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Biochemical and Pharmacological Properties of a New Proton Pump Inhibitor, 2-Amino-4,5-dihydropyrido[1,2-a]thiazolo [5,4-g] benzimidazole (YJA20379-5)

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This study was designed to determine biochemical and pharmacological properties of a newly synthesized benzimidazole derivative, 2-amino-4,5-dihydropyrido [1,2-a] thiazolo [5,4-g] benzimidazole (YJA20379-5) *in vitro* and *in vivo*. In the leaky membrane vesicles of pig gastric mucosa, YJA20379-5 inhibited the K^+-stimulated H^+,K^+-ATPase activity in a concentration- and time-dependent manner, with IC_{50} values being 43 μM and 31 μM at pH 6.4 and 7.4, respectively. YJA20379-5, given intraduodenally, had a potent inhibitory effect on the gastric acid secretion in pylorus-ligated rats. The ED_{50} value for acid secretion was 15.4 mg/kg. YJA20379-5, administered orally, also suppressed gastric damages induced by water-immersion stress, indomethacin and ethanol, and duodenal damage induced by mepirizole in rats; the ED_{50} values were 17.6, 4.7, 3.0 and 18.7 mg/kg, respectively. Furthermore, repeated oral administration of YJA20379-5 accelerated the spontaneous healing of acetic acid-induced gastric ulcers in rats. It is concluded that the antisecretory activity of YJA20379-5 appears to be associated with inhibition of H^+,K^+-ATPase, while its antigastric and antiduodenal lesion activities are primarily related to the antisecretory effect.

Key words : Gastric mucosa, H^+,K^+-ATPase, Acid secretion, Antisecretory effect

INTRODUCTION

Peptic ulcer is a heterogeneous and multifactorial disease. In most types of mucosal ulceration, there is an acute or sustained loss of equilibrium between an aggressive factor, particularly gastric acid, and mucosal defense (Caldwell and McCallum, 1991). Acid appears to be a mediator common to most forms of peptic ulcer. Therefore, the current treatment approach to these conditions aims to suppress acid secretion and protect the mucosa from acid and peptic attack. Available means of therapeutic regulation of acid secretion include modification of neural influences by means of surgery and alteration of parietal cell second-messenger levels by receptor antagonists such as antimuscarinics or antihistaminics and by acid pump inhibitors (Hirschowitz *et al.*, 1995). The gastric H^+,K^+-ATPase has been shown to be the primary pump for acid secretion in the stomach (Reenstra *et al.*, 1986). The enzyme catalyzes a one-for-one exchange of cytoplasmic H^+ for luminal K^+ (Sachs *et al.*, 1976). Therefore, the inhibition of H^+,K^+-ATPase, which mediates the terminal step of acid secretion, would be the most effective way of controlling gastric acid secretion. Recently, many agents have been developed as gastric H^+,K^+-ATPase inhibitors. Omeprazole, a substituted benzimidazole and a prototypical proton pump inhibitor, is remarkably effective and specific for the inhibition of H^+,K^+-ATPase (Elander *et al.*, 1986; Fellinius *et al.*, 1981; LaMattina *et al.*, 1990). It was found that omeprazole prevented the formation of acutely-induced gastroduodenal lesions in animals (Yamamoto *et al.*, 1984) and promoted the healing of peptic ulcers in humans (Classen *et al.*, 1985; Tytgat *et al.*, 1985).

In the present study, we investigated the effects of 2-amino-4,5-dihydro-pyrido [1,2-a]thiazolo[5,4-g]benzimidazole (YJA20379-5) on H^+,K^+-ATPase activity in gastric microsomes, gastric acid secretion, the formation of the acute experimentally induced gastroduodenal damages, and the healing of the chronic gastric ulcer induced in rats. Omeprazole was used as a reference drug.

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MATERIALS AND METHODS

Compounds

YJA20379-5 (Fig. 1) was synthesized by Yung-Jin

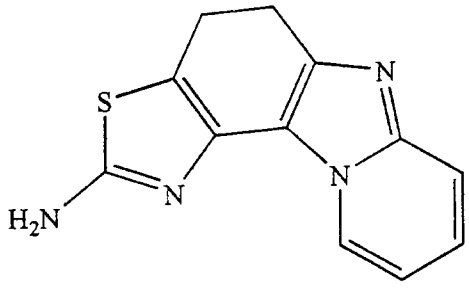

Fig. 1. The structural formula for YJA20379-5.

Pharm. Co., Ltd., Korea. Omeprazole was obtained from Chong Kun Dang Pharm. Co., Ltd., Korea. For *in vitro* studies, the test compounds were dissolved in dimethyl sulfoxide (DMSO) (International Specialty Chemical, Chicago, Illinois, USA). For *in vivo* studies involving oral administration, test compounds were suspended in 1% carboxymethyl cellulose solution (CMC, w/v). In measuring the gastric acid secretion, the compounds were suspended in 1% CMC containing 0.2% $NaHCO_3$ (w/v) and the pH was adjusted to 9.0 with 2 N NaOH.

Preparation of H^+,K^+-ATPase-enriched pig gastric vesicles

Gastric microsomal vesicles were prepared from pig fundic mucosa by modification of the method of Saccomani *et al.* (1977). Briefly, tissue was homogenized in isotonic medium and a microsomal fraction was obtained by differential centrifugation. This material was separated on a discontinuous density gradient and the fraction at the interface between the 0.25 M sucrose and 0.25 M sucrose plus 7% Ficoll layers was taken. The interface fraction was diluted in two volumes of homogenization buffer, spun down and freeze-dried overnight. The lyophilized vesicles were resuspended in homogenization buffer and stored at -70°C. Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as the standard.

H^+,K^+-ATPase assay

ATPase was preincubated in 5 mM imidazole buffer (pH 6.4 or 7.4) containing various concentrations of inhibitors for 30 min. The enzyme activity was determined at 37°C in the presence of 2 mM $MgCl_2$, 2 mM Na_2ATP, and 20 mM imidazole buffer (pH 7.4), with or without 10 mM KCl. After incubation for 15 min at 37°C, the reaction was terminated by adding 1 mL of ice-cold 22% trichloroacetic acid. The inorganic phosphate hydrolysed from ATP was measured by the method of Fiske and Subbarow (1924).

To investigate the effect of preincubation time on the inhibition of H^+,K^+-ATPase, gastric microsomal vesicles were preincubated for designated times (0, 4, 10, 20, 30 and 60 min) in 5 mM imidazole buffer (pH 6.4) with or without 100 μM of YJA20379-5, and then H^+,K^+-ATPase activity was measured as described above.

Gastric acid secretion

Male Sprague-Dawley rats (180-220 g) were deprived of food for 24 hr with access to water *ad libitum*. Gastric acid secretion was determined using the pylorus ligation technique (Shay *et al.*, 1954). Under ether anesthesia, the abdomen was incised and the pylorus ligated. Test compounds (YJA20379-5:3, 10 and 30 mg/kg or omeprazole:3, 6 and 12 mg/kg) or vehicle were intraduodenally administered immediately after the ligation. 4 hr after the pylorus ligation, the rats were killed by cervical dislocation. The gastric contents were collected and analyzed for acidity. Acidity was determined by titration against 0.01 N NaOH to an endpoint of 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 and sacrificed. The stomach of each rat was removed and inflated by injecting 10 mL of 3% formalin for 10 min to fix the inner and outer layers of the gastric wall. This formalin treatment was performed in all subsequent experiments. The stomach was then incised along the greater curvature and examined for lesions in the glandular portion. Test compounds (YJA 20379-5 or omeprazole: 3, 10 and 30 mg/kg) or vehicle were administered orally 30 min before the water immersion.

Indomethacin-induced gastric lesions

Female Sprague-Dawley rats (160~180 g) were deprived of food but allowed free access to water for 48 hr before the experiments. Indomethacin (35 mg/kg, Sigma, St. Louis, MO, USA), suspended in saline containing a few drops of Tween 80, was given subcutaneously to rats. The rats were sacrificed 7 hr later, and the stomach was examined for lesions in the glandular portion. Test compounds (YJA20379-5 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-Dawley rats (180~200 g) were deprived of food but allowed free access to water for 24 hr before the experiments. The rats were treated orally with 1 mL absolute ethanol/200 g body weight (Hayman, England, U.K.). The rats were sacrificed 1.5 hr after

the ethanol treatment. Following removal, the stomach was examined for lesions in the glandular portion. Test compounds (YJA20379-5: 1, 3, and 10 mg/kg or omeprazole: 3, 10 and 30 mg/kg) or vehicle were administered orally 30 min before the ethanol treatment.

Mepirizole-induced duodenal ulcers

Non-fasted male Sprague-Dawley rats (180~200 g) were used in the study. Mepirizole (250 mg/kg, Sigma, St. Louis, Mo, USA), suspended in 1% CMC was administered orally to rats, which were then deprived of food and water. The rats were sacrificed 24 hr later and examined for ulcers in the duodenum. Test compounds (YJA20379-5: 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 hr before injection of acetic acid into the submucosal layer. Under ether anesthesia, 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. After operation, the animals were maintained on rat chow and water *ad libitum*. Test compounds (YJA 20379-5 or omeprazole: 10, 30 and 100 mg/kg) or vehicle were administered orally twice a day (9:00 AM, 6:00 PM) for 8 consecutive days from the next day after surgery. The rats were killed 16 hr after the final administration of test compound or vehicle, and the stomachs were examined for ulcers.

Ulcer 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 used as the lesion index. The areas (mm^2) of the mepirizole-induced duodenal ulcers and acetic acid-induced gastric ulcers were also measured and summed per stomach, and the total used as the ulcer index.

Analysis of data

A Duncan multiple range test was employed to determine the statistical significance of the data at the levels of $P<0.05$ and $P<0.01$. ED_{50} values (the doses that inhibit gastric acid and prevent the formation of the gastric and duodenal damage by 50%) were calculated by the probit method (Finney, 1971). The IC_{50} values (the concentration that inhibits H^+,K^+-ATPase activity by 50%) were determined for each compound from plots of percentage inhibition versus concentration.

RESULTS

Effect of YJA20379-5 on gastric H^+,K^+-ATPase

YJA20379-5 potently inhibited H^+,K^+-ATPase activity isolated from the pig stomach in a concentration dependent manner (Fig. 2). The IC_{50} values of YJA20379-5 were 43 μM and 31 μM at pH 6.4 and 7.4, respectively. Omeprazole also inhibited the H^+,K^+-ATPase activity; the IC_{50} values were 20 μM and 61 μM at pH 6.4 and 7.4, respectively. The specific activity of H^+,K^+-ATPase in the control group was 36 μmol Pi/mg prot-

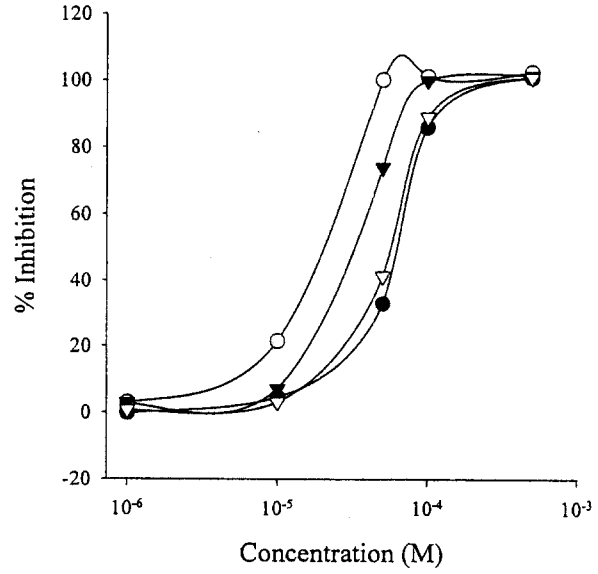

Fig. 2. Effects of YJA20379-5 on H^+,K^+-ATPase activity of the lyophilized pig gastric microsomes. YJA20379-5 (▽, ▼) and omeprazole (○, ●) at pH 6.4 and 7.4. Enzyme activity without an inhibitor was taken as 100%.

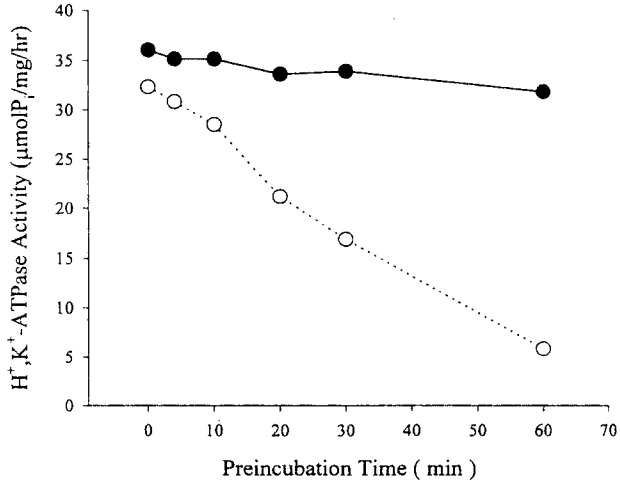

Fig. 3. The preincubation time dependence on the inhibition of the H^+,K^+-ATPase by YJA20379-5 at pH 7.4 (●: control, ○: 100 μM YJA20379-5).

ein/hr.

The inhibition of the H^+,K^+-ATPase activity by YJA 20379-5 was increased in proportion to the length of the preincubation time. The H^+,K^+-ATPase activities at 0, 4, 10, 20, 30 and 60 min after preincubation with YJA20379-5 were 90, 88, 82, 64, 51 and 21% of the control value, respectively (Fig. 3).

Effect of YJA20379-5 on gastric acid secretion

In the control groups, the basal gastric acid secretion (acid output) was 3.42±0.50 mEq/kg/4 hr (mean ±S.E.). YJA20379-5, given intraduodenally at doses of 3, 10 and 30 mg/kg, dose-dependently inhibited gastric acid secretion in pylorus-ligated rats following intraduodenal administration (Fig. 4). The ED_{50} value was 15.4 mg/kg. Intraduodenal administration of omeprazole at doses of 3, 6 and 12 mg/kg inhibited gastric acid secretion, with an ED_{50} value of 2.4 mg/kg.

Effect of YJA20379-5 on water immersion stress-induced gastric lesions

Water-immersion stress for 7 hr produced several linear and dotted erosions in the glandular stomach, and the mean lesion index in vehicle treated rats was 55.6±1.9 mm (n=8). YJA20379-5, administered orally, dose-dependently inhibited production of these lesions; the percentage inhibitions of the lesion index at doses of 3, 10 and 30 mg/kg were 7.6, 44.8 and 59.7%, respectively. The ED_{50} value of YJA20379-5 was 17.6 mg/kg. Oral administration of omeprazole also significantly inhibited the lesion formation; the percentage inhibitions of the lesion index at doses of 3, 10 and 30 mg/kg were 36.7, 76.1 and 96.6%, respectively. The

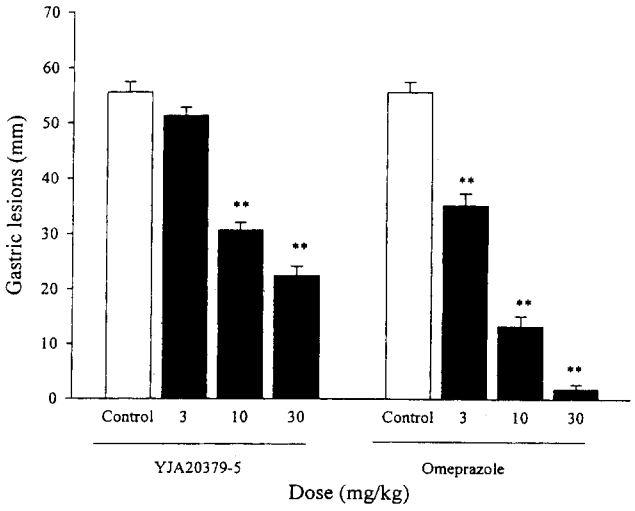

Fig. 5. Effects of YJA20379-5 and omeprazole on water-immersion stress-induced gastric lesions in rats. Each value represents the mean±S.E. $^{**}P<0.01$, vs. gastric lesion index of the vehicle-treated control group.

ED_{50} value of omeprazole was 4.4 mg/kg. The effect of YJA20379-5 was less potent than that of omeprazole (Fig. 5).

Effect of YJA20379-5 on indomethacin-induced gastric lesions

Indomethacin produced multiple lesions in the glandular stomach 7 hr after the treatment; the mean lesion index in vehicle treated rats was 25.3±3.4 mm (n=6). YJA20379-5, administered orally, dose-dependently inhibited formation of these lesions; the percentage inhibitions of the lesion index at doses of 1, 3 and 10 mg/kg were 25.0, 31.9 and 69.4%, respective-

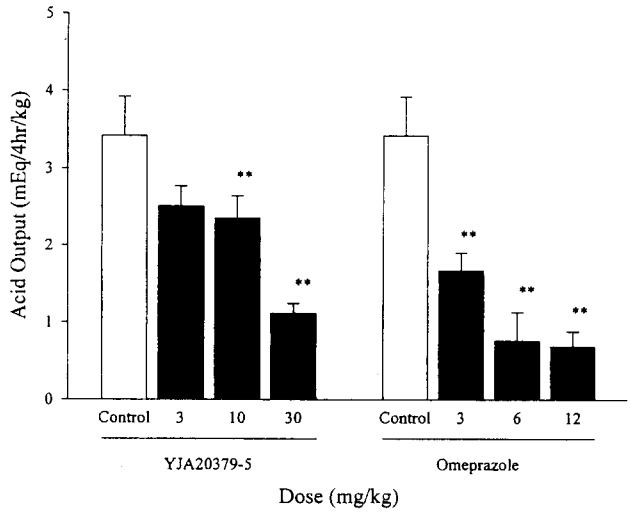

Fig. 4. Inhibitory effects of YJA20379-5 and omeprazole on gastric acid secretion in pylorus-ligated rats. Each value represents the mean±S.E. $^{**}P<0.01$, vs. acid output in of the vehicle-treated control group.

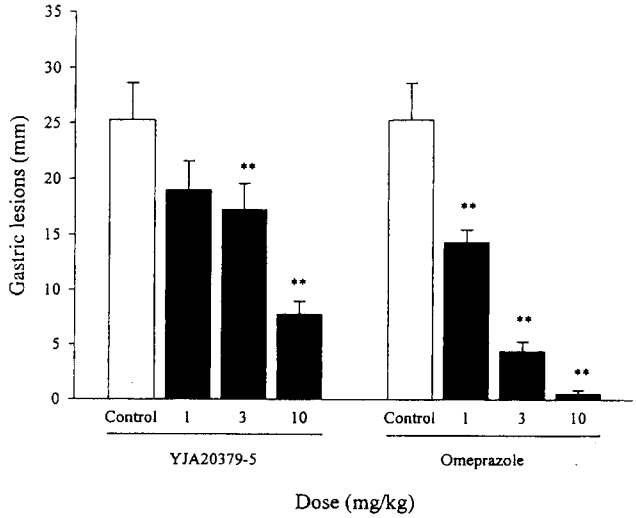

Fig. 6. Effects of YJA20379-5 and omeprazole on indomethacin-induced gastric lesions in rats. Each value represents the mean±S.E. $^{**}P<0.01$, vs. gastric lesion index of the vehicle-treated control group.

ly. The ED_{50} value of YJA20379-5 was 4.7 mg/kg. Oral administration of omeprazole also significantly inhibited the indomethacin-induced lesions; the percentage inhibitions of the lesion index at doses of 1, 3 and 10 mg/kg were 43.5, 82.6 and 97.8%, respectively. The ED_{50} value of omeprazole was 1.2 mg/kg (Fig. 6). The effect of YJA20379-5 was less potent than that of omeprazole.

Effect of YJA20379-5 on ethanol-induced gastric lesions

When absolute ethanol was given orally to control animals, hemorrhagic band-like lesions were produced in the glandular portion of the stomach; the mean lesion index in vehicle treated rats was 84.4±9.2 mm (n=8). YJA20379-5, administered orally, dose-dependently inhibited lesion formation; the percentage inhibitions of the lesion index at doses of 1, 3 and 10 mg/kg were 6.5, 64.3 and 90.7%, respectively. The ED_{50} value of YJA20379-5 was 3.0 mg/kg. Oral administration of omeprazole also significantly inhibited lesion production; the percentage inhibitions of the lesion index at doses of 3, 10 and 30 mg/kg were 0.5, 34.2 and 72.7%, respectively. The ED_{50} value of omeprazole was 17.1 mg/kg (Fig. 7). The effect of YJA20379-5 was 5 times more potent than that of omeprazole.

Effect of YJA20379-5 on mepirizole-induced duodenal ulcers

Mepirizole produced one or two penetrating ulcers in the proximal duodenum; the mean ulcer index in vehicle treated rats was 13.4±2.5 mm^2 (n=8). YJA 20379-5, administered orally, dose-dependently inhibited formation of these ulcers; the percentage inhibitions of the ulcer index at the doses of 3, 10 and 30 mg/kg were 30.4, 40.9 and 56.5%, respectively. The ED_{50} value of YJA20379-5 was 18.7 mg/kg. Oral administration of omeprazole also significantly inhibited ulcer formation; the percentage inhibitions of the ulcer index at doses of 1, 3 and 10 mg/kg were 23.1, 50.9, and 82.9%, respectively. The ED_{50} value of omeprazole was 2.8 mg/kg (Fig. 8). The effect of YJA20379-5 was less potent than that of omeprazole.

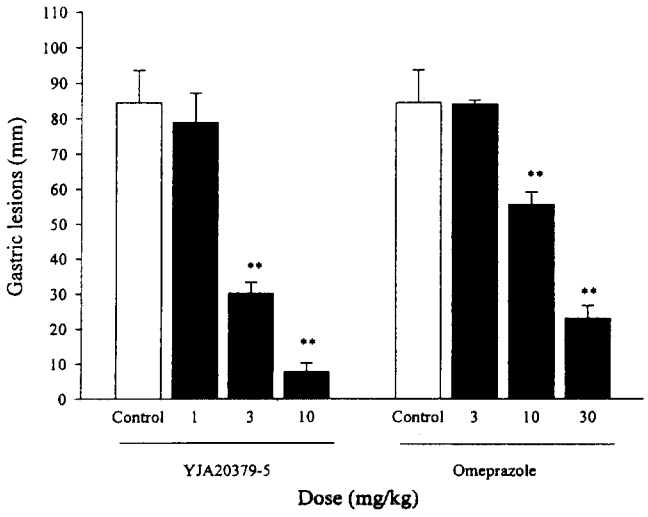

Fig. 7. Effects of YJA20379-5 and omeprazole on ethanol-induced gastric lesions in rats. Each value represents the mean ±S.E. $^{**}P<0.01$, vs. gastric lesion index of the vehicle-treated control group.

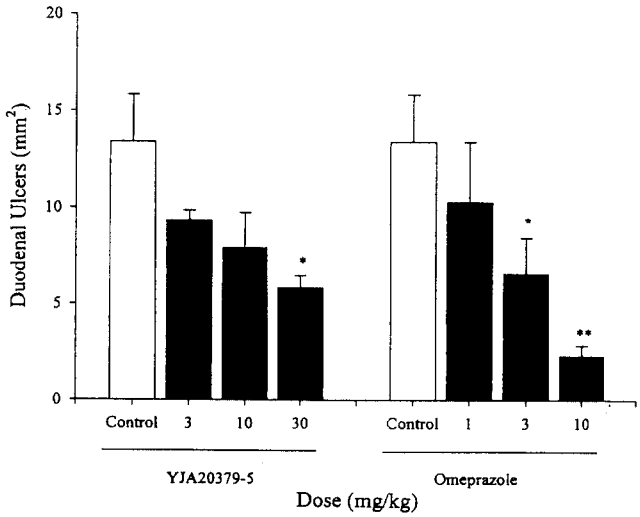

Fig. 8. Effects of YJA20379-5 and omeprazole on mepirizole-induced duodenal ulcers in rats. Each value represents the mean±S.E. $^{*}P<0.05$, $^{**}P<0.01$, vs. duodenal ulcer index of the vehicle-treated control group.

Effect of YJA20379-5 on acetic acid-induced gastric ulcers

The submucosal injection of 30% acetic acid (0.02

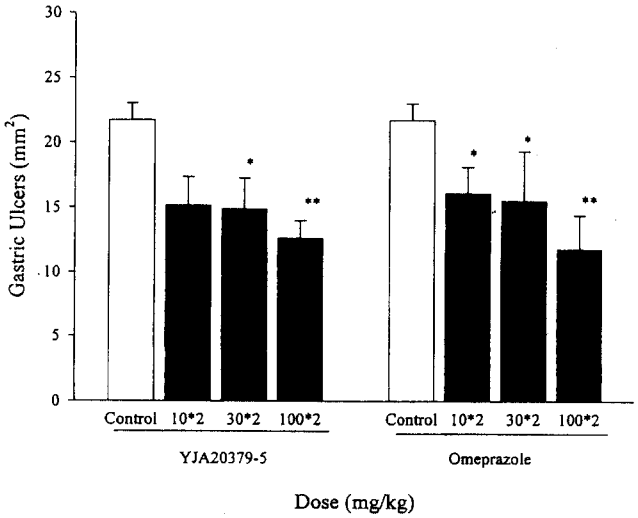

Fig. 9. Effects of repeated administration of YJA20379-5 and omeprazole on the spontaneous healing of acetic acid-induced gastric ulcers in rats. Each value represents the mean ±S.E. $^{*}P<0.05$, $^{**}P<0.01$, vs. gastric ulcer index of the vehicle-treated control group.

mL) induced a visible and consistent ulcer in the stomach; the mean ulcer index in vehicle treated rats was 21.8 ± 1.3 mm^2 (n=10). YJA20379-5, administered orally twice a day for 8 days, dose-dependently accelerated the healing of the ulcer; the percentage inhibitions of the ulcer index at 20, 60 and 200 mg/kg/day were 30.3, 31.6 and 41.9%, respectively. Omeprazole also dose-dependently accelerated the healing of the ulcers; the percentage inhibition of the ulcer index at these same doses was 24.5, 27.1, and 44.0% respectively (Fig. 9). The healing effect of YJA20379-5 was similar to that of omeprazole.

DISCUSSION

YJA20379-5, a novel proton pump inhibitor, inhibited the pig gastric H^+,K^+-ATPase, and had antisecretory and antigastroduodenal lesion activities in rats. The inhibitory effect of YJA20379-5 on H^+,K^+-ATPase was concentration-dependent and not markedly affected by pH condition (6.4 or 7.4). The IC_{50} values at neutral and acidic environment were 43 μM and 31 μM, respectively. These slight difference between conditions may reflect an instability of YJA20379-5 in acidic media. In contrast, the inhibitory activity of omeprazole was about 3 times more potent at pH 6.4 compared with that obtained at pH 7.4. These data were consistent with previous results (Wallmark *et al.*, 1984) which showed that the activity of omeprazole is pH dependent.

In assessing the nature of H^+,K^+-ATPase inactivation by YJA20379-5, it was found that the inhibitory effect of YJA20379-5 was time-dependently increased by preincubation with the enzyme. These data indicate that YJA20379-5 may bind to the enzyme irreversibly since irreversible inhibition is characterized by a progressive increase with time, with no equilibrium set up between enzyme and inhibitor (Dixon and Webb, 1979; Hioki *et al.*, 1990).

In *in vivo* studies using pylorus-ligated rats, the antisecretory activity of YJA20379-5 was about 6 times less potent compared with that of omeprazole. Considering that YJA20379-5 inhibited H^+,K^+-ATPase activity *in vitro* with a similar potency to omeprazole and that the inhibition of gastric H^+,K^+-ATPase is closely related to antisecretory activity (Wallmark *et al.*, 1985), these results may involve several possibilities, e.g. poor bioavailability or instability in gastric acid. Further investigation would elucidate the correct reasons of this result.

Despite the weak antisecretory activity compared with that of omeprazole, YJA20379-5 markedly inhibited the gastric mucosal lesions induced by ethanol in rats. The inhibitory effect of YJA20379-5 was 5 times more potent than that of omeprazole, based on ED_{50} value. It is previously reported that necrotizing agent-induced gastric lesions are not suppressed by the inhibition of acid secretion, but rather by cytoprotective effects (Yamada *et at.*, 1996; Yuki *et al.*, 1995). Therefore this result suggests that YJA20379-5 may have a potent cytoprotective activity on the gastric mucosa of rats. YJA20379-5 also significantly inhibited the production of indomethacin-induced gastric lesions. This effective dose of YJA20379-5 was lower than that for its antisecretory effect in pylorus-ligated rats. These results indicate that the preventive effects of YJA20379-5 could not be explained solely by its antisecretory effects. On the other hand, YJA20379-5 could not inhibit effectively the water-immersion stress-induced gastric lesion and mepirizole-induced duodenal ulcer. These results could be due to the weak suppression of gastric acid secretion following oral administration of YJA20379-5.

The acetic acid-induced chronic ulcer model in rats is often used to evaluate the curative effect of drugs on the healing of ulcers, since the ulcers persist for more than 1 month (Tanaka *et al.*, 1989). YJA20379-5 markedly accelerated the healing of acetic acid-induced gastric ulcers in rats. Omeprazole also accelerated the healing of the gastric ulcers and the potency was similar to that of YJA20379-5. Although the mechanism of action of YJA20379-5 responsible for the accelerated healing of experimental ulcers was not determined, both the inhibition of gastric acid secretion and the protection of the mucosa seem to be involved in promoting the repair.

Taken together, these results suggest that a new proton pump inhibitor, YJA20379-5 exerts curative and preventive effects on gastric ulcers by suppressing acid secretion through inhibition of H^+,K^+-ATPase and possibly protecting the gastroduodenal mucosa.

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