

Biochemical and Pharmacological Properties of a Newly Synthesized Proton Pump (H^+/K^+ -ATPase) Inhibitor, TY-11345 in Experimental Animals

Takaji Yamaguchi, Kazuyuki Aihara, Shin-ichi Yamada, Sen-ichi Narita and Kentaro Kogi

R & D Department, Toa Eiyo Ltd., Iizaka, Fukushima 960-02, Japan

Received December 7, 1992 Accepted May 1, 1993

ABSTRACT—We investigated the effects of the newly synthesized proton pump inhibitor TY-11345, (\pm)-2-[(4-methoxy-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-9-yl)sulfinyl]-1*H*-benzimidazole sodium salt, on gastric mucosal proton pump (H^+/K^+ -ATPase) activity, gastric acid secretion and gastro-duodenal lesions in experimental animals. TY-11345 potently inhibited H^+/K^+ -ATPase activity in isolated rabbit gastric mucosal microsomes; and the inhibitory effect was enhanced under weak acid conditions, the IC_{50} (concentrations that inhibit the enzyme activity by 50%) being 5.8 μ M and 9.9 μ M at pH 6.0 and pH 7.4, respectively. In Ghosh & Schild rats, intravenous injection of TY-11345 significantly inhibited gastric acid secretion stimulated by tetragastrin; the effect of TY-11345 was twice as potent as that of omeprazole. In pylorus ligated rats, TY-11345 inhibited basal gastric acid secretion by both the intraduodenal and oral routes, with ED_{50} values of 1.2 and 4.0 mg/kg, respectively. These effects were 9 and 5 times more potent than those of omeprazole, respectively. Moreover, the antisecretory effect of TY-11345 persisted for more than 24 hr in pylorus ligated rats. In experimental ulcer models, TY-11345 prevented the formation of water-immersion stress, ethanol or indomethacin-induced gastric lesions and mepirizole-induced duodenal lesions in rats. The antiulcer effects of TY-11345 were 3 to 15 times more potent than those of omeprazole. These results suggest that TY-11345 has potent antisecretory and antiulcer effects which are exerted by suppression of H^+/K^+ -ATPase activity in gastric parietal cells, so that TY-11345 should be useful for the clinical treatment of peptic ulcer diseases.

Keywords: TY-11345, Omeprazole, H^+/K^+ -ATPase inhibitor, Antisecretory effect, Antiulcer effect

Gastric acid has been demonstrated to be a major factor in peptic ulcer diseases. In 1967, Forte et al. demonstrated the existence of a K^+ -stimulated ATPase in gastric mucosal microsomes prepared from rabbits during their investigations on the production and secretory mechanisms of gastric acid (1). Lee et al. reported the mechanisms of the ATPase responsible for generation of the hydrogen ion gradient at the luminal surface of gastric parietal cells (2). These observations indicated that the enzyme involved in the final step of acid secretion is the H^+/K^+ -ATPase "proton pump".

In 1981, Fellenius et al. demonstrated that substituted benzimidazole inhibited the H^+/K^+ -ATPase and suppressed gastric acid secretion (3). This work led to the discovery of the proton pump inhibitor omeprazole. Omeprazole has a pyridine ring, CH_2SO and a benzimida-

zole ring as fundamental structural elements. This compound is activated under acid conditions to form a sulfenamide which modifies the SH substrate of H^+/K^+ -ATPase (4, 5).

TY-11345, (\pm)-2-[(4-methoxy-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-9-yl)sulfinyl]-1*H*-benzimidazole sodium salt, is a new benzimidazole derivative with a cycloheptenopyridine ring, and our previous study has revealed that TY-11345 has a potent inhibitory effect on H^+/K^+ -ATPase activity, and the effect is enhanced by decreasing the pH of the medium (6). Our present study shows the antisecretory and antiulcer properties of TY-11345 in experimental animals and compares these results with the properties of omeprazole.

MATERIALS AND METHODS

Test compounds

TY-11345 (Fig. 1) and omeprazole were synthesized by Toa Eiyo. For the *in vitro* study, these compounds were dissolved in dimethyl sulfoxide (DMSO) and then diluted with 10 mM imidazole buffer (pH 6.0 or 7.4), with the final concentration of DMSO being less than 1%. For the *in vivo* studies, these compounds were dissolved in polyethylene glycol 400 (PEG) and then diluted with 0.56 mg/ml NaHCO₃ (less than 10% PEG) for intravenous administration or suspended in 0.5% carboxymethyl cellulose sodium salt (CMC-Na) solution combined with 0.2% NaHCO₃ for oral and intraduodenal administration. TY-11345 and omeprazole were administered in a volume of 2 ml/kg body weight in the *in vivo* studies.

H⁺/K⁺-ATPase activity in gastric mucosal microsomes

Preparation of rabbit gastric membranes enriched in H⁺/K⁺-ATPase: Gastric H⁺/K⁺-ATPase was purified from the parietal cell-rich fraction of the rabbit stomach in accordance with the method of Saccomani et al. (7). The stomach of Japanese white rabbits (2.5 to 3.5 kg) was dissected out and washed quickly with ice-cold 3 M NaCl. The fundic mucosa was removed from the underlying muscular layer and homogenized in 10 volumes of ice-cold Tris-HCl/sucrose buffer (20 mM/250 mM, pH 7.4) by a Teflon-glass homogenizer. The resulting homogenates were centrifuged at 9,000 × *g* for 10 min. The pellets were suspended in a twofold volume homogenate buffer and centrifuged again under the same conditions. The resulting supernatants of the two centrifugations were combined and recentrifuged at 105,000 × *g* for 60 min. The microsomal pellets were resuspended in the 250 mM sucrose and layered over 7.5% Ficoll (w/w) in 250 mM sucrose. Centrifugation was carried out in an SRP 28A swing rotor (Hitachi, Tokyo) at 25,000 rpm for 4 hr. The

light microsomal bands at the interface between the 250 mM sucrose and Ficoll were collected. The preparations were stored at -80°C until use. Protein content was measured by the commercially available protein assay kit (Bio Rad, Richmond, CA, USA) using bovine serum albumin as the standard.

Assay procedure: Rabbit gastric H⁺/K⁺-ATPase was measured as described by Saccomani et al. (7). Briefly, membrane protein (80 µg protein) was preincubated for 3 to 30 min at 37°C in an assay medium consisting of 10 mM imidazole buffer (pH 6.0 or 7.4) and various concentrations of TY-11345 or omeprazole (final volume of 0.5 ml). The enzyme reaction was started by adding 0.5 ml of a solution containing 4 mM MgCl₂, 4 mM ATP, 2 × 10⁻⁵ M valinomycin and 80 mM imidazole buffer (pH 7.4), with or without 20 mM KCl. The reaction was stopped after a 15-min incubation at 37°C by placing the tubes in ice-slush and adding 1 mM ice-cold 12% trichloroacetic acid. Inorganic phosphate produced from ATP hydrolysis was measured by a commercially available assay reagent for inorganic phosphate (Iatron-Ma701 Pi, Iatron, Tokyo).

Measurement of gastric acid secretion

Tetragastrin-stimulated secretion (Ghosh & Schild rats): A modified method based on the technique of Ghosh and Schild was utilized (8). Male Sprague-Dawley rats (213–364 g) were deprived of food but allowed free access to water for 24 hr prior to the experiments. Rats were anesthetized with urethane (1.25 g/kg, *i.p.*). A polyethylene tube was inserted into the trachea to facilitate spontaneous breathing. A midline laparotomy was then performed, and the stomach was exteriorized. Through an incision in the forestomach, the gastric contents were gently washed out with saline. A double-lumen cannula (outer: Tygon with a diameter of 7 mm, inner: polyethylene with diameter of 2 mm) was inserted into the forestomach and secured by a ligature at the forestomach. The pylorus was ligated, and saline at room temperature was infused through the inner cannula at a rate of 1.0 ml/min and drained from the outer tube. The gastric secretion was stimulated by a constant infusion of tetragastrin (Mect, Tokyo; 30 µg/kg/hr, *i.v.*). The gastric effluent was collected at 10-min intervals. The acid output (in microequivalents per min) was determined by titration of the perfusate with 0.01 N NaOH to pH 7.0 with an automatic titrator (AUT-201, Toa Denpa, Tokyo). TY-11345 (0.3, 0.5 and 1.0 mg/kg), omeprazole (0.3, 0.5 and 1.0 mg/kg) or vehicle were administered from the femoral vein after the gastric acid secretion reached a plateau.

Basal secretion (pylorus ligated rats): Male Sprague-Dawley rats (232–267 g) were deprived of food but allowed free access to water for 24 hr prior to the experiments. While under ether anesthesia, the abdomen was

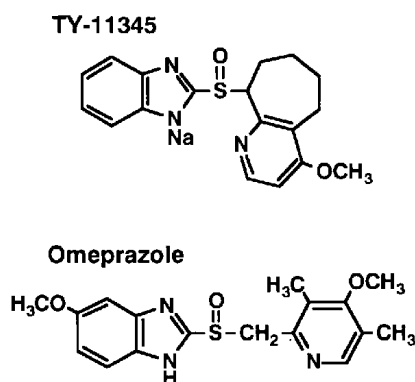


Fig. 1. Chemical structures of TY-11345 and omeprazole.

opened and the pylorus ligated. Test compounds or vehicle were administered intravenously (TY-11345: 0.1, 0.3 and 1.0 mg/kg; omeprazole: 1.0, 3.0 and 10 mg/kg); intraduodenally (TY-11345: 1, 3 and 10 mg/kg; omeprazole: 3, 10 and 30 mg/kg), immediately after the ligation; or orally, at 0.5 (3, 10 and 30 mg/kg) or 19 hr (10, 30 and 100 mg/kg) before the ligation. Five hours after the pylorus ligation, the rats were sacrificed. The gastric contents were collected and analyzed for the volume and acidity. The acid concentration in a 1-ml aliquot of the gastric juice was determined by an automatic titrator against 0.1 N NaOH to pH 7.0. The total acid output was calculated as the volume times the acid concentration.

Acute experimental lesions

Water-immersion stress-induced gastric lesions: Male Sprague-Dawley rats (190–273 g) were deprived of food but allowed free access to water for 24 hr prior to the experiments. The rats were placed in a restraint cage, and then immersed vertically to the level of the xiphoid process in a water bath (23 °C) for 6 hr and sacrificed (9). The stomach of each rat was removed and inflated by injecting 8 ml of 1% formalin to fix the inner and outer layers of the gastric wall. 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 (TY-11345: 0.3, 1.0 and 3.0 mg/kg; omeprazole: 3, 10 and 30 mg/kg) or vehicle were administered orally at 30 min before the stress load.

Indomethacin-induced gastric lesions: Male Sprague-Dawley rats (178–285 g) were deprived of food but allowed free access to water for 24 hr prior to the experiments. Indomethacin, suspended in 0.5% CMC-Na, was orally given in a dose of 10 mg/kg (10). The rats were sacrificed 6 hr later, and the stomach was examined for lesions in the glandular portion. Test compounds (TY-11345: 0.3, 1.0 and 3.0 mg/kg; omeprazole: 3, 10 and 30 mg/kg) or vehicle were administered orally at 30 min before the indomethacin treatment.

Ethanol-induced gastric lesions: Male Sprague-Dawley rats (215–315 g) were deprived of food but allowed free access to water for 24 hr prior to the experiments. The rats were each orally given 1 ml absolute ethanol (11). The rats were sacrificed at 1 hr after receiving the ethanol, and then the stomach was examined for lesions in the glandular portion. Test compounds (3, 10 and 30 mg/kg) or vehicle were administered orally at 30 min before the ethanol treatment.

Mepirazole-induced duodenal ulcers: Male Sprague-Dawley rats (215–285 g) were used. Mepirazole, suspended in 0.5% CMC-Na, was administered orally at 200 mg/kg to rats, which were then deprived of food and

water (12). The rats were sacrificed 24 hr later and examined for ulcers in the duodenum. Test compounds (TY-11345: 0.3, 1 and 3 mg/kg \times 2; omeprazole: 1, 3 and 10 mg/kg \times 2) or vehicle were administered orally twice, at 30 min before and 9 hr after the mepirazole treatment.

Chronic experimental ulcers

Acetic acid-induced gastric ulcers: Male Sprague-Dawley rats (192–218 g) were used. Prior to the experiments, the abdomens of ether anesthetized rats were incised and the anterior portion of the stomach exposed. Then, 0.03 ml of 20% 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 rats were maintained on rat chow and allowed free access to water (13). Test compounds (TY-11345: 3, 10 and 30 mg/kg; omeprazole: 30 mg/kg) or vehicle were administered orally from the first day after the operation for 14 consecutive days, once daily, to the rats with gastric ulcers. The rats were sacrificed at 24 hr after the final administration of drugs, and their 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 used as the lesion index. The areas (mm²) of the mepirazole-induced duodenal ulcers and acetic acid-induced ulcers were also measured and summed per stomach, and used as the ulcer index.

Statistics

Data are expressed as the mean \pm S.E. A Williams multiple comparison test was employed to determine the statistical significance of the data at the level of $P < 0.05$. ED₅₀ values (the doses that inhibit gastric acid output 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 in rabbit gastric mucosal microsomes

The H⁺/K⁺-ATPase activity in the control group was 38.9 ± 1.2 μ mol Pi/hr/mg protein (n=5). TY-11345 potentially inhibited the H⁺/K⁺-ATPase activity of purified rabbit gastric mucosal microsomes in a concentration-dependent manner. The IC₅₀ values (concentrations that inhibit the enzyme activity by 50%) were 5.8 μ M and 9.9 μ M at pH 6.0 and pH 7.4, respectively. Omeprazole also inhibited H⁺/K⁺-ATPase activity, and the IC₅₀ values were 16.1 μ M and 201.9 μ M at pH 6.0 and 7.4, respective-

ly. The effects of TY-11345 were about 3 and 20 times more potent than that of omeprazole (Fig. 2). The inhibitory effects of TY-11345 and omeprazole depended on the preincubation time. Nearly peak effects of TY-11345 and omeprazole were attained at 10 min and 30 min, respectively. However, for the 30 min preincubation, the inhibitory effects of TY-11345 and omeprazole at concentrations of 10 μ M and 30 μ M, respectively, were almost equal at these concentrations and the values were about 85% (Fig. 3).

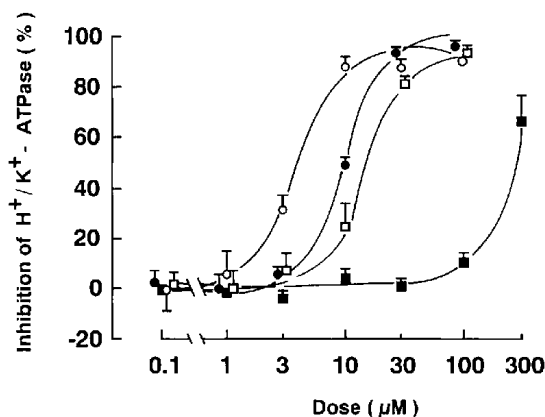


Fig. 2. Inhibitory effects of TY-11345 and omeprazole on H^+/K^+ -ATPase activity at pH 6.0 or pH 7.4 in rabbit gastric microsomes. TY-11345 or omeprazole was preincubated with H^+/K^+ -ATPase for 30 min, and the ATPase assay was initiated by adding ATP. ○: TY-11345 (pH 6.0), ●: omeprazole (pH 6.0), □: TY-11345 (pH 7.4), ■: omeprazole (pH 7.4). Each point represents the mean \pm S.E. for 5 experiments.

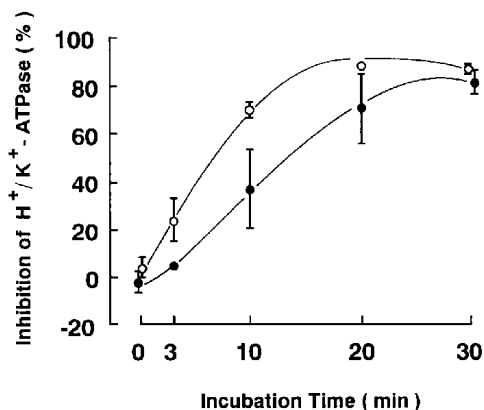


Fig. 3. Time course of inhibitory effects of TY-11345 (10 μ M, ○) and omeprazole (30 μ M, ●) on H^+/K^+ -ATPase activity in rabbit gastric microsomes at pH 6.0. The enzyme assay was started by adding ATP immediately or 3, 10, 20 and 30 min after the addition of test compounds. Each point represents the mean \pm S.E. for 3 experiments.

Effects on gastric acid secretion

Tetragastrin-stimulated secretion (Ghosh & Schild rats): The antisecretory activity of TY-11345 was evaluated by screening against tetragastrin-stimulated acid secretion in rats and by comparing the results with those obtained with omeprazole. Basal secretion in acute fistula rats was almost negligible, the acid output being 1.83 ± 0.02 μ Eq/10 min ($n=5$). Within 1.5 hr after the beginning of tetragastrin infusion, the acid secretion attained a fairly stable plateau, the acid output being 10.51 ± 1.20 μ Eq/10 min. Intravenous administration of TY-11345 at 0.3 to 1.0 mg/kg potently suppressed tetragastrin-stimulated gastric acid secretion for at least 180 min. The ED_{50} value of TY-11345 in the periods be-

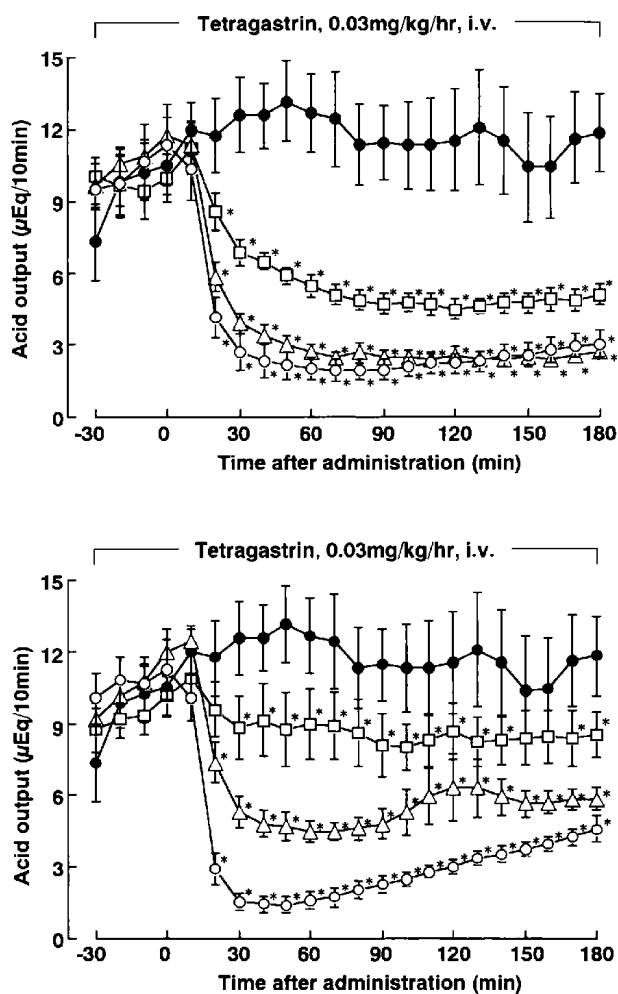


Fig. 4. Effects of TY-11345 (upper panel) and omeprazole (lower panel) on tetragastrin-stimulated gastric acid secretion in Ghosh & Schild rats. Test compounds were administered intravenously at doses of 0.3 mg/kg (□), 0.5 mg/kg (△) and 1.0 mg/kg (○), respectively. The control group (●) was administered the vehicle instead of the test compounds. Each point represents the mean \pm S.E. for 5 rats. Statistically significant differences from the values for the control (vehicle) are indicated by * ($P < 0.05$).

tween 30 to 60 min was 0.39 mg/kg, so the effect of TY-11345 was approximately as potent as that of omeprazole. The ED_{50} value of TY-11345 in the periods between 150 to 180 min was 0.22 mg/kg, so the effect of TY-11345 was approximately twice as potent as that of omeprazole (Fig. 4).

Basal secretion (pylorus ligated rats): Ligation of the pylorus for 5 hr produced an accumulation of gastric juice; the volume, acidity and acid output were 4.65 ± 0.29 ml/stomach, 94.5 ± 2.5 mEq/l and 0.457 ± 0.039 mEq/5 hr ($n=34$), respectively. TY-11345, given intravenously at 0.1, 0.3 and 1.0 mg/kg immediately after the ligation, dose-dependently inhibited the gastric secre-

tion (volume, acid concentration and acid output). Omeprazole, given intravenously at 1, 3 and 10 mg/kg, also dose-dependently inhibited the volume, acid concentration and acid output. The effect of TY-11345 was more potent than that of omeprazole (Fig. 5). TY-11345, given intraduodenally at 1, 3 and 10 mg/kg immediately after the ligation, dose-dependently inhibited the gastric secretion. The inhibition of acid output was complete when TY-11345 was administered in the dose of 10 mg/kg. Omeprazole also dose-dependently inhibited the volume, acid concentration and acid output. However, the degree of inhibition by omeprazole was weaker compared to that of TY-11345. The ED_{50} values of TY-11345 and omepra-

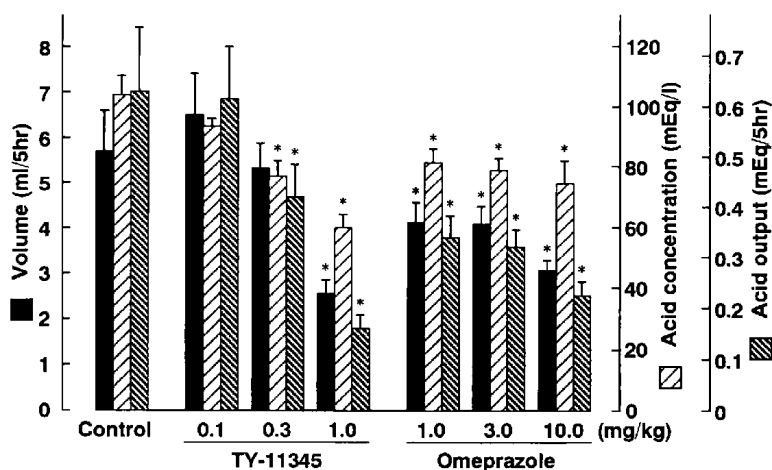


Fig. 5. Inhibitory effects of TY-11345 and omeprazole on gastric acid secretion in pylorus-ligated rats. The test compounds were administered intravenously immediately after pylorus ligation. Five hours after ligation, the gastric contents were collected and analyzed for volume, acid concentration and acid output. Each column represents the mean \pm S.E. for 8 rats. Statistically significant differences from the values for the control (vehicle) are indicated by * ($P < 0.05$).

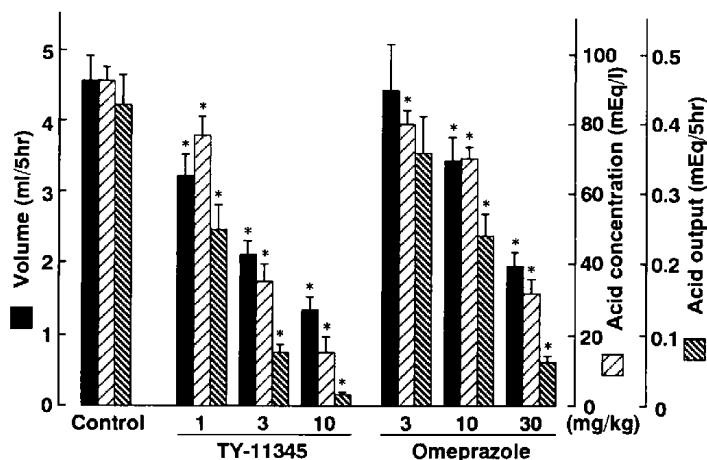


Fig. 6. Inhibitory effects of TY-11345 and omeprazole on gastric acid secretion in pylorus-ligated rats. The test compounds were administered intraduodenally immediately after pylorus ligation. Five hours after ligation, the gastric contents were collected and analyzed for volume, acid concentration and acid output. Each column represents the mean \pm S.E. for 8 rats. Statistically significant differences from the values for the control (vehicle) are indicated by * ($P < 0.05$).

zole were 1.2 mg/kg and 10.2 mg/kg, respectively. The effect of TY-11345 was 9 times more potent than that of omeprazole (Fig. 6). TY-11345 and omeprazole, administered orally, also inhibited the gastric secretion. The potencies of both compounds were reduced in comparison to those of the respective compounds by intraduodenal administration. The ED_{50} values of TY-11345 and omeprazole were 4.0 mg/kg and 20.2 mg/kg, respectively (Fig. 7). When each compound was administered orally at 19 hr before the ligation, TY-11345 and omeprazole still showed a significant reduction of the acid output. The ED_{50} values of TY-11345 and omeprazole were 12.7 mg/kg and 41.1 mg/kg, respectively (Fig. 8).

Effects on acute experimental lesions

Water-immersion stress-induced gastric lesions: Water-immersion stress for 6 hr produced several linear and dotted lesions in the glandular stomach, the mean lesion index in the vehicle-administered rats was 17.9 ± 2.4 mm ($n=13$). TY-11345, administered orally at 0.3, 1 and 3 mg/kg, dose-dependently inhibited these lesions; the inhibitory percentage of the lesion index at each dose was 31%, 37% and 84%, respectively. The ED_{50} value of TY-11345 was 0.92 mg/kg. Omeprazole also significantly inhibited the lesions. The ED_{50} value of omeprazole was 7.12 mg/kg. The effect of TY-11345 was 8 times more potent than that of omeprazole (Table 1).

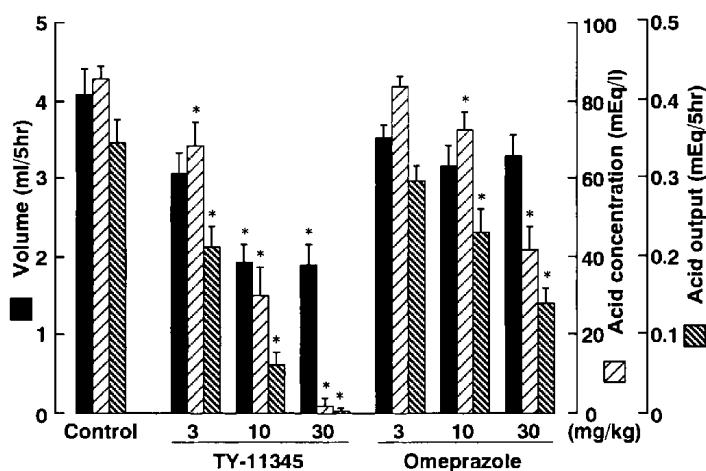


Fig. 7. Inhibitory effects of TY-11345 and omeprazole on gastric acid secretion in pylorus-ligated rats. The test compounds were administered orally 0.5 hr before pylorus ligation. Five hours after ligation, the gastric contents were collected and analyzed for volume, acid concentration and acid output. Each column represents the mean \pm S.E. for 10 rats. Statistically significant differences from the values for the control (vehicle) are indicated by * ($P < 0.05$).

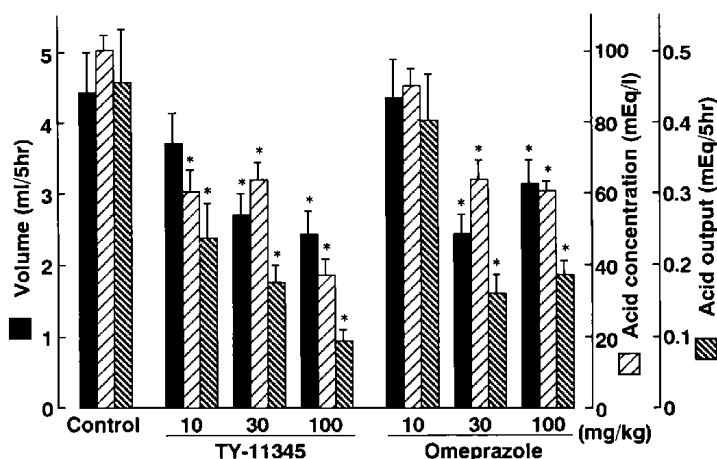


Fig. 8. Inhibitory effects of TY-11345 and omeprazole on gastric acid secretion in pylorus-ligated rats. The test compounds were administered orally 19 hr before pylorus ligation. Five hours after ligation, the gastric contents were collected and analyzed for volume, acid concentration and acid output. Each column represents the mean \pm S.E. for 8 rats. Statistically significant differences from the values for the control (vehicle) are indicated by * ($P < 0.05$).

Table 1. Comparison of ED₅₀ values of TY-11345 and omeprazole in antiulcer activities

	No. of rats	ED ₅₀ (mg/kg)	
		TY-11345	Omeprazole
Acute gastric lesion			
Water-immersion stress	13	0.92 (0.43— 1.91)	7.12 (4.56—10.43)
Indomethacin	9	0.64 (0.05— 1.62)	9.72 (5.30—17.09)
Ethanol	15	10.5 (8.78—12.55)	12.5 (10.60—14.78)
Acute duodenal ulcer			
Mepirizole	9	0.90 (0.55— 1.40)	2.84 (1.29— 5.86)
Chronic gastric ulcer			
Acetic acid	12	16.5 (13.58—20.29)	53.9%*

The ED₅₀ values were calculated from the dose-inhibition relationships for 9 to 15 rats by the probit method. Numbers in parentheses represent the 95% fiducial limits of the ED₅₀ values. The compounds were administered orally once at 0.5 hr before stress, indomethacin and ethanol. In the case of mepirizole-induced duodenal ulcers, the compounds were administered orally twice at 0.5 hr before and 9 hr after mepirizole. In the case of acetic acid-induced gastric ulcers, the compounds were administered orally once daily for 14 consecutive days. * expresses the healing acceleration of ulcer compared with the control group at the dose of 30 mg/kg/day.

Indomethacin-induced gastric lesions: Indomethacin produced multiple lesions in the glandular stomach 6 hr after the treatment; the mean lesion index in the vehicle-administered rats was 10.5 ± 1.1 mm ($n=9$). TY-11345, administered orally at 0.3, 1 and 3 mg/kg, dose-dependently inhibited the development of these lesions; the inhibitory percentage of the lesion index at each dose was 36%, 52% and 87%, respectively. The ED₅₀ value of TY-11345 was 0.64 mg/kg. Omeprazole also dose-dependently inhibited the lesions. The ED₅₀ value of omeprazole was 9.72 mg/kg. The effect of TY-11345 was 15 times more potent than that of omeprazole (Table 1).

Ethanol-induced gastric lesions: Ethanol produced hemorrhagic band-like lesions in the glandular stomach 1 hr after the treatment; the mean lesion index in the vehicle-administered rats was 80.5 ± 6.4 mm ($n=15$). TY-11345, administered orally at 3, 10 and 30 mg/kg, dose-dependently inhibited the development of these lesions; the inhibitory percentage of the lesion index at each dose was 10%, 46% and 88%, respectively. The ED₅₀ value of TY-11345 was 10.5 mg/kg. Omeprazole also dose-dependently inhibited the lesions. The ED₅₀ value of omeprazole was 12.5 mg/kg. The effect of TY-11345 was similar to that of omeprazole (Table 1).

Mepirizole-induced duodenal ulcers: Mepirizole produced one or two penetrating ulcers in the proximal duodenum, the mean ulcer index in the vehicle-administered rats was 14.8 ± 2.5 mm² ($n=9$). TY-11345, administered orally twice at 0.3, 1.0 and 3.0 mg/kg, dose-dependently inhibited the development of these ulcers, the inhibitory percentage of the ulcer index at each dose was 8%, 64% and 87%, respectively. The ED₅₀ value of TY-

11345 was 0.90 mg/kg. Omeprazole also dose-dependently inhibited the ulcers. The ED₅₀ value of omeprazole was 2.84 mg/kg. The effect of TY-11345 was 3 times more potent than that of omeprazole (Table 1).

Effect on chronic experimental ulcers

Acetic acid-induced gastric ulcers: The submucosal injection of acetic acid solution produced visible and consistent ulcers in the stomach; the mean ulcer index in the vehicle-administered rats was 6.76 ± 1.14 mm² ($n=12$). Oral administration of TY-11345, at 10 and 30 mg/kg once daily, for 14 days, dose-dependently accelerated the healing of the ulcers, the inhibitory percentage of the ulcer index at each dose was 52% and 82%, respectively. The ED₅₀ value of TY-11345 was 16.5 mg/kg/day. However, omeprazole tended to accelerate the healing at the dose of 30 mg/kg once a daily, for 14 days. The healing effect of TY-11345 was superior to that of omeprazole (Table 1).

DISCUSSION

The newly synthesized proton pump inhibitor TY-11345 exhibited a potent antiseecretory effect, and it markedly prevented the gastric and duodenal mucosal lesions and accelerated the healing of acetic acid ulcers in various experimental animals.

Previous reports have shown that the proton pump inhibitors, such as omeprazole, exist predominantly in their neutral base form at physiological pH, and they show their inhibitory effect when transformed into the active form sulfenamide, which modifies the SH group of H⁺/K⁺-ATPase under acid conditions in the parietal cells (4,

14). In the *in vitro* study, TY-11345 potently inhibited H^+/K^+ -ATPase activity in isolated rabbit gastric mucosal microsomes in acidic solution (pH 6.0). At pH 6.0, the IC_{50} values of TY-11345 and omeprazole were $5.8 \mu M$ and $16.1 \mu M$, respectively. Our results for omeprazole confirmed the findings of Wallmark et al. (15), who found that the activity of omeprazole is pH-dependent; the efficacy of omeprazole was potent when the pH of the medium was 6.0, but it became weak at pH 7.4. TY-11345 also showed a pH-dependent response; the effect of TY-11345 was about 3 times more potent compared with that of omeprazole at pH 6.0. In addition, the nearly peak effect of TY-11345 was attained more rapidly than that of omeprazole, when compared with the time course of H^+/K^+ -ATPase inhibition. Furthermore, from nuclear magnetic resonance studies, we have data confirming the transformation of TY-11345 into the sulfenamide form under the acidic condition (data not shown). From these data, we can deduce that TY-11345 is likely to be transformed into the SH-reactive form, because it contains a cycloheptenopyridine ring as a fundamental structural element.

In the *in vivo* study, TY-11345 significantly suppressed both the basal and stimulated acid secretion. In Ghosh & Schild rats, the effect of TY-11345 for tetragastrin stimulated acid secretion was twice or 3 times more potent than that of omeprazole when they were administered intravenously. These results indicated that the potent antiseecretory effect of TY-11345 was correlated with the inhibition of H^+/K^+ -ATPase; the inhibitory effect of TY-11345 on H^+/K^+ -ATPase was also 3 times more potent *in vitro* compared to that of omeprazole. In pylorus ligated rats, TY-11345 also showed the antiseecretory effect, and the effect by intravenous, intraduodenal or oral routes was 4 to 9 times more potent than that of omeprazole. Thus, TY-11345 showed the potent antiseecretory effect by the various routes. When compared with intraduodenal administration, the oral potency of TY-11345 was 3 times lower in pylorus ligated rats. Larsson et al. reported that the antiseecretory effect of omeprazole administered orally was attenuated compared with that administered intraduodenally (16). Since TY-11345 is unstable at acidic pH, partial degradation may have occurred in the stomach, before absorption, thereby resulting in a lower systemic bioavailability and effectiveness by the oral route. On the other hand, it should be noted that TY-11345 persistently suppressed gastric acid secretion 24 hr after the administration in pylorus ligated rats. This long and potential antisecretory effect would be a beneficial property for the clinical use of this drug for diseases with acid-induced damage.

As expected from its potent antisecretory effect, TY-11345 had a potent inhibitory effect on various types of acutely-induced gastric lesions; i.e., water-immersion-

stress-, indomethacin-induced gastric lesions. TY-11345 also prevented mepirizole-induced duodenal ulcers, which are presumed to be induced by the inflow of accumulated gastric juice into the proximal duodenum and attenuate defensive mechanism. These lesions are also prevented by histamine- H_2 -blocking agents (17, 18). These findings suggest that the pathogenesis of these lesions is related to gastric acid secretion. The anti-lesion effects of TY-11345 were 3–15 times more potent than that of omeprazole. We observed that the ED_{50} values of TY-11345 on these experimental lesions were considerably lower than that on acid output in pylorus ligated rats. Therefore, TY-11345 seems to prevent lesion formation mainly by a potent and long-acting antisecretory activity and partly by unknown mechanisms. Yamamoto et al. (17) reported 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. They indicated that omeprazole had a mucosal protective effect unrelated to the reduction of acid secretion.

TY-11345 administered orally also prevented mucosal lesions caused by absolute ethanol. This lesion model is often used to determine the cytoprotective property of drugs, as these lesions are not prevented by antisecretory agents but by low doses of prostaglandins (11). Omeprazole is also known to have a cytoprotective action (19, 20). We also confirmed the cytoprotective action of omeprazole. It is considered that the cytoprotective mechanism of omeprazole is the maintenance of gastric mucosal blood flow (21), mucus secretion (22) or mucosal oxygenation (23); however the accurate mechanism remains unknown, except that it is unrelated to the biosynthesis of mucosal prostaglandins or gastric acid inhibition (19, 24).

TY-11345 at 30 mg/kg significantly accelerated the spontaneous healing of chronic gastric ulcers induced by acetic acid, and omeprazole tended to accelerate the healing of ulcers at the same dose as TY-11345. Yamamoto et al. reported that omeprazole at 100 mg/kg twice a day accelerated the healing of ulcers (17).

These results taken together suggest that the new proton pump inhibitor TY-11345 has an excellent curative and preventive effect on gastric and duodenal ulcers, mainly by suppressing acid secretion through inhibition of H^+/K^+ -ATPase and partly by protecting the gastroduodenal mucosa, and will be a beneficial use for the treatment of human peptic ulcers and diseases with acid-induced damage.

Acknowledgments

We wish to thank Dr. S. Okabe, Department of Applied Pharmacology, Kyoto Pharmaceutical University, Kyoto, Japan, for critical reading of the manuscript.

REFERENCES

- 1 Forte, J.G., Forte, G.M. and Saltman, P.: K^+ -stimulated phosphatase of microsomes from gastric mucosa. *J. Cell. Physiol.* **69**, 293–304 (1967)
- 2 Lee, J., Simpson, G. and Scholest, P.: An ATPase from dog gastric mucosa: changes of outer pH in suspensions of membrane vesicles accompanying ATP hydrolysis. *Biochem. Biophys. Res. Commun.* **60**, 825–832 (1974)
- 3 Fellenius, E., Berglinde, T., Sachs, G., Olbe, L., Elander, B., Sjöstrand, S.E. and Wallmark, B.: Substituted benzimidazoles inhibit gastric acid secretion by blocking $(H^+ + K^+)$ ATPase. *Nature* **290**, 159–161 (1981)
- 4 Im, W.B., Sih, J.C., Blakeman, D.P. and McGrath, J.P.: Omeprazole, a specific inhibitor of gastric $(H^+ - K^+)$ -ATPase, is a H^- -activated oxidizing agent of sulfhydryl groups. *J. Biol. Chem.* **260**, 4591–4597 (1985)
- 5 Beil, W., Staar, U., Schuenemann, P. and Sewing, K.F.: Omeprazole, SCH 28080 and doxepin differ in their characteristics to inhibit hydrogen ion/potassium ATPase driven proton accumulation by parietal cell membrane vesicles. *Biochem. Pharmacol.* **37**, 4487–4493 (1988)
- 6 Yamaguchi, T., Aihara, K. and Kogi, K.: Antisecretory and antiulcer profiles of TY-11345, a new proton pump inhibitor. *Japan. J. Pharmacol.* **58**, Supp. I, 351P (1992)
- 7 Saccomani, G., Stewart, H.B., Shaw, D., Lewin, M. and Sachs, G.: Characterization of gastric mucosal membranes IX. Fractionation and purification of K^+ -ATPase containing vesicles by zonal centrifugation and free flow electrophoresis technique. *Biochim. Biophys. Acta* **465**, 311–330 (1977)
- 8 Ghosh, M.N. and Schild, H.B.O.: Continuous recording of gastric acid secretion in the rat. *Br. J. Pharmacol.* **13**, 54–58 (1958)
- 9 Takagi, K. and Okabe, S.: The effect of drugs on the production and recovery process of the stress ulcer. *Japan. J. Pharmacol.* **18**, 9–18 (1968)
- 10 Kolfshoten, A.A., Hagelen, F., Hillen, F.C., Jager, L.P., Zandberg, P. and Nordwijk, J.: Protective effect of prostaglandins against ulcerogenic activity of indomethacin during different stages of erosion development in rat stomach: role of acid and bicarbonate secretion. *Dig. Dis. Sci.* **28**, 1127–1132 (1983)
- 11 Robert, A., Nezamis, J.E., Lancaster, C. and Hanchar, A.J.: Cytoprotection by prostaglandins in rats. *Gastroenterology* **77**, 433–443 (1979)
- 12 Okabe, S., Ishihara, Y., Inoo, H. and Tanaka, H.: Mepirizole-induced duodenal ulcers in rats and their pathogenesis. *Dig. Dis. Sci.* **27**, 242–249 (1982)
- 13 Takagi, K., Okabe, S. and Saziki, R.: A new method for the production of chronic gastric ulcer in rats and the effect of several drugs on its healing. *Japan. J. Pharmacol.* **19**, 418–426 (1969)
- 14 Wallmark, B., Brandstrom, A. and Larsson, H.: Evidence for acid-induced transformation of omeprazole into an active inhibitor of $(H^+ + K^+)$ -ATPase within the parietal cell. *Biochim. Biophys. Acta* **778**, 549–558 (1984)
- 15 Wallmark, B., Lorentzon, P. and Larsson, H.: The mechanism of action of omeprazole – a survey of its inhibitory actions in vitro. *Scand. J. Gastroenterol.* **20**, Supp. **108**, 37–51 (1985)
- 16 Larsson, H., Carlsson, E., Junggren, U., Olbe, L., Sjöstrand, S.E., Skånberg, I. and Sundell, G.: Inhibition of gastric acid secretion by omeprazole in the dog and rat. *Gastroenterology* **85**, 900–907 (1983)
- 17 Yamamoto, O., Okada, Y. and Okabe, S.: Effects of a proton pump inhibitor, omeprazole, on gastric secretion and gastric and duodenal ulcers or erosions in rats. *Dig. Dis. Sci.* **29**, 349–401 (1984)
- 18 Okabe, S., Takeuchi, K., Urushidani, T. and Takagi, K.: Effects of cimetidine, a histamine H_2 -receptor antagonist, on various experimental gastric and duodenal ulcers. *Am. J. Dig. Dis.* **22**, 677–684 (1977)
- 19 Mattsson, H., Andersson, K. and Larsson, H.: Omeprazole provides protection against experimentally induced gastric mucosal lesions. *Eur. J. Pharmacol.* **91**, 111–114 (1983)
- 20 Okabe, S., Miyake, H. and Awane, Y.: Cytoprotective effects of NC-1300 and omeprazole on HCl-ethanol-induced gastric lesions in rats. *Japan. J. Pharmacol.* **42**, 123–133 (1986)
- 21 Holm, L.: Gastric mucosal blood flow and mucosal protection. *J. Clin. Gastroenterol.* **10**, Supp. **1**, S114–S119 (1988)
- 22 Ohara, S., Murayama, N., Kuwata, H., Ishihara, K. and Hotta, K.: Effects of omeprazole on rat gastric mucus glycoproteins with acetylsalicylic acid-induced gastric damage. *Arch. Int. Pharmacodyn. Ther.* **296**, 192–201 (1988)
- 23 Kawano, S., Tanimura, H., Sato, N., Tsuji, S., Takei, Y., Ogihara, T., Nagano, K., Fusamoto, H. and Kamada, T.: Effects of proton pump inhibitor on gastric mucosa hemodynamics and tissue oxygenation in anesthetized rats. *Eur. J. Pharmacol.* **211**, 55–60 (1992)
- 24 Konturek, S.J., Brzozowski, T. and Radecki, T.: Protective action of omeprazole, a benzimidazole derivative, on gastric mucosal damage by aspirin and ethanol in rats. *Digestio* **27**, 159–164 (1983)