Effect of DS-4574, a Novel Peptidoleukotriene Antagonist with Mast Cell Stabilizing Action, on Acute Gastric Lesions and Gastric Secretion in Rats

Yoshiaki Tabuchi and Yoichi Kurebayashi

Exploratory Research Laboratories III, Daiichi Pharmaceutical Co., Ltd., 16-13, Kiukasai 1-chome, Edogawa-ku, Tokyo 134, Japan

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ABSTRACT—DS-4574 is a peptidoleukotriene antagonist with mast cell stabilizing activity. In the present study, we studied the effects of this compound on gastric secretion and various acute gastric lesions in rats. Intraduodenal administration of DS-4574 at doses of 5 to 10 mg/kg significantly and dose-dependently inhibited gastric acid secretion in pylorus-ligated rats, but a further increase in the dose up to 50 mg/kg did not cause any further inhibition. Shay ulceration in response to pylorus ligation was dose-dependently prevented by DS-4574 (10–25 mg/kg, i.d.). Water-immersion restraint stress- and aspirin-induced gastric ulcers were also significantly prevented in a dose-related manner by oral pre-treatment with DS-4574 (10–50 mg/kg). The lower doses of DS-4574 (1–10 mg/kg, p.o.) significantly and dose-dependently protected the gastric mucosa against the necrotizing action of either absolute ethanol or concentrated hydrochloric acid, indicating that this compound possesses a potent gastroprotective activity. These antulcer and gastric protective effects of DS-4574 were more potent than those of cimetidine used as a reference drug. These findings suggest that DS-4574 is useful for peptic ulcer therapy, as well as for the therapy of various allergic diseases, including asthma.

Keywords: DS-4574, Cimetidine, Gastric ulcer, Gastric acid secretion

DS-4574 (6-(2-cyclohexylethyl)-[1,3,4]thiadiazolo[3,2-a]-1,2,3-triazolo[4,5-d]pyrimidin-9(3H)-one) is a newly synthesized compound which was found to possess both peptidoleukotriene antagonism and mast cell stabilizing activity during a drug screening program in our laboratory (1, 2). Available data have shown that DS-4574 effectively inhibits various types of experimental asthma models through its dual actions, indicating that this compound is useful for the therapy of allergic airway diseases (2). Recently, leukotrienes, especially peptidoleukotrienes, have been supposed to be related to the pathogenesis of peptic ulcer diseases as proulcerogenic factors because of their adverse effects on gastric pepsin secretion, motility, circulation and transgastric potential difference (3). In view of these evidence together with the well-established pathological roles of histamine in the gastric lesion formation (4), it is conceivable that DS-4574 may offer therapeutic benefits in the treatment of peptic ulcer diseases. In the present study, we examined the effects of this compound on gastric secretion and various experimental acute gastric lesions in rats, and compared them with those of cimetidine, a representative histamine H2-antagonist used in clinical practice.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley (Japan SLC, Shizuoka, Japan) and male Donryu (Nihon Rat Co., Saitama, Japan) rats, 5- to 10-week-old (150–320 g), were used in the present experiments. The animals were housed in raised mesh-bottom cages to prevent coprophagy, and they were maintained on standard laboratory chow (F-2, Funabashi Farm, Chiba, Japan) and tap water ad libitum. Before the experiments, they were deprived of food but allowed free access to water, unless otherwise stated.

Drugs

DS-4574 and a reference drug, cimetidine, were synthesized in our laboratory. They were suspended in
0.5% carboxymethylcellulose solution, and administered orally or intraduodenally. The injection volume was kept constant at 5 ml/kg of body weight, and an equal volume of the vehicle was administered to the control rats.

**Induction of Shay ulcer**

Donryu rats, 6-week-old, were fasted for 48 hr before the experiments. Under light ether anesthesia, the animals were laparotomized, and the pylorus was ligated according to the method of Shay et al. (5). The drugs were injected intraduodenally immediately after the pylorus ligation. Ten hours later, they were sacrificed by an overdose of ether and their stomachs were removed, cut along the greater curvature and put on a filter paper. Each sample was examined for the presence of forestomach ulceration and the severity of the injury was graded into degrees as an ulcer index as follows: 0: no lesion, 1: hemorrhagic suffusion, 2: 1-5 small ulcers (>3 mm diameter), 3: more than 5 ulcers or 1 ulcer of marked size, 4: many ulcers of marked size, 5: perforated ulcer.

**Induction of water-immersion restraint stress-induced ulcer**

Water-immersion restraint stress ulcer was induced according to the method of Takagi and Okabe (6). In brief, nonfasted Donryu rats (10-week-old) were placed in the individual restraint cage and immersed vertically to the level of the xiphoid process in a water bath maintained at 21°C. Seven hours after the stress imposition, they were sacrificed by an overdose of ether, and the gastric mucosal lesions were evaluated. The length (mm) of each necrotic lesion was measured and summed per stomach. The sum was used as an ulcer index.

**Induction of aspirin-induced ulcer**

Donryu rats (6-week-old) were starved overnight and treated with the drugs orally at 30 min before an oral administration of 300 mg/kg of aspirin (Ebisu, Osaka, Japan). Five hours after administration of aspirin, the gastric mucosal lesions were evaluated as described above.

**Induction of ethanol- and HCl-induced ulcers**

Sprague-Dawley rats (6-week-old) were fasted overnight before the experiments. Either absolute ethanol or 0.6 N HCl was administered orally in a volume of 1 ml, and the animals were sacrificed 1 hr later. The drugs were given orally at 30 min before the ulcer induction. The ulcer index was evaluated as described above.

**Gastric secretion study**

Donryu rats (6-week-old), fasted overnight before use, were anesthetized with ether, and a midline laparotomy was made. Subsequently, the pylorus was ligated by the method of Shay et al. (5), and the drugs were injected intraduodenally immediately after the pylorus ligation. Four hours later, the stomachs were removed, and the gastric contents were collected. The samples were centrifuged at 3,000 rpm for 20 min, and the volume of the supernatant was measured. The acidity was titrated with 0.1 N NaOH to pH 7.0 by an autoburette (HSM-10A, Toa Electronics, Tokyo, Japan).

**Statistical analyses**

The data are reported as the means ± S.E. of from 8 to 10 rats per group. Statistical analyses for parametrical data were carried by Dunnett's multiple comparison test for unpaired variables, and P-values less than 0.05 were regarded to indicate a significant difference. For non-parametrical data, the U-test of Mann-Whitney was employed, and statistical significance was evaluated as described above.

**RESULTS**

**Effect of DS-4574 on Shay ulcer**

As shown in Table 1, DS-4574 (10–50 mg/kg, i.d.) dose-dependently inhibited Shay ulcers induced by pylorus ligation. The protective effect of DS-4574 was statistically significant at doses above 25 mg/kg, the inhibition of ulceration being 68% (P < 0.01) at the highest dose (50 mg/kg). Cimetidine also suppressed the ulcerative response to pylorus ligation at intraduodenal doses of 50 and 100 mg/kg.

**Effect of DS-4574 on water-immersion restraint stress-induced ulcer**

Pretreatment of rats with DS-4574, at oral doses ranging from 10 to 50 mg/kg, significantly prevented gastric mucosal lesions induced by 7-hr water-immersion restraint stress (Table 2). The antulcer activity of DS-4574 increased with its doses, and the inhibition at the highest dose was 80% (P < 0.01). Orally administered cimetidine at doses of 100 and 200 mg/kg also significantly inhibited the development of stress-induced gastric lesions in a dose-dependent manner.
Effect of DS-4574 on aspirin-induced ulceration

Table 3 summarizes the inhibitory action of DS-4574 on aspirin-induced gastric ulcers. Oral administration of DS-4574 in a dose range of 10 to 50 mg/kg dose-dependently prevented gastric mucosal ulceration in response to application of 300 mg/kg of aspirin (p.o.), and the inhibition by this compound was statistically significant at doses above 25 mg/kg. Also, oral pretreatment with cimetidine at doses of 50 and 100 mg/kg significantly and dose-dependently inhibited aspirin-induced gastric erosions.

Each drug was administered intraduodenally immediately after the pylorus ligation. Rats were sacrificed 10 hr after the pylorus ligation. Each figure represents the mean ± S.E. *: P < 0.05, **: P < 0.01 vs. control.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>N Ulcer index (score)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>8</td>
<td>4.0 ± 0.4</td>
</tr>
</tbody>
</table>
| DS-4574  | 10           | 8                     | 2.5 ± 0.4       | 38
|          | 25           | 8                     | 1.5 ± 0.4**     | 63
|          | 50           | 8                     | 1.3 ± 0.3**     | 68
| Cimetidine | 50          | 8                     | 2.4 ± 0.4*      | 40
|          | 100          | 8                     | 1.0 ± 0.3**     | 75

Effect of DS-4574 on ethanol-induced ulcer

As shown in Table 4, gastric mucosal hemorrhagic necrosis induced by an oral instillation of absolute ethanol was significantly inhibited by pretreatment with DS-4574 at all doses tested (from 1 to 30 mg/kg, p.o.), and the inhibition by this compound was statistically significant at doses above 25 mg/kg. Also, oral pretreatment with cimetidine at doses of 50 and 100 mg/kg significantly and dose-dependently inhibited aspirin-induced gastric erosions.

Effect of DS-4574 on HC1-induced ulcer

As summarized in Table 5, pretreatment with DS-4574 at all doses tested (from 1 to 30 mg/kg, p.o.) significantly protected the gastric mucosa against the damaging action of 0.6 N HC1. The inhibition by this compound of macroscopic mucosal necrosis was statistically significant at doses above 3 mg/kg, and practically complete at doses of 10 mg/kg and higher. In contrast, cimetidine showed no protective effect even at a dose of 100 mg/kg (p.o.).
Effect of DS-4574 on gastric secretion

The effects of DS-4574 and cimetidine on gastric secretion in pylorus-ligated rats are shown in Table 6. Intraduodenal injection of DS-4574 in a dose range of 5 to 50 mg/kg resulted in dose-related and significant reductions in both the volume of gastric juice and acid output, but the pH value and acidity of the juice were hardly affected by this agent even at the highest dose (50 mg/kg). On the other hand, treatment with cimetidine at intraduodenal doses of 50 and 100 mg/kg significantly decreased the volume, acid output and acidity of the gastric juice and increased the pH value.

DISCUSSION

DS-4574 is, at least, a dually effective compound, being a competitive specific peptidoleukotriene antagonist and an inhibitor of mast cell mediator release. In isolated guinea pig ileum, DS-4574 antagonized the contraction induced by LTC₄, LTD₄ and LTE₄ with IC₅₀ values of 3.5 × 10⁻⁷, 2.0 × 10⁻⁷ and 2.6 × 10⁻⁷ M, respectively (1). In passive cutaneous anaphylaxis, this compound given intravenously and orally induced dose-dependent inhibition with ID₅₀ values of 0.55 and 2.8 mg/kg, respectively (2). On the other hand, the high safety of this compound was inferred from the results of various toxicity studies (LD₅₀ value in rats: >5,000 mg/kg) (M. Kato et al., unpublished data).

The results of the present study clearly show that DS-4574 possesses potent antiulcer and antisecretory activities in rats. In pylorus-ligated rats, intraduodenal treatment with DS-4574 affected neither the pH nor the acidity of gastric juice, but significantly decreased the secretion volume, thereby resulting in a significant reduction in the acid output. In contrast, cimetidine increased the pH and reduced the volume, acidity and acid output. The mode of the antisecretory action of DS-4574 is thus apparently different from that of cimetidine whose action depends upon the histamine H₂-receptor antagonist activity (7). It has been reported that a high concentration of DS-4574 (10⁻⁴ M) caused only 20 and 18% inhibition of the ileal submaximal contraction induced by acetylcholine (3 × 10⁻⁴ M) and histamine (2 × 10⁻⁷ M), respectively (1). Therefore, the blockade of cholinergic or histaminergic receptor is an unlikely mechanism for the antisecretory action of this compound. In a separate rat study, DS-4574 (50 mg/kg, i.d.) was found to significantly suppress both carbachol- and pentagastrin-induced gastric hyperacidity, but did not affect the response to histamine (8). In the separate study, DS-4574 dose-dependently inhibited histamine release into the stomach juice of pylorus-ligated rats stimulated by pentagastrin or carbachol (8). Furthermore, a representative peptidoleukotriene antagonist (FPL-55712) has been reported to lack an inhibitory effect on gastric acid secretion in rats (9). It is, therefore, conceivable that the suppressed gastric secretion after DS-4574 treatment is mediated by the inhibition of endogenous histamine release from histamine-storing cells such as mast cells and enterochromaffin-like (ECL) cells in the stomach, as has been proposed for other mast cell stabilizers such as disodium cromoglycate and FPL-52694 (10, 11).

Gastric acid plays a crucial role in the gastric ulcerative response to pylorus ligation, water-immersion restraint stress or aspirin injection. Actually, histamine H₂-blockers (12), cholinolytics (7) or vagotomy (13) are capable of inhibiting these ulcers. The ability of DS-4574 to inhibit acid secretion contributes, at least partly, to its antiulcer activity. The magnitude of reduction in acid output in response to DS-4574 reached a plateau level at 10 mg/kg (i.d.), whereas the antilesion activ-

Table 6. Effects of DS-4574 and cimetidine on gastric secretion in pylorus-ligated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Volume (ml/100 g b.w.) (%)</th>
<th>pH</th>
<th>Acidity (mEq/l) (%)</th>
<th>Acid output (μEq/100 g b.w.) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>3.3 ± 0.4</td>
<td>1.3 ± 0.0</td>
<td>89 ± 3</td>
<td>296 ± 39</td>
</tr>
<tr>
<td>DS-4574</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>8</td>
<td>2.5 ± 0.5</td>
<td>1.3 ± 0.1</td>
<td>85 ± 5</td>
<td>217 ± 48</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>8</td>
<td>1.7 ± 0.2***</td>
<td>1.4 ± 0.1</td>
<td>74 ± 4</td>
<td>129 ± 16**</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>8</td>
<td>1.7 ± 0.2***</td>
<td>1.3 ± 0.0</td>
<td>75 ± 3</td>
<td>130 ± 18**</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>8</td>
<td>2.0 ± 0.3*</td>
<td>1.3 ± 0.0</td>
<td>76 ± 6</td>
<td>158 ± 35*</td>
</tr>
<tr>
<td>Cimetidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>8</td>
<td>1.8 ± 0.3***</td>
<td>1.7 ± 0.1</td>
<td>53 ± 3**</td>
<td>101 ± 22**</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>8</td>
<td>1.9 ± 0.3***</td>
<td>1.9 ± 0.3*</td>
<td>54 ± 7**</td>
<td>112 ± 24**</td>
</tr>
</tbody>
</table>

Each drug was administered intraduodenally immediately after the pylorus ligation. Four hours later, the animals were sacrificed, and the gastric juice was collected. Each figure represents the mean ± S.E. *: P < 0.05, **: P < 0.01 vs. control. *: Inhibition %.
ities of this compound to pylorus ligation, water-immersion restraint stress and aspirin increased with its doses up to 25 and 50 mg/kg, respectively, by either the oral or intraduodenal route. Therefore, other mechanisms independent of an antisecretory property are also involved in the antiulcer activity of DS-4574.

Our data also show that pretreatment of rats with DS-4574 strongly protected the gastric mucosa against the necrotizing insults of hydrochloric acid and ethanol. Such protection has been demonstrated after administration of prostaglandins, and is generally designated gastric "cytoprotection" which differs certainly from the antiulcerogenic action associated with inhibition of gastric acid secretion (14). The present gastroprotective activity of DS-4574 against the necrotizing insults seems to be independent of its antisecretory action because a large protection was observed at the dose of 1 mg/kg which hardly affected acid secretion, and the potency of antilesion activity at the maximally-effective antisecretory dose (10 mg/kg) was much greater than that of the antisecretory activity. Furthermore, in a separate study, we confirmed that pretreatment with indomethacin (5 mg/kg, s.c.) did not affect the protective effect of DS-4574 against ethanol-induced injury (Y. Tabuchi et al., unpublished observation), indicating that the protection mechanism by this compound is not due to a stimulation of endogenous prostaglandin synthesis by DS-4574.

Evidence has accumulated indicating the pathological role of mucosal mast cell degranulation in gastric ulcer formation. Several mast cell stabilizing agents effectively prevented stress-induced gastric ulceration (11, 15). An ulcerogenic dose of aspirin induced mucosal mast cell degranulation with a concomitant reduction in gastric mucosal content of histamine (16). Furthermore, a significant amount of histamine is released in the gastric mucosa after damage with concentrated ethanol (17), and mepyramine, a representative histamine H1-receptor antagonist, significantly suppressed ethanol-induced gastric hyperemia (18). The gastric mucosal protection by meciadanol (19) and zinc sulfate (17) against ethanol injury was suggested to be due to their inhibitory effects on histamine release from mucosal mast cells. These facts suggest that mast cell stabilizing activity is an important factor in understanding the mechanisms by which DS-4574 exerted its antiulcer and gastroprotective activities in rats.

On the other hand, recent attention has been focused on the role of lipoygenase products, especially peptido-leukotrienes, in the pathogenesis of gastrointestinal injury. Although this problem is still controversial (20–22), it has been demonstrated that a considerable amount of leukotriene C4 is generated from the gastric tissue in various pathological conditions (9, 21), and that peptido-leukotrienes act as pro-ulcerogenic factors through their unfavorable effects on the gastric mucosal circulation, transgastric potential difference, pepsin secretion and motility (3). Several peptido-leukotriene antagonists and 5-lipoxygenase inhibitors were shown to possess antiulcer and gastroprotective activities in rats (9, 18, 23). It is thus likely that the peptido-leukotriene antagonist activity of DS-4574 is also responsible, at least in part, for both its antiulcer and gastroprotective activities.

In conclusion, the present results demonstrate that DS-4574 possesses potent antisecretory, antiulcer and gastroprotective activities. These are due to combined effects of peptido-leukotriene antagonism, mast cell stabilizing and undetermined potencies of the compound. The antiulcer and gastroprotective effects of DS-4574 are comparable to or more potent than those of cimetidine, suggesting the possible therapeutic benefits of this compound as a new type of antiulcer drug.

REFERENCES
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