg alcian blue/g tissue.

6) Determination of gastric alkaline secretion: The alkaline secretion was measured according to the method of Garner and Flemstrom (8). Rats weighing between 300 and 400 g were fasted for 24 hr and anesthetized with urethane (1.2 g/kg, i.p.). The abdomen was exposed through a midline incision and a silicone tube passed into the stomach through a cut in the duodenum. The tube was exteriorized via a stab wound in the right flank before the abdomen was closed. A polyethylene catheter was passed down the esophagus into the stomach, and the tip of the catheter was positioned just beyond the cardia. This catheter was held in place by ligation around the cardia and the esophagus in the neck region. The esophageal catheter and the silicone tube were connected with three way taps to form a closed circuit.

The following two solutions were used: a control solution of 150 mM NaCl adjusted to pH 2.0 with HCl (acid saline) and a test solution containing TA-2711 in acid saline. The stomach was instilled with 5.5 to 6.0 ml of each solution via the esophageal catheter every 20 min. The pH and P⁰C₀₂ of the recovered instillates were determined using a blood-gas analyzer (ABL 30, Radiometer), and total HCO₃⁻ output (μmoles/hr) was calculated.

4. Statistical analysis

ED₅₀ (doses protecting gastric mucosa against necrotizing agents by 50%) values with 95% confidence limits were estimated from the linear regression analysis of log dose on percent protection.

Statistical significance was determined by one-way analysis of variance followed by Bonferroni's method. Data on alkaline secretion and gastric PD were analyzed by the paired t-test. A 'P' value of less than 0.05 was regarded as significant.

Results

1. Protective effect against gastric mucosal lesions

Oral administration of TA-2711 at doses of 12.5 to 200 mg/kg prevented the formation of gastric mucosal lesions produced by ethanol, 0.6 N HCl, 0.2 N NaOH and boiling water in a dose-related manner. As summarized in Table 1, the doses of TA-2711 that produced 50% protection (ED₅₀) against ethanol, HCl, NaOH and boiling water were 24.0, 58.2, 16.4 and 101.1 mg/kg, respectively. The protective effect of TA-2711 against all of these irritants was more potent than that of cetraxate. Furthermore, the effect of TA-2711 against ethanol-, NaOH- and boiling water-induced gastric lesions was more potent than that of sucralfate. Both cetraxate and sucralfate up to 200 mg/kg provided no significant protection against boiling water-induced gastric lesions.

Figure 1 shows the time course of the protective effect of TA-2711 (25–200 mg/kg, p.o.) against ethanol-induced gastric lesions. TA-2711 had a rapid onset of action, with a maximal effect attained 30 min after administration at all doses. At the highest dose of 200
Table 1. ED\textsubscript{50} values of oral TA-2711, cetraxate and sucralfate for protecting gastric mucosa against ethanol (99.5%), HCl (0.6 N), NaOH (0.2 N) and boiling water in rats

<table>
<thead>
<tr>
<th>Irritants</th>
<th>TA-2711 (mg/kg, p.o.)</th>
<th>Cetraxate (mg/kg, p.o.)</th>
<th>Sucralfate (mg/kg, p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>24.0 (11.3–39.7)</td>
<td>160.8 (104.0–300.2)</td>
<td>121.6 (84.8–314.7)</td>
</tr>
<tr>
<td>HCl</td>
<td>58.2 (23.3–127.5)</td>
<td>112.5 (62.1–221.2)</td>
<td>33.5 (23.6–51.8)</td>
</tr>
<tr>
<td>NaOH</td>
<td>16.4 (8.3–28.3)</td>
<td>110.9 (54.4–148.2)</td>
<td>109.5 (36.6–442.1)</td>
</tr>
<tr>
<td>Boiling water</td>
<td>101.1 (33.7–291.8)</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

Each irritant (1 ml/rat) was given orally 30 min after the test drugs. The stomach was removed 1 hr after the irritant. Six to 14 animals were used for each dose.

mg/kg group, significant protection was sustained for 5 hr after dosing.

2. Effect on gastric mucosal PGE\textsubscript{2} and PD: TA-2711 at an oral dose of 100 mg/kg significantly increased gastric mucosal PGE\textsubscript{2} level (Table 2A), whereas this drug up to 200 mg/kg hardly affected the gastric mucosal PD (Table 2B). Oral sodium lauryl sulfate at 25 mg/kg increased the mucosal PGE\textsubscript{2} level, and the effect was almost equal to that of TA-2711 (Table 2A). On the other hand, sodium lauryl sulfate at 25 mg/kg caused a marked drop in gastric PD unlike TA-2711 (Table 2B).

3. Effect on gastric mucosal defensive factors

1) Gastric mucosal blood flow: Oral administration of TA-2711 (100 mg/kg) and 16,16-dimethyl PGE\textsubscript{2} (10 \mu g/kg) had no significant effect on the basal blood flow of the gastric mucosa until 90 min after dosing in anesthetized rats (data not shown). Indomethacin (10 mg/kg, s.c.) also showed no significant effect on the gastric blood flow. As shown in Fig. 2, intragastric application of 99.5% ethanol produced a sustained decrease in gastric mucosal blood flow. TA-2711, given orally at 50 mg/kg 30 min before ethanol, prevented the decrease in blood flow induced by ethanol; and rather, at the higher dose of 100 mg/kg, the blood flow increased after ethanol. Oral 16,16-dimethyl PGE\textsubscript{2} at 10...
Table 2. Effect of TA-2711 and sodium lauryl sulfate on gastric mucosal PGE₂ level and gastric PD in rats

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Dose mg/kg, p.o.</th>
<th>n</th>
<th>PGE₂ ng/g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>31.2±9.2</td>
</tr>
<tr>
<td>TA-2711</td>
<td>100</td>
<td>6</td>
<td>109.6±15.0*</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>25</td>
<td>6</td>
<td>101.0±26.1*</td>
</tr>
</tbody>
</table>

A: Gastric mucosal PGE₂ level in rats. The stomach was removed 1 hr after oral administration of test drugs. *P<0.05, compared with control. B: Gastric mucosal PD in anesthetized rats. Physiological saline solution (0.15 M NaCl) or the test drug was instilled into the stomach in a volume of 5 ml/kg. *P<0.05, compared with before.

Fig. 3. Effect of TA-2711 (100 mg/kg, p.o.) on ethanol-induced decrease in gastric mucosal blood flow with or without indomethacin (IND: 10 mg/kg, s.c.) in anesthetized rats. IND was administered 30 min before TA-2711. The gastric mucosal blood flow (GMBF) immediately before ethanol (99.5%, 5 ml/kg) treatment was designated as 100%. Each point represents the mean±S.E. (n=6). *P<0.05, compared with the control; #P<0.05, compared with TA-2711.

2) Amount of mucus adherent to the gastric mucosa: As shown in Fig. 4, TA-2711, 1 hr after oral administration at 25 to 100 mg/kg, dose-dependently increased the amount of gastric mucus, and the rate of increase at 100 mg/kg was 53%. Cetraxate at 100 mg/kg, p.o., also increased gastric mucus 1 hr after dosing. TA-2711 tended to increase the mucus level 3 hr after dosing, but the increase was not statistically significant.

Intravenous administration of TA-2711 at 30 and 100 mg/kg did not influence gastric mucus determined 30 min after the administration (data not shown).

3) Alkaline secretion: As shown in Fig. 5, intragastric instillation of TA-2711 at concentrations of 2.5 and 5.0 mg/ml significantly increased the secretion of HCO₃⁻. The increase persisted during the presence of TA-2711 in the gastric lumen.

Discussion

TA-2711, given orally, prevented the gastric mucosal lesions produced by a variety of necrotizing agents such as ethanol, a strong acid, a strong base and boiling water. ED₅₀ values of TA-2711 against different irritants
Fig. 4. Effect of TA-2711 and cetraxate on the amount of mucus adherent to the gastric mucosa in rats. The stomach was removed 1, 3 and 7 hr after oral administration of test drugs, and the amount of mucus was measured by alcian blue binding to the glandular portion. Each column represents the mean±S.E. (n=10-14). *P<0.05, compared with control.

Fig. 5. Effect of TA-2711 on gastric $\text{HCO}_3^-$ secretion in anesthetized rats. Horizontal bar indicates the duration of intragastric application of TA-2711. Each point represents the mean±S.E. (n=5-6). *P<0.05, compared with the value immediately before the application of TA-2711.

ranged from 16 to 100 mg/kg, depending on the irritant. On the whole, the protective effect of TA-2711 against these necrotizing agents was more potent than that of cetraxate and sucralfate, both of which are known to exhibit gastric mucosal protective activity (9, 10). The mode of the protective property of TA-2711 against different necrotizing agents was similar to that of PGE$_2$ reported by Robert et al. (3). A previous study has suggested that the anti-ulcer activity of TA-2711 partly depends on endogenous prostaglandins (1). In the present study, it was found that TA-2711, at a protective dose of 100 mg/kg, p.o., increases PGE$_2$ content in the gastric mucosa. Accordingly, the mucosal protective effect of TA-2711 against different irritants may be mediated by mucosal prostaglandins such as PGE$_2$.

TA-2711 has been indicated to exert its effects mainly by a local action (1). In the present study, TA-2711 had a rapid onset of protective action against ethanol lesions, with a maximal effect attained 30 min after oral dosing. The result may further support the local property of this drug.

It has been reported that so-called mild irritants protect gastric mucosa against strong
irritants through the increased synthesis of mucosal prostaglandins (11, 12). Mild irritants such as 10–20% ethanol, 0.15–0.35 N HCl, 0.5–1 M NaCl and 20 mM taurocholic acid are known to cause both an increase in gastric mucosal prostaglandins and a slight damage to the gastric mucosa detected by the fall in transmucosal PD in rats (13–17). In the present study, it was found that TA-2711, at a mucosal protective dose, increased the gastric mucosal PGE2 level, but did not produce any change in transmucosal PD. Sodium lauryl sulfate, a detergent, increased the mucosal PGE2 to a level comparable to that by TA-2711, but it caused a marked fall in transmucosal PD. In addition, oral dosing of TA-2711 even at a high dose of 1,000 mg/kg did not produce any damage to the gastric mucosa in rats (Y. Onoda et al., unpublished observation). Therefore, it seems that the property of TA-2711 is different from that of these mild irritants.

TA-2711, as well as 16,16-dimethyl PGE2, prevented the ethanol-induced decrease in gastric mucosal blood flow, and the effect of TA-2711 was suppressed by the pretreatment with indomethacin. Recently it has been pointed out that the disturbance of the mucosal microcirculation appears to be an important early step in ethanol-induced gastric lesion formation (11), and the maintenance of mucosal blood flow by endogenous or exogenous prostaglandins plays a major role in the prostaglandin protective mechanism (19, 20). Therefore, it is likely that the protective effect of TA-2711 against ethanol-induced gastric lesions is ascribed to the maintenance of mucosal blood flow mediated by endogenous prostaglandins. In the present study, 16,16-dimethyl PGE2 as well as TA-2711 did not augment the basal gastric mucosal blood flow. Similar results with 16,16-dimethyl PGE2 in anesthetized rats have been reported by Leung et al. (19) and Piham et al. (21). On the other hand, in the group pretreated with TA-2711 at 100 mg/kg, the mucosal blood flow turned into an increase after ethanol challenge. Production of a great amount of vasodilating prostaglandins in the gastric mucosa might explain the mechanism of the increase in mucosal blood flow after ethanol in TA-2711-treated rats, because the increase was inhibited by the pretreatment with indomethacin.

TA-2711, given orally, increased the amount of mucus adherent to the gastric mucosa, and the effect was almost equal to that of cetraxate, which has been reported to have stimulating effects on the gastric mucus production (22). Furthermore, TA-2711 was found to have a stimulating effect on gastric mucosal HCO3− secretion by intragastric instillation in situ. As exogenous or endogenous prostaglandins have been known to stimulate secretion of gastric mucus and alkali (2), it seems likely that these effects of TA-2711 are also mediated by endogenous prostaglandins. In addition, antipepsin activity of TA-2711 (1) might contribute to the increase in mucus gel, since pepsin is physiologically involved in the degradation of the mucus layer (23). The increasing effect of TA-2711 on gastric mucus and alkaline secretion may enhance mucosal protection, particularly against the damaging effect of gastric acid and pepsin (24).

These results suggest that TA-2711 enhances mucosal defense by increasing mucus gel and alkaline secretion and maintaining mucosal blood flow, and protects the gastric mucosa against various stimuli. It is considered that the effects of this drug are mediated by mucosal prostaglandins such as PGE2.

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References


