Effects of YJA20379-4 on Gastric Secretion, *Helicobacter pylori* Growth and Various Gastric and Duodenal Lesions in Rats

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Effects of a newly synthesized antiulcer agent, YJA20379-4, on gastric proton pump (H⁺/K⁺-ATPase) activity, *Helicobacter pylori* (H. pylori) growth, gastric acid secretion, and gastro-duodenal lesions, were examined in comparison with those of omeprazole. YJA20379-4 markedly inhibited the H⁺/K⁺-ATPase activity in a concentration-dependent manner and the inhibitory effect was increased under a weak acidic condition; the IC₅₀ values were 32 and 81 μM at pH 6.4 and 7.4, respectively. The inhibition was completely antagonized by 0.5 mM dithiothreitol (DTT). In addition, YJA20379-4 showed a significant anti-*H. pylori* activity determined by the agar dilution method. The value of minimum inhibitory concentration (MIC, 3.9—11.7 μg/ml) was at least 3 times more potent than that of omeprazole. In pylorus ligated rats, YJA20379-4 inhibited basal gastric acid secretion when administered by the intraduodenal route (ED₅₀, 23.6 mg/kg). In experimental ulcer models, YJA20379-4 administered by the oral route dose-dependently prevented the development of gastro-duodenal lesions in rats. Moreover, repeated administration of YJA20379-4 promoted the healing of gastric ulcers induced by acetic acid. On the basis of the data obtained, it is suggested that YJA20379-4 has a wide spectrum of antiulcer activities, and its mode of antiulcer actions is dependent on the inhibition of H⁺/K⁺-ATPase activity and *H. pylori* growth and the enhancement of a mucosal defense. Thus, YJA20379-4 might prove to be a beneficial therapy for gastritis and peptic ulcer diseases.

**Key words** YJA20379-4; proton pump inhibitor; *Helicobacter pylori*; acid secretion; gastro-duodenal lesion

It is generally accepted that peptic ulcers are caused by an imbalance between two sets of conditions: the presence of acid/pepsin output and the presence of predisposing factors, collectively thought of as a reduction of the mucosal defense. Consequently, antiulcer therapy has been mainly directed toward these two factors. But, besides those two factors, it has recently been found that *Helicobacter pylori* (*H. pylori*) can also play a significant role in the pathogenesis of peptic ulcers. Therefore, current therapy continues to have, as one of its major goals, the control of *H. pylori* as well as H⁺/K⁺-ATPase, acid secretion and a subsequent reversal of mucosal damage and inflammation.

Omeprazole, a H⁺/K⁺-ATPase inhibitor (proton pump inhibitor), is believed to be a drug that possesses all of the above properties. Despite these advantages, however, omeprazole has a significant drawback. That is, omeprazole covalently binds with cystein residues in H⁺/K⁺-ATPase and reacts irreversibly so that parietal cell function is not recovered within its life cycle. The development of a reversible proton pump inhibitor, therefore, which overcomes the side effects associated with the irreversibility of omeprazole is highly desirable, and at present, studies of the reversibility of YJA20379-4 are under investigation.

In this study, the effects of YJA20379-4, a newly synthesized benzimidazole derivative, were investigated on H⁺/K⁺-ATPase and *H. pylori* growth. Its effects on gastric acid secretion, antiulcer and cytoprotection were also examined and compared with those of omeprazole.

**MATERIALS AND METHODS**

**Test Compounds** YJA20379-4, 2-[3-(2,3-dihydro-1H- pyrrolo[1,2-a]benzimidazolyl)sulfinyl]-5-methyl-1H-benzimidazole and omeprazole (Fig. 1) were synthesized by Yung-Jin Pharmaceutical Company. For the *in vitro* studies, test-compounds were dissolved in dimethyl sulfoxide (DMSO) at an appropriate concentration and then diluted with 10 mM imidazole buffer (pH 6.4 or 7.4) for H⁺/K⁺-ATPase activity or with distilled water for the MIC test. For the *in vivo* studies involving oral administration, these compounds were suspended in 1% carboxymethyl cellulose sodium salt solution.

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**Fig. 1. Chemical Structures of YJA20379-4 and Omeprazole**

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(CMC, w/v). In the measurement of gastric acid secretion involving intraduodenal administration, these compounds were suspended in 1% CMC containing 0.2% NaHCO₃ (w/v) and the pH was adjusted to 9.0 with 2 N NaOH.

**Preparation of Rabbit Gastric Membranes Enriched in H⁺/K⁺-ATPase** Gastric H⁺/K⁺-ATPase was purified from the parietal cell-rich fraction of rabbit stomach as described by Saccomani et al.⁶ The stomach of New Zealand white rabbit (2—3 kg) was dissected out and washed quickly in tap water. The fundic mucosal surface was immersed with 1 M NaCl, and most of the surface epithelial cells were removed with a slide glass. The fundic mucosa was removed from the underlying muscular layer and homogenized in 10 volumes of an ice-cold solution of Tris–HCl/sucrose buffer (20 mM/250 mM, pH 7.4) by a Teflon-glass homogenizer. The resulting homogenates were centrifuged at 12000 rpm for 10 min and the supernatant was collected. The remaining pellets were resuspended in 2 volumes of homogenate buffer and centrifuged again under the same conditions. The resulting supernatants of the two centrifugations were combined and recentrifuged at 27000 rpm for 60 min. The crude microsomal pellets were resuspended in 250 mM sucrose and layered over 7% Ficoll (w/w) in 250 mM sucrose. Centrifugation was carried out in a Sorvall F-28/36 rotor centrifuge (Du Pont, U.S.A.) at 25000 rpm for 2 hr. The light microsomal bands at the interface between the 250 mM sucrose and Ficoll were carefully collected. The vesicle preparations were stored at −80°C until use.

Protein concentration was determined by the Lowry method using bovine serum albumin as a standard.

**Assay Procedures** Rabbit gastric H⁺/K⁺-ATPase activity was determined as described by Saccomani et al.⁶ The enzyme protein (80 μg) was preincubated at 37°C for 30 min in a medium consisting of 10 mM imidazole buffer (pH 6.4 or 7.4) and various concentrations of YJA20379-4 or omeprazole (final volume of 0.5 mL). Following preincubation, the enzyme reaction was started by adding 0.5 mL of a mixture containing 4 mM MgCl₂, 4 mM ATP, and 80 mM imidazole buffer (pH 7.4), with or without 10 mM KCL. The reaction was stopped after 15 min by the addition of 1 mL of ice-cold 24% trichloroacetic acid. Inorganic phosphate formed from ATP hydrolysis was determined by the method of Fiske and Subbarow.⁶

**Effect of Dithiothreitol (DTT) on the Inhibition** Gastric H⁺/K⁺-ATPase was preincubated in 10 mM imidazole buffer (pH 7.4) containing various concentrations of both DTT (0—0.5 mM) and YJA20379-4 (100 μM) for 30 min at 37°C, and the assay for H⁺/K⁺-ATPase was begun.

**Bacterial Strains and Growth Conditions** H. pylori NCTC 11637 (type strain), 11638, 12385 and 11916 were purchased from the National Collection of Type Cultures. Clinical isolates were kindly provided by Dr. Young-Chil Ha (Seoul National University, Seoul, Korea). The identification of H. pylori strains was based on standard biochemical tests.⁷ Frozen stocks were prepared from overnight cultures of H. pylori grown in brucella broth (BBL, U.S.A.) containing 5% fetal bovine serum (FBS), with shaking at 130 rpm on a rotary shaker (Jeio Tech) in a sealed jar (Forma Scientific, Inc.) under microaerobic conditions. H. pylori from this overnight culture was stored in a deep freezer with 15% glycerol and 10% FBS.

A fresh culture was prepared by directly plating the frozen stock on blood agar base #2. Plates were incubated for 4—5 d at 37°C in a microaerobic atmosphere. Colonies collected from the plates were incubated into 250 mL brucella broth supplemented with 5% FBS in a 1000 mL flask for culturing H. pylori in large quantities. The culture flasks were incubated with shaking at 130 rpm in a microaerobic atmosphere for 3 d. Purity control was carried out at each stage of growth by gram staining, hippurate hydrolysis, nitrate and nitrite reduction, urease, alkaline phosphatase and catalase test.

**Determination of MICs by the Agar Dilution Method** H. pylori strains were grown on blood agar base #2 supplemented with 5% FBS at 37°C for 3—5 d under microaerobic conditions, and suspended in brucella broth with 5% FBS to give the turbidity equivalent to McFarland standard no. 0.5; this resulted in suspensions containing approximately 5 × 10⁸ CFU/mL. The bacterial suspensions were applied to blood agar base #2 plates containing two-fold serial dilutions of antiulcer agents by a multi-point inoculator capable of delivering 1 μL samples. The plates were incubated at 37°C in microaerobic environments. After 3 d of incubation, there was satisfactory growth of all strains tested on the control plates. Readings were performed after 3 d unless otherwise specified. MICs were defined as the lowest concentrations of the test compounds inhibiting visible bacterial growth.

**Measurement of Basal Gastric Acid Secretion** Male Sprague-Dawley rats (180—220 g) were fasted but allowed free access to water for 24 hr prior to the experiment. Under ether anesthesia, the abdomen was incised and the pylorus ligated. Test compounds (YJA20379-4: 3, 10, and 30 mg/kg or omeprazole: 3, 6, and 12 mg/kg) or vehicle were intraduodenally administered immediately after the ligation. Four hours after the pylorus ligation, the rats were sacrificed by cervical dislocation. The gastric contents were collected and analyzed for volume and acidity. Acidity was determined against 0.01 N NaOH to pH 7.0.

**Water-Immersion Stress-Induced Gastric Lesions** Male Sprague-Dawley rats (180—200 g) were deprived of food but allowed free access to water for 24 hr before the experiments. The rats were placed in a restraint cage, then immersed vertically to the level of the xiphoid process in a water bath (21—23°C) for 7 hr, then sacrificed.⁸ The stomach of each rat was removed and inflated by injecting 10 mL of 3% formalin to fix the inner and outer layers of the gastric wall for 10 min. This formalin treatment was performed in all of the following experiments. Subsequently, the stomach was incised along the greater curvature and examined for lesions in the glandular portion. Test compounds (YJA20379-4 or omeprazole: 3, 10 and 30 mg/kg) or vehicle were administered orally at 30 min before the stress load.

**Indomethacin-Induced Gastric Lesions** Female SpragueDawley rats (160—180 g) were deprived of food but allowed free access to water for 48 hr before the experiment. Indomethacin (Sigma, St. Louis, MO, U.S.A.), suspended in Tween-saline (one or two drops), was given subcutaneously in a dose of 35 mg/kg to rats.⁹ The rats were sacrificed 7 hr later, and the stomach removed and examined for lesions in the glandular portion. Test compounds (YJA20379-4 or omeprazole: 1, 3 and 10 mg/kg) or vehicle were administered orally 30 min before the indomethacin treatment.

**Ethanol-Induced Gastric Lesions** Male Sprague-Daw-
ley rats (180—200 g) were deprived of food but allowed free access to water for 24 h before the experiment. The rats received 1 ml of absolute ethanol by oral gavage. One and half hours after the ethanol treatment the rats were killed and the stomach examined for lesions in the glandular portion. Test compounds (YJA20379-4 or omeprazole: 3, 10 and 30 mg/kg) or vehicle were administered orally 30 min before the ethanol treatment.

**Mepirizole-Induced Duodenal Ulcers** Male Sprague-Dawley rats (180—200 g) were used. Mepirizole (Sigma, St. Louis, MO, U.S.A.), suspended in 1% CMC was administered orally at 200 mg/kg to rats, which were then deprived of food and water. The rats were sacrificed 24 h later and examined for ulcers in the duodenum. Test compounds (YJA20379-4: 3, 10, and 30 mg/kg or omeprazole: 1, 3 and 10 mg/kg) or vehicle were administered orally 30 min before the mepirizole treatment.

**Acetic Acid-Induced Gastric Ulcers** Male Sprague-Dawley rats (200—220 g) were used. In producing gastric ulcers, animals were fasted for 5 h before the injection of acetic acid into the submucosal layer. In ether anesthetized rats, the abdomen was incised and the anterior portion of the stomach exposed. Then, 0.02 ml of 30% acetic acid (v/v) was injected into the submucosal layer at the junction of the fundus and antrum, about 1 cm proximal to the pylorus. Postoperatively, the animals were maintained on rat chow and water ad libitum. Test compounds (YJA20379-4 or omeprazole: 10, 30 and 100 mg/kg) or vehicle were orally administered twice daily (9:00 AM, 6:00 PM) commencing the day after surgery for 8 consecutive days to rats with gastric ulcers. The rats were sacrificed 16 h after the final administration of drugs, and the stomachs examined for ulcers.

**Ulc er or Lesion Index** The length (mm) of each lesion induced by water-immersion stress, indomethacin or ethanol was measured macroscopically and summed per stomach, and the total was used as the lesion index. The areas (mm²) of the mepirizole-induced duodenal ulcers and acetic acid-induced gastric ulcers were also measured and summed per stomach, and the total was used as the ulcer index.

**Statistics** All data are expressed as the mean±S.E. A Duncan Multiple Range test was used to determine the statistical significance of the data at the levels of p<0.05 and p<0.01. ED₅₀ values (the doses that inhibit gastric acid and prevent the formation of the gastric and duodenal lesions by 50%) were calculated by the probit method.

**RESULTS**

**Effects on the Activity of H⁺/K⁺-ATPase** Both YJA20379-4 and omeprazole caused a concentration-dependent inhibition of H⁺/K⁺-ATPase activity in isolated rabbit gastric mucosa (Fig. 2). The concentrations of YJA20379-4 which inhibited 50% (IC₅₀) of H⁺/K⁺-ATPase activity in the presence of 10 mM KCl were 32 and 81 μM at pH 6.4 and 7.4, respectively. Those of omeprazole were 25 and 93 μM at pH 6.4 and 7.4, respectively.

**Effect of DTT on the Inhibition** The inhibitory effect of both YJA20379-4 and omeprazole on gastric H⁺/K⁺-ATPase activity was prevented by simultaneous incubation with DTT. DTT almost completely abolished the effect of YJA20379-4 and omeprazole at a concentration of 0.5 mM (Fig. 3).

**Characteristics of H. pylori Strains** All of the bacteria were confirmed to be Gram (−), slender curved or spiral rods (data not shown). As shown in Table 1, all of the 5 isolates tested were found to share identical characteristics with those found in 4 reference strains, including the nomenclature type strain NCTC 11637. They failed to grow aerobically, but did grow microaerobically. They were found positive for catalase, alkaline phosphatase, and urease, but reduced neither nitrate nor nitrite. All of the strains tested were negative for hippurate hydrolysis.

**Activities of Antiulcer Agents against H. pylori** The
Table 1. Biochemical Characteristics of 9 Strains of *H. pylori*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NCTC 11637</th>
<th>11638</th>
<th>11916</th>
<th>12385</th>
<th>Clinical isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic growth</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microaerobic growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Nitrate reduction</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrite reduction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*a* Type strain, +: positive, -: negative.

Table 2. Activities of Various Antiulcer Agents against *H. pylori* Strains

<table>
<thead>
<tr>
<th>Agents</th>
<th>MIC (µg/ml)<em>a</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>1.9—62.5</td>
</tr>
<tr>
<td>YJA20379-4</td>
<td>1.9—62.5</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.00025—0.64</td>
</tr>
</tbody>
</table>

*a* MICs were determined by the agar dilution method on brucella agar.

Table 3. Comparison of ED<sub>50</sub> Values of YJA20379-4 and Omeprazole in Antisecretory and Antiulcer Activities

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (mg/kg)*b</th>
<th>YJA20379-4</th>
<th>Omeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pylorus-ligated rats (Acute-gastric lesion)</td>
<td>7</td>
<td>23.6</td>
<td>2.4</td>
</tr>
<tr>
<td>Water-immersion stress</td>
<td>8</td>
<td>8.1</td>
<td>4.4</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>7</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Ethanol (Acute duodenal ulcer)</td>
<td>7</td>
<td>14.9</td>
<td>17.1</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>8</td>
<td>14.4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*a* The ED<sub>50</sub> values were calculated from the dose-inhibition relationships for 7 to 8 rats by the probit method.

The data represent the mean±S.E. for 7 rats. *p<0.05, **p<0.01, vs. the values for the vehicle-treated control group.

![Inhibitory Effects of YJA20379-4 and Omeprazole on Gastric Acid Secretion in Pylorus-Ligated Rats](chart)

MICs of 2 antiulcer agents and amoxicillin for 9 strains of *H. pylori* were determined by the agar dilution method. Table 2 shows the ranges of MICs and the concentrations required to inhibit 50 and 90% of the strains, which were determined after 3 d of incubation. All of the plates for the MIC tests were further incubated for 2 additional days, but there was no change in the MICs.

Amoxicillin was active against all of the strains tested and the two proton pump inhibitors, YJA20379-4 and omeprazole, also showed considerable activities. The activity of YJA20379-4 was at least 3 times more potent than that of omeprazole.

Effects on Gastric Acid Secretion Basal gastric acid secretion in pylorus-ligated rats was 3.1±1.3 mEq/kg/4 h (mean±S.E.). YJA20379-4 (3—30 mg/kg) dose-dependently inhibited basal acid secretion in pylorus-ligated rats by intraduodenal treatment (Fig. 4). The ED<sub>50</sub> value was 23.6 mg/kg (Table 3). Omeprazole inhibited basal gastric acid secretion with an ED<sub>50</sub> value of 2.4 mg/kg.

**Water-Immersion Stress-Induced Gastric Lesions** Water-immersion stress for 7 h produced several linear and dotted lesions in the glandular stomach, and the mean lesion index in the vehicle administered rats was 49.1±9.9 mm (n=8). YJA20379-4, administered orally at 3, 10, and 30 mg/kg, dose-dependently inhibited these lesions; the percentage inhibition of the lesion index at each dose was 29.0, 32.1, and 62.5%, respectively. The ED<sub>50</sub> value of YJA20379-4 was 18.1 mg/kg (Table 3). Omeprazole also significantly inhibited the lesions; the percentage inhibition of the lesion index at each dose was 36.7, 76.1, and 96.66%, respectively. The ED<sub>50</sub> value of omeprazole was 4.4 mg/kg. The effect of YJA20379-4 was less potent than that of omeprazole (Fig. 5).

**Indomethacin-Induced Gastric Lesions** Indomethacin produced multiple lesions in the glandular stomach 7 h after the treatment; the mean lesion index in the vehicle administered rats was 31.6±4.7 mm (n=7). YJA20379-4, administered orally at 1, 3, and 10 mg/kg, inhibited these lesions dose-dependently; the percentage inhibition of the lesion index at each dose was 51.1, 69.1, and 76.2%, respectively. The ED<sub>50</sub> value of YJA20379-4 was 0.8 mg/kg (Table 3). Omeprazole also statistically inhibited lesion formation; the percentage inhibition of the lesion index at each dose was 43.5, 80.6, and 97.8%, respectively. The ED<sub>50</sub> value of omeprazole was 1.2 mg/kg (Fig. 6). The effect of YJA20379-4 was slightly greater than that of omeprazole.

**Ethanol-Induced Gastric Lesions** When absolute ethanol was given to the control animals, hemorrhagic band-like lesions were produced in the glandular portion of the stom-
The mean lesion index in the control animals was 87.4 ± 12.7 mm (n = 7). YJA20379-4, administered orally at 3, 10, and 30 mg/kg, dose-dependently inhibited these lesions; the percentage inhibition of the lesion index at each dose was 20.7, 32.2, and 70.1%, respectively. The ED₅₀ value of YJA20379-4 was 14.9 mg/kg (Table 3). Omeprazole also significantly inhibited the lesions; the percentage inhibition of the lesion index at each dose was 0.5, 34.2, and 72.7%, respectively. The ED₅₀ value of omeprazole was 17.1 mg/kg (Fig. 7). The effect of YJA20379-4 was a slightly greater than that of omeprazole.

**Mepirizole-Induced Duodenal Ulcers** Mepirizole produced one to two penetrating ulcers in the proximal duodenum; the mean ulcer index in the control animals was 13.4 ± 4.2 mm² (n = 8). YJA20379-4, administered orally at 3, 10, and 30 mg/kg, caused a dose-dependent inhibition of the ulceration; the percentage inhibition of the ulcer index at each dose was 21.1, 52.4, and 58.3%, respectively. The ED₅₀ value of YJA20379-4 was 14.4 mg/kg (Table 3). Omeprazole also significantly inhibited the ulcers; the percentage inhibition of the ulcer index at each dose was 23.1, 50.9, and 82.9%, respectively. The ED₅₀ value of omeprazole was 2.8 mg/kg (Fig. 8). The effect of YJA20379-4 was 5 times less potent than that of omeprazole.

**Acetic Acid-Induced Gastric Ulcers** The submucosal injection of 30% acetic acid (0.02 ml) induced visible and consistent ulcers in the stomach; the mean ulcer index in the vehicle-administered rats was 21.82 ± 2.2 mm² (n = 9). YJA20379-4, given orally twice daily for 8 d, accelerated the healing of the ulcers in a dose-dependent manner; the percentage inhibition of the ulcer index at 20, 60, and 200 mg/kg/d was 2.6, 32.1, and 35.0%, respectively. Omeprazole also dose-dependently accelerated the healing of the ulcers; the percentage inhibition of the ulcer index at each same dose was 24.5, 27.1, and 44.0%, respectively (Fig. 9). The healing effect of YJA20379-4 was similar to that of omeprazole.

**DISCUSSION**

The experiments demonstrated that YJA20379-4 is a po-
tent inhibitor of H⁺/K⁺-ATPase, *H. pylori* growth and acid secretion, and markedly inhibits the production of gastric and duodenal lesions. The healing of acetic acid ulcers in rats was also accelerated. Previous reports⁹,¹⁰ have shown that the proton pump inhibitor, omeprazole, exists predominantly in its neutral base form at physiological pH, and it exerts its inhibitory effect by interacting with the SH group of H⁺/K⁺-ATPase when transformed into the active sulfenamide form in the acid space of the parietal cells.

In the *in vitro* studies, YJA20379-4 pH-dependently inhibited H⁺/K⁺-ATPase activity in isolated rabbit gastric mucosal microsomes; YJA20379-4, as with omeprazole, exhibited more enhanced inhibition (about 3 times) under a weak acidic condition (pH 6.4) compared with that at a neutral pH (pH 7.4). Moreover, the inhibitory effect of YJA20379-4 on the H⁺/K⁺-ATPase was almost completely protected by the concurrent incubation of the enzyme with a high concentration of DTT (0.5 mM). These results suggest that the inhibitory action of YJA20379-4, like omeprazole, may occur through a covalent disulfide linkage with the cysteine residues of gastric H⁺/K⁺-ATPase. YJA20379-4 also showed marked *in vitro* activity against *H. pylori*, which has been recognized as the etiologic factor of chronic gastritis and a major factor in the development of peptic ulcers. In particular, the antibacterial activity of YJA20379-4 against this organism was 3 times more potent than that of omeprazole, suggesting that YJA20379-4 might offer a greater advantage in the therapy of peptic ulcers.

In the *in vivo* studies, though less potent than that of omeprazole, YJA20379-4 significantly suppressed basal gastric acid secretion when administered intraduodenally. As expected from its significant antisecretory effect, YJA20379-4 had a potent effect on various types of acutely-induced gastric lesions (water-immersion stress-, indomethacin-, and ethanol-induced gastric lesions); an especially marked effect was observed in indomethacin-induced lesions. In addition, YJA20379-4 prevented mepergoride-induced duodenal ulcers, which are presumed to be induced by the flow of accumulated gastric juice into the proximal duodenum and subsequently by an attenuated defensive mechanism. These lesions are also prevented by histamine-H₂-blocking agents ⁺⁴,⁺⁵.

These findings suggest that gastric acid secretion is crucially involved in the pathogenesis of these lesions.

A report⁶ showed that orally administered omeprazole significantly prevented experimental gastric mucosal lesions, but the low dose which inhibited gastric lesions was less effective on basal acid secretion. It was also suggested that omeprazole had a mucosal protective effect unrelated to the reduction of acid secretion; that is, a certain amount of gastric acid was necessary to produce the ulceration, but the breakdown of defensive mechanism of the mucosa was a much more important factor in the pathogenesis of ulcers. To determine the cytoprotective property of a drug on the weakened defensive mechanisms of the mucosa, therefore, we tested the effect of YJA20379-4 on mucosal lesions caused by absolute ethanol since these lesions are not prevented by antisecretory agents but by a low dose of prostaglandins.⁷ The results demonstrate that YJA20379-4, like omeprazole, markedly protected the gastric mucosa against ethanol (ED₅₀: 14.9 mg/kg), which has been indicated to increase vascular permeability in the gastric mucosa and subsequently cause gastric mucosal injury.⁸ Based on these data, that the cytoprotective effect of YJA20379-4 occurred at a dose markedly lower than that inhibiting gastric secretion, it was considered that the cytoprotective effect of YJA20379-4 might be independent of its antisecretory effect. Furthermore, in comparison with omeprazole, it was apparent that YJA20379-4 had the potential to accelerate the spontaneous healing of chronic gastric ulcers induced by acetic acid.

In summarizing the present results, it is suggested that the new proton pump inhibitor, YJA20379-4, has an excellent preventive effect on gastric and duodenal ulcers, which depends not only on its antisecretory effect through the inhibition of H⁺/K⁺-ATPase but also on its enhancement of gastroduodenal mucosal defensive factors. Besides, YJA20379-4 has potent inhibitory action against *H. pylori* that is central to the treatment of peptic ulcer diseases. Although further detailed investigations are required, taking these above results into account, we concluded that YJA20379-4 would be clinically useful for the treatment of peptic ulcers and diseases with acid-induced damage by virtue of its potent inhibition of H⁺/K⁺-ATPase, *H. pylori* and acid secretion, and because of its antiulcer and cytoprotective effects.

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