Effect of OPC-12759, a Novel Antiulcer Agent, on Chronic and Acute Experimental Gastric Ulcer, and Gastric Secretion in Rats

Katsuya YAMASAKI, Hironobu ISHIYAMA, Takashi IMAIZUMI, Toshimi KANBE and Youichi YABUUCHI

2nd Tokushima Institute of New Drug Research, 'Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima 771-01, Japan

Accepted December 13, 1988

Abstract—The antiulcer effects of OPC-12759, a novel antiulcer agent were compared with those of cetraxate in various experimental ulcer models and on gastric secretion in rats. OPC-12759 (0.3–30 mg/kg, b.i.d., p.o.) significantly accelerated the healing rate of acetic acid-induced gastric ulcer in a dose-dependent manner, while cetraxate did not. When administered orally at 0.3–30 mg/kg, b.i.d., for 7 days, pretreatment with OPC-12759 (0.3–30 mg/kg, b.i.d., p.o.) prevented the formation of acute gastric ulcers, induced by: restraint water immersion stress, aspirin, indomethacin, histamine, serotonin, platelet activating factor (PAF) and DDC. Cetraxate showed antiulcer activity against a part of the OPC-12759-positive gastric ulcer models. Given intraperitoneally at the single dosing range of 10–100 mg/kg, OPC-12759 inhibited the formation of these acute gastric ulcer models. OPC-12759 administered orally at 0.3–30 mg/kg, b.i.d., for 7 days did not inhibit basal gastric secretion in pylorus ligated rats. The results indicated that OPC-12759 possesses wide spectrum antiulcer activity as compared with cetraxate.

Materials and Methods

Animals

Male Wistar or Wistar/ST rats weighing between 150–250 g were used as the experi-
mental animal. The rats were housed in cages with mesh bottoms to prevent coprophagia and were kept in a 12L:12D light regimen at 24±1 °C. The animals were fed on a standard diet and allowed access to water ad libitum. Prior to the induction of gastric mucosal lesions or the determination of gastric secretion, the animals were fasted, but allowed water, for a 24 or 48 hr period.

Production of acetic acid-induced gastric ulcer

Acetic acid-induced gastric ulcers were produced according to the method of Okabe and Pfeiffer (5). The animals were anesthetized with ether and a subserosal injection of 20 µl 30% acetic acid was made to the border of the corpus and antrum in the ventral wall. The test drugs were given orally, twice daily, for the first 8 days and once on the 9th day, following production of the ulcer. Four hours after the final administration of the test drug, the rats were sacrificed and their stomachs removed.

Production of acute gastric ulcer

The different models of acute gastric ulcer were produced as follows:

Restraint water immersion stress-induced gastric ulcer: Rats were restrained in stainless steel cages and immersed up to their xiphoid in a water bath maintained at 23±1 °C, according to the method of Takagi et al. (6). After 7 hr of this exposure, the rats were sacrificed.

Aspirin-induced gastric ulcer: Aspirin suspended in 0.5% carboxymethylcellulose sodium salt (CMC) was given as a single oral dose of 200 mg/kg. The rats were sacrificed 5 hr after aspirin dosing.

Indomethacin-induced gastric ulcer: Indomethacin dissolved in 3% NaHCO₃ was administered in a single oral dose of 30 mg/kg. The rats were sacrificed 5 hr after indomethacin dosing.

Histamine-induced gastric ulcer: Histamine dihydrochloride dissolved in physiological saline was given intraperitoneally at a dose of 300 mg/kg. Five hours later, the rats were sacrificed.

Serotonin-induced gastric ulcer: Serotonin creatinine sulfate dissolved in physiological saline was injected subcutaneously at a dose of 30 mg/kg. Five hours after administration, the rats were sacrificed.

PAF-induced gastric lesion: PAF dissolved in saline was administered intravenously at a dose of 5µg/kg. The rats were sacrificed 1 hr after PAF injection.

DDC-induced gastric antral ulcer (7): The rats were anesthetized with ether, a midline laparotomy was made and the pylorus ligated. Diethyldithiocarbamate (DDC) dissolved in saline was given subcutaneously at a dose of 800 mg/kg, and a 1 ml oral dose of 0.1 N HCl was given to each rat. The rats were sacrificed 7 hours after the treatment with DDC.

Fixation and evaluation of gastric mucosal damage: Stomachs removed were fixed for 10 min in 3% neutral buffered formalin. The stomach was cut along the greater curvature and the gastric mucosal surface was examined by a rater, unaware of the experimental protocol, for evidence of ulcer and/or necrotic and hemorrhagic lesions. The area (in the case of acetic acid-, serotonin- and DDC-induced gastric lesion) or the length (in the case of stress-, aspirin-, indomethacin- and histamine-induced gastric lesion) of the damage in each animal was summed planimetrically, and the total was used as the ulcer or lesion index. In the case of PAF-induced lesion, the extent of the lesion area on the gastric mucosa was measured with a computer aided digital image processing system (LA-500, PIAS Japan). The damaged area was expressed as the percentage of the surface epithelium showing gross damage on macroscopic inspection of the whole corpus mucosa.

Administration of test compounds: In the oral study, OPC-12759 and cetraxate were administered twice daily (9:00 and 17:00) for 7 days. Thirty minutes after the final dose on day 8, each ulcerogenic procedure was performed. In the intraperitoneal study, the test compound was administered in a single dose 30 minutes before ulcerogenic treatment, and in the case of DDC-induced gastric antral ulcer, the compounds were given twice, once immediately after the ligation of the pylorus and again 3 hr later.

Determination of gastric secretion

Pylorus ligated rats: Laparotomy was followed by ligation of the pylorus under ether anesthesia, and the abdomen was then sutured. The gastric contents were collected
5 hours after pylorus ligation; and the volume, pH, total acidity and pepsin activity were determined. Total acidity was estimated by titration of gastric contents with 0.01 N NaOH using an automatic titrator (Radiometer) to an endpoint pH of 7.0. Pepsin activity was determined by a modification of the method of Anson (8). OPC-12759 and cetraxate were given orally, b.i.d., for 7 days and once at 30 min prior to pylorus ligation on day 8.

**Stomach perfused rats:** An in vivo system for stomach perfusion in rats was prepared according to the method of Ghosh and Schild (9). The stomach was perfused continuously with saline containing 0.25 mM NaOH, at a rate of 1 ml/min, using a peristatic pump. The perfusate was passed over a glass electrode pH meter which recorded the pH on a chart recorder. Histamine (1 mg/kg/hr), tetragastrin (8 µg/kg/hr) or carbachol (10 µg/kg/hr) dissolved in saline were infused intravenously throughout the experiment as a secretagogue. In the preliminary experiment, it was confirmed that this dose of each secretagogue was the supramaximal one for induction of gastric acid output in this stomach perfusion method. After confirmation of maximal and stable acid production by each secretagogue, the test drug was injected intraperitoneally.

**Drugs**

OPC-12759 was synthesized by Otsuka Pharmaceutical Co., Ltd. Other drugs used were as follows: aspirin (Nakarai Chemical Co., Ltd.); indomethacin and platelet activating factor (PAF) (Sigma Chemical Co., St. Louis, MO, U.S.A.); serotonin creatinine sulfate, histamine dihydrochloride, and diethylthiocarbamate (DDC) (Wako Pure Chemical Co., Ltd.); tetragastrin (San-a Pharmaceutical Co., Ltd.); carbachol (Tokyo Chem. Ind. Co., Ltd.); and cetraxate (Neuer, Daiichi Pharmaceutical Co., Ltd.).

OPC-12759 suspended in 0.5% carboxymethylcellulose sodium salt (CMC) and cetraxate suspended in saline were given in a volume of 4 ml/kg body weight. The control animals received 0.5% CMC or saline.

**Statistical analysis**

The healing ratio of acetic acid-induced gastric ulcer in the drug treated groups was calculated as follows:

\[
\text{Healing ratio (\%)} = \frac{\mu g \text{ ulcer index of control group} - \mu g \text{ ulcer index of drug treated group}}{\mu g \text{ ulcer index of control group}} \times 100
\]

The percent inhibition of acute gastric ulcer models in the drug treated group was calculated as follows:

\[
\text{Percent inhibition (\%)} = \frac{\mu g \text{ ulcer index of control group} - \mu g \text{ ulcer index of drug treated group}}{\mu g \text{ ulcer index of control group}} \times 100
\]

The statistical evaluations for multiple group comparison of parametric data were done by analysis of variance followed by Dunnett's test. A difference of P<0.05 is regarded as significant.

**Results**

**Effect on the healing process of chronic gastric ulcer:** OPC-12759 given orally at doses

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Ulcer index (mm²)</th>
<th>Healing ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>16</td>
<td>9.7±0.6</td>
<td>—</td>
</tr>
<tr>
<td>OPC-12759</td>
<td>0.3</td>
<td>16</td>
<td>7.4±0.9</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16</td>
<td>7.2±0.9</td>
<td>25.8</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>16</td>
<td>5.5±0.5*</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>16</td>
<td>6.8±0.5*</td>
<td>29.9</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>16</td>
<td>7.7±0.5</td>
<td>—</td>
</tr>
<tr>
<td>Cetraxate</td>
<td>50</td>
<td>16</td>
<td>8.4±0.7</td>
<td>—</td>
</tr>
</tbody>
</table>

OPC-12759 or cetraxate was administered orally to the rats twice daily (9:00 and 17:00) for 8 consecutive days from the day following the formation of acetic acid-ulcer. The rats were sacrificed 4 hr after the final dose at 9:00 on day 9, and the stomach was removed. Each of the data represents the percent healing of the control healing. *P<0.05.
Table 2. Effect of OPC-12759 and cetraxate on the formation of various acute gastric ulcer models in rats

<table>
<thead>
<tr>
<th>Model</th>
<th>Route</th>
<th>OPC-12759 Dose (mg/kg)</th>
<th>% Inhibition</th>
<th>Cetraxate Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.3</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Stress</td>
<td>p.o.</td>
<td>4.0</td>
<td>14.5</td>
<td>-6.4</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>51.0*</td>
<td>57.2*</td>
<td>62.1*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>i.p.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>p.o.</td>
<td>40.4</td>
<td>51.2</td>
<td>21.9</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>21.2</td>
<td>1.3</td>
<td>22.3</td>
</tr>
<tr>
<td>Histamine</td>
<td>p.o.</td>
<td>9.4</td>
<td>-5.3</td>
<td>48.8*</td>
</tr>
<tr>
<td>Serotonin</td>
<td>i.p.</td>
<td>20.9</td>
<td>41.8</td>
<td>1.6</td>
</tr>
<tr>
<td>PAF</td>
<td>i.p.</td>
<td>-8.1</td>
<td>57.0</td>
<td>80.2*</td>
</tr>
<tr>
<td>DDC</td>
<td>p.o.</td>
<td>37.9</td>
<td>41.4</td>
<td>65.5*</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>8.0</td>
<td>60.0</td>
<td>68.0*</td>
</tr>
</tbody>
</table>

In the oral study, OPC-12759 and cetraxate were administered twice daily (9:00 and 17:00) for one week. Thirty minutes after the final dose on day 8, each ulcerogenic procedure was performed, as described in the text. In the intraperitoneal study, the test compound was administered in a single dose 30 minutes before ulcerogenic treatment. PAF: platelet activating factor, DDC: diethyldithiocarbamate. Significant differences from the control were obtained at *: P<0.05 by Dunnett’s test, N=7–8.

Table 3. Effect of OPC-12759 and cetraxate on gastric secretion in pylorus ligated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Volume (ml/5 hr)</th>
<th>pH</th>
<th>T.A. (µEq/ml)</th>
<th>A.O. (µEq/5 hr)</th>
<th>P.A. (mgTyr/ml)</th>
<th>P.O. (mgTyr/5 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.3</td>
<td>7</td>
<td>6.9±1.4</td>
<td>1.38±0.06</td>
<td>75.6±5.4</td>
<td>557±140</td>
<td>11.8±1.1</td>
<td>85.7±19.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
<td>7.2±0.7</td>
<td>1.25±0.02</td>
<td>85.3±5.0</td>
<td>628±88</td>
<td>11.5±0.5</td>
<td>83.6±9.5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>7</td>
<td>7.6±1.2</td>
<td>1.29±0.03</td>
<td>80.3±7.0</td>
<td>634±126</td>
<td>9.6±0.5</td>
<td>73.7±12.8</td>
</tr>
<tr>
<td>OPC-12759</td>
<td>100</td>
<td>7</td>
<td>7.1±0.9</td>
<td>1.23±0.02</td>
<td>88.0±4.8</td>
<td>708±135</td>
<td>11.2±0.6</td>
<td>87.7±14.2</td>
</tr>
<tr>
<td>Cetraxate</td>
<td>7</td>
<td>100</td>
<td>7.1±0.9</td>
<td>1.23±0.02</td>
<td>87.6±5.0</td>
<td>635±100</td>
<td>12.1±0.4</td>
<td>86.8±10.6</td>
</tr>
</tbody>
</table>

OPC-12759 and cetraxate were given orally, b.i.d., for 7 days and once at 30 min prior to pylorus ligation on day 8. Gastric contents were collected 5 hr after the ligation. T.A.: total acidity, A.O.: acid output, P.A.: pepsin activity, P.O.: pepsin output.
Table 4. Effect of OPC-12759 on acid secretion induced by various secretagogues in stomach perfused rat

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Histamine (1 mg/kg/hr, i.v.)</th>
<th>Tetragastrin (8 μg/kg/hr, i.v.)</th>
<th>Carbachol (10 μg/kg/hr i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>pH of perfusate</td>
<td>N</td>
<td>pH of perfusate</td>
</tr>
<tr>
<td>Pre-value</td>
<td>5</td>
<td>5.99±0.11</td>
<td>5</td>
<td>5.97±0.17</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>3.46±0.07</td>
<td>5</td>
<td>3.65±0.16</td>
</tr>
<tr>
<td>OPC-12759</td>
<td>10</td>
<td>3.58±0.09</td>
<td>5</td>
<td>3.35±0.12</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.61±0.13</td>
<td>5</td>
<td>3.45±0.07</td>
</tr>
</tbody>
</table>

Acid production was induced by constant intravenous infusion of histamine (1 mg/kg/hr), tetragastrin (8 μg/kg/hr) or carbachol (10 μg/kg/hr), respectively. a: pH of stomach perfusate before each secretagogue stimulation. b: pH of stomach perfusate after the treatment with each secretagogue.

Effect of OPC-12759 on Experimental Gastric Ulcer

OPC-12759 had no significant effect on gastric basal secretions in pylorus ligated rats in terms of volume, acid output and pepsin output. Similarly, cetrazate at 100 mg/kg p.o., b.i.d., for 7 days also had little effect on these parameters (Table 3).

In the stomach perfused rats, there was a decrease in the pH of the perfusate triggered by the infusion of histamine (1 mg/kg/hr, i.v.), tetragastrin (8 μg/kg/hr, i.v.) or carbachol (10 μg/kg/hr, i.v.). OPC-12759 at doses of 1, 10 and 100 mg/kg, i.p. did not significantly affect the pH (Table 4).

Discussion

The present results show that OPC-12759 has a significant promoting effect on the healing of pre-existing gastric ulcer in the rat. Additionally, pretreatment with OPC-12759 has been demonstrated to have a significant protective effect against the formation of variety of experimentally induced gastric ulcers. This protective effect has been shown not to be associated with an inhibition in either basal or stimulated gastric secretion.

The mechanism of genesis of peptic ulcer is generally accepted to be related to a correlation of defensive factors and aggressive factors, as proposed in the balance theory of Shay and Sun (10). Although the mode of antiulcer action of OPC-12759 has, as yet, not been fully elucidated, it has previously been demonstrated to act as a stimulant of the biosynthesis of PGE₂-like substances in the rat gastric mucosa (2). There are several reports on the relationship between PGs and the healing of acetic acid-induced ulcer stating that exogenous PGs have a trophic effect on gastric ulcer production.
effect on the gastric mucosa (11, 12). Aspirin, which has been demonstrated to suppress PG biosynthesis (13), has also been shown to inhibit the healing of acetic acid-induced ulcer (14), suggesting that endogenous PGs are deeply involved in the healing of these ulcers. Consequently, the action of OPC-12759 in increasing endogenous PG content (2) may be responsible for its accelerated ulcer healing effect. Results of gastric quantitative studies of endogenous PG content performed with regard to peptic ulcer patients are in conflict. The gastric mucosal PG level in patients with gastric ulcer was lower than the normal volunteers (15, 16). There was no difference between the levels found in the patients with duodenal ulcer and those of the normal volunteers (16–18). These findings raise the possibility that OPC-12759, in causing an increment in gastric mucosal PG content, may prove to be a useful anti-gastric ulcer agent. The effective oral dose of OPC-12759 against acute gastric ulcer was similar for all the experimental models used in this study, and it was equivalent to the effective oral dose for chronic gastric ulcer. This may indicate that the preventive effect of OPC-12759 on acute gastric ulcer formation may be due to the same mechanism by which the therapeutic effect of chronic ulcer is accelerated.

The pathogenesis of experimental models of acute gastric ulcer have been generally accepted to be as follows. The restraint water immersion stress ulcer develops as the results of: vagus nerve excitement which increases gastric secretion (19) and gastric motility (20), the diminution of gastric mucus (21), and the alteration in the microcirculation of the gastric mucosa (22). Aspirin- and indomethacin-induced gastric ulcers are caused by suppression of prostaglandin biosynthesis (23, 24) and disruption of the gastric mucosal barrier (24, 25). Histamine-induced gastric ulcer develops as a result of a marked vascular disturbances in the gastric wall (26). Therefore, both a decrease in defensive factors and/or an increase in aggressive factors are involved in the pathogenesis of the ulcer models described. OPC-12759, when administered intraperitoneally at doses of 10, 30 and 100 mg/kg, had little effect on the reduction of the pH of gastric perfusate induced by the secretagogues histamine, carbachol or tetragastrin in stomach perfused rats, thus suggesting that proamipide does not have H2-antihistamine, antimuscarine or antigastrin receptor activity. Oral administration of OPC-12759 at 0.3, 3 and 30 mg/kg, b.i.d. for 7 days in pylorus ligated rats also had no significant effect on gastric secretion. From this, it is suggested that the antiulcer activity of OPC-12759 is the result of the stimulation of gastric mucosal defensive mechanisms, rather than the suppression of aggressive mechanisms.

Cetraxate, used as the reference drug in this study, has been shown to increase mucosal blood flow (4). While cetraxate had antiulcer activity against only a few gastric ulcer models, OPC-12759 at low doses demonstrated an antiulcer activity equal to or more potent than cetraxate for a greater number of ulcer models. This broader range of activity suggests that mechanisms other than increased mucosal blood flow may also be involved in the antiulcer effect of OPC-12759.

The increased PGE2 levels observed in the gastric mucosa after treatment with OPC-12759 (2) might explain its antiulcer effect against stress or chemically induced gastric ulcer models, as used in this study. However, it has been reported that exogenous PGE2 and dmPGE2 when given orally or intravenously failed to prevent PAF-induced gastric lesions, and yet several doses of them caused an increase in PAF-induced gastric damage (27). This suggests that some mechanisms other than stimulation of prostaglandin synthesis may be involved.

It is unlikely that gastric acid secretion is involved in the pathogenesis of the serotonin-induced gastric lesion (28). In this case, the lesion develops as a result of the marked effect of serotonin on the peripheral circulation (29). Platelet aggregation followed by the formation of fibrinous thrombi has been observed in the injured mucosal area in the serotonin injected rats (29). Recently PAF was identified by Rosam et al. (30) as a novel mediator in the pathogenesis of gastrointestinal damage, and it has been reported that PAF produces superoxide anion radicals
in guinea pig polymorphonuclear leucocytes (31). DDC, which chelates Cu** from the superoxide dismutase (SOD) protein which scavenges the superoxide anion (32), decreased endogenous SOD activity in gastric mucosa at 800 mg/kg, s.c. (33). OPC-12759 significantly inhibited gastric mucosal lesions caused by the increase in free radicals following treatment with PAF or DDC. Cetraxate, however, failed to prevent the formation of these lesions. Although the mechanism of action of OPC-12759 on gastric ulcers induced by increased O2- is still unclear, its ability to reduce the formation of lesions caused by PAF and DDC suggests that it may, at least in part, have a unique mode of action by reducing the activity of superoxide radicals. In conclusion, OPC-12759 has been shown to be equally effective in both the healing of acetic acid-induced chronic ulcer and the prevention of the formation of a number of models of acute gastric ulcer. OPC-12759 did not inhibit the gastric acid secretion at the anti-ulcer dose. The results of the present study suggest that the antifuler activity of OPC-12759 may be partly related to an augmenting effect on the gastric mucosal defense mechanisms.

References
1 Uchida, M., Tabusa, F., Komatsu, M., Morita, S., Kanbe, T. and Nakagawa, K.: Studies on 2(1H)-quinolinone derivatives as gastric antulcer active agents. 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid and related compounds. Chem. Pharm. Bull. (Tokyo) 33, 3775-3786 (1985)
5 Okabe, S. and Pfeiffer, C.J.: Chronicity of acetic acid ulcer in the rat stomach. Dig. Dis. 17, 619-628 (1972)
17 Konturek, S.J., Obstulowicz, W., Sito, E., Oleksy, J., Wilkon, S. and Dembinska-Kiec, A.: Distribution of prostaglandins in gastric and duodenal mucosa of healthy subjects and


20 Garrick, T., Leung, F.W., Buack, S., Hirabayashi, K. and Guth, P.H.: Gastric motility is stimulated but overall blood flow is unaffected during cold restraint in the rat. Gastroenterology 91, 141–149 (1986)


