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# Biochemical and Pharmacological Characteristics of 3-Butyryl-8-methoxy-4-[(2-thiophenyl)amino]quinoline, a New Proton-pump Inhibitor, in Rabbit Gastric Microsomes and in Rats

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**Abstract**

We have investigated the properties of the newly synthesized proton-pump inhibitor, 3-butyryl-8-methoxy-4-[(2-thiophenyl)amino]quinoline (YJA20379-6), on gastric mucosal proton-pump ($H^+/K^+$-ATPase) activity, gastric acid secretion and gastroduodenal lesions in experimental rats.

YJA20379-6 markedly inhibited $H^+/K^+$-ATPase activity in rabbit isolated gastric mucosal microsomes, confirming its classification as a proton-pump inhibitor. The inhibitory efficacy of YJA20379-6 on the proton pump was approximately 14-times higher than that of omeprazole at pH 7·4. YJA20379-6 given intraduodenally had a potent inhibitory effect on gastric secretion in pylorus-ligated rats (ED50 22·9 mg $kg^{-1}$) but was less active than omeprazole. Pretreatment of rats with YJA20379-6 dose-dependently protected the gastric mucosa from damage induced by water-immersion stress, indomethacin and absolute ethanol, and the duodenal mucosa from damage induced by mepirizole. Repeated administration of YJA20379-6 also dose-dependently accelerated the spontaneous healing of acetic acid-induced gastric ulcers.

These results suggest that YJA20379-6 has potent anti-secretory and anti-ulcer effects which are exerted by suppression of $H^+/K^+$-ATPase activity in gastric parietal cells. YJA20379-6 might be useful for the clinical treatment of peptic ulcer diseases.

The aetiology of peptic ulcer diseases is complex and multifactorial (Caldwell & McCallum 1991; Vaezi et al 1995). It is widely accepted, however, that peptic ulcers are caused by an imbalance between aggressive factors (acid and pepsin) and defensive mucosal factors (mucus, bicarbonate and blood flow). Consequently, anti-ulcer therapy has been mainly directed toward inhibition of the aggressive factors and enhancement of protective defensive factors (Kauffman 1985; Goldschmiedt et al 1989). An ideal anti-ulcer drug will, therefore, not only suppress gastric secretion but also have cytoprotective properties.

Yungjin Pharmaceutical are endeavouring to develop new compounds with potent anti-secretory and cytoprotective effects. 3-Butyryl-8-methoxy-4-[(2-thiophenyl)amino]quinoline (YJA20379-6) is a new potent inhibitor of $H^+/K^+$-ATPase activity. To determine the anti-secretory and anti-ulcer activity of this compound, the effect of YJA20379-6 on gastric acid secretion in pylorus-ligated rats and on gastric and duodenal lesions induced by indomethacin, water-immersion and restraint stress, absolute ethanol and mepirizole in rats was investigated. The effect of YJA20379-6 on the spontaneous healing of acetic acid-induced gastric ulcers was also studied. Simultaneous experiments were performed with omeprazole, a benzimidazole derivative which inhibits gastric secretion in man and laboratory animals by inhibition of $H^+/K^+$-ATPase in parietal cells (Wallmark et al 1986; Adams et al 1988) and is thus called a proton-pump inhibitor. This agent also has a potent cytoprotective effect in rats (Yamamoto et al 1984). However, because omeprazole binds covalently to the $H^+/K^+$-ATPase, reversal of its action requires either in-situ reduction of the disulphide bond formed or de-novo synthesis of $H^+/K^+$-ATPase

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(Im et al 1985). Omeprazole has been used clinically to treat patients with peptic ulcer diseases and is more effective than $H_2$-receptor antagonists for treatment of duodenal ulcers (Lauritsen et al 1985; Bardhan et al 1986; Archambault et al 1988), at least equally effective against benign gastric ulcer (Lauritsen et al 1988), and more effective for treatment of reflux oesophagitis (Klinkenberg-knol et al 1987; Havelund et al 1988; Bate et al 1990). In this work the biochemical and pharmacological properties of YJA20379-6 in experimental animals have been determined and compared with those of omeprazole.

## Materials and Methods

*Test compounds*

YJA20379-6 and omeprazole (Figure 1) were synthesized by Yungjin Pharmaceutical. For in-vitro studies test compounds were dissolved in dimethylsulphoxide (DMSO; International Specialty Chemical, Chicago, IL) and then diluted with 10 mM imidazole buffer (pH 7·4); the final concentration of DMSO was less than 1%. For in-vivo studies with oral administration the test compounds were suspended in 1% (w/v) sodium carboxymethylcellulose solution. For measurement of gastric acid secretion the compounds were suspended in 1% (w/v) sodium carboxymethylcellulose solution containing 0·2% (w/v) $NaHCO_3$ and the pH was adjusted to 9·0 with 2 M NaOH.

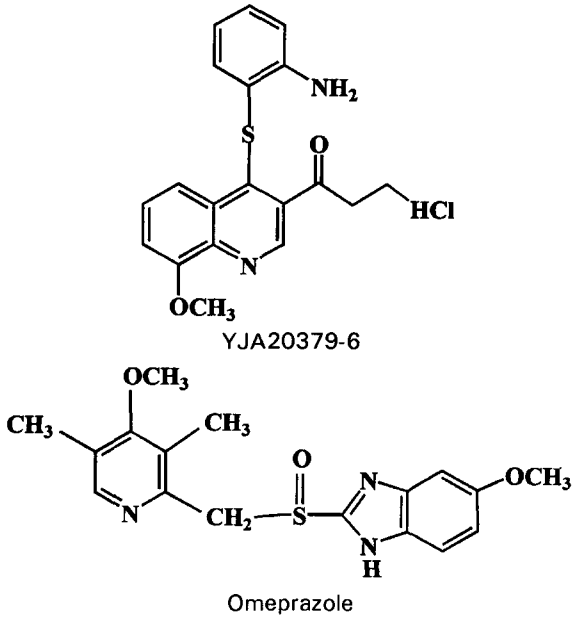


Figure 1. The chemical structures of YJA20379-6 and omeprazole.

*Biochemical studies*

*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 (1977). The stomach of New Zealand white rabbits, 2–3 kg, was dissected out and washed quickly in tap water. The fundic mucosal surface was immersed in 1 M NaCl and most of the surface epithelial cells removed with a glass slide. The fundic mucosa removed from the underlying muscular layer was suspended in approximately 10 vol of an ice-cold solution of Tris-HCl (20 mM)–sucrose (250 mM) buffer, pH 7·4.

Homogenization was performed with a Teflon-glass homogenizer (Wheaton, USA). The resulting homogenates were centrifuged at 8000 *g* for 10 min. The pellets were washed once and the combined supernatant was centrifuged at 105000 *g* 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 performed with a Sorvall F-28/36 rotor (DuPont, USA) at 105000 *g* for 2 h. 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 method of Lowry et al (1971) using bovine serum albumin as standard.

*Assay procedures.* Gastric $H^+/K^+$-ATPase activity was determined as described by Saccomani et al (1977). The enzyme protein (approx. 80 μg) was pre-incubated in 10 mM imidazole buffer (pH 7·4) containing different concentrations of either YJA20379-6 or omeprazole (final volume 0·5 mL). The pre-incubation time was 30 min at 37 °C and the enzyme reaction was started by adding 0·5 mL of a mixture containing 4 mM $MgCl_2$, 4 mM ATP and 80 mM imidazole buffer (pH 7·4), with or without 20 mM KCl. After incubation for 15 min at 37 °C, the reaction was stopped by addition of ice-cold 24% trichloroacetic acid (1 mL). Inorganic phosphate formed by hydrolysis of ATP was determined by the method of Fiske & Subbarow (1925).

*Pharmacological studies*

*Measurement of basal gastric acid secretion (pylorus-ligated rats).* Male Sprague–Dawley rats, 180–220 g, were fasted for 24 h before the experiment but allowed free access to water. Under ether anaesthesia, the abdomen was incised and the

pylorus ligated. Test compounds YJA20379-6 (3, 10 or 30 mg $kg^{-1}$) or omeprazole (3, 6 or 12 mg $kg^{-1}$) or vehicle were administered intraduodenally immediately after ligation. Rats were killed by cervical dislocation 4 h after pylorus ligation. The gastric contents were collected and the volume and acidity determined. Acidity was determined with a pH meter (Orion, USA) against 0·01 M NaOH to pH 7·0.

*Water-immersion stress-induced gastric lesions.* Male Sprague–Dawley rats, 180–200 g, were fasted, but allowed free access to water, for 24 h before the experiment. The rats were placed in a restraint cage, immersed vertically to the level of the xiphoid process in a water bath (21–23 °C) for 7 h and then killed. The stomach of each rat was removed and inflated for 10 min by injection of formalin (3%, 10 mL) to fix the inner and outer layers of the gastric wall. This formalin treatment was performed in all the experiments. The stomach was subsequently incised along the greater curvature and examined for lesions in the glandular portion. YJA20379-6 or omeprazole (3, 10 or 30 mg $kg^{-1}$) or vehicle were administered orally 30 min before stressing.

*Indomethacin-induced gastric lesions.* Female Sprague–Dawley rats, 160–180 g, were fasted, but allowed free access to water, for 48 h before the experiment. Indomethacin (Sigma, St Louis, MO) suspended in Tween-saline was given subcutaneously at a dose of 35 mg $kg^{-1}$. The rats were killed 7 h later and the stomach examined for lesions in the glandular portion. YJA20379-6 or omeprazole (1, 3 or 10 mg $kg^{-1}$) or vehicle were administered orally 30 min before indomethacin treatment.

*Ethanol-induced gastric lesions.* Male Sprague–Dawley rats, 180–200 g, were fasted, but allowed free access to water, for 24 h before the experiments. The rats were dosed orally with absolute ethanol (Hayman, UK; 1 mL) then killed 1·5 h later and the stomach examined for lesions in the glandular portion. YJA20379-6 or omeprazole (3, 10 or 30 mg $kg^{-1}$) or vehicle were administered orally 30 min before ethanol treatment.

*Mepirizole-induced duodenal ulcers.* Mepirizole (Sigma) suspended in 1% sodium carboxymethylcellulose was administered orally (200 mg $kg^{-1}$) to male Sprague–Dawley rats, 180–200 g, which were then deprived of food and water. The rats were killed 24 h later and examined for ulcers in the duodenum. YJA20379-6 (3, 10 or 30 mg $kg^{-1}$) or omeprazole (1, 3, or 10 mg $kg^{-1}$) or vehicle were administered orally 30 min before mepirizole treatment.

*Acetic acid-induced gastric ulcers.* Male Sprague–Dawley rats, 200–220 g, were fasted for 5 h before injection of acetic acid into the submucosal layer. The abdomen of ether-anaesthetized rats was incised, the anterior portion of the stomach exposed, and acetic acid (30% v/v; 0·02 mL) was injected into the submucosal layer at the junction of the fundus and antrum, approximately 1 cm proximal to the pylorus. After the operation the animals had free access to rat chow and water. YJA20379-6 or omeprazole (10, 30 or 100 mg $kg^{-1}$) or vehicle were then administered orally twice daily (0900 and 1800 h) commencing the day after surgery for eight consecutive days. The rats were killed 16 h after the final administration of drugs and the stomachs examined for ulcers.

*Ulcer or lesion index*

The length (mm) of each lesion induced by water-immersion stress, indomethacin or ethanol was measured macroscopically, summed for each 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 for each stomach, and the total used as the ulcer index.

*Statistics*

Data are expressed as means ± s.e.m. In biochemical studies one-way analysis of variance was performed at each drug concentration and pairs of results were compared by means of Duncan's multiple-range test. In pharmacological studies Duncan's multiple-range test was also employed 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%) and 95% confidence limits were calculated by the probit method.

## Results

*Effects on $H^+/K^+$-ATPase activity in rabbit gastric mucosal microsomes*

YJA20379-6 and omeprazole concentration-dependently inhibited $H^+/K^+$-ATPase activity in rabbit isolated gastric mucosa (Figure 2). The concentration of YJA20379-6 which inhibited 50% of $H^+/K^+$-ATPase activity (IC50) in the presence

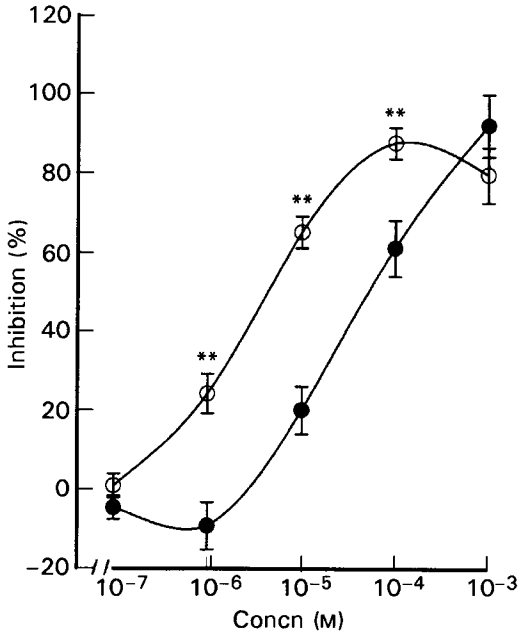


Figure 2. The inhibitory effects of ○ YJA20379-6 and ● omeprazole on $H^+/K^+$-ATPase activity at pH 7·4 in rabbit gastric microsomes. YJA20379-6 or omeprazole was pre-incubated with $H^+/K^+$-ATPase for 30 min and the ATPase assay was initiated by adding 4 mM ATP. Each point represents the mean ± s.e.m. of results from three experiments. $^{**}P < 0.01$, significantly different from inhibition of $H^+/K^+$-ATPase in the omeprazole-treated group.

of 20 mM KCl was $4.17 \times 10^{-6}$ M at pH 7·4. The IC50 for omeprazole was $5.75 \times 10^{-5}$ M at pH 7·4.

*Effects on gastric acid secretion*

Basal gastric acid secretion in pylorus-ligated rats was $3.27 \pm 0.81$ mEq kg$^{-1}$/4 h (mean ± s.e.m.). YJA20379-6 (3–30 mg kg$^{-1}$) dose-dependently inhibited basal acid secretion in pylorus-ligated rats after intraduodenal administration (Table 1). The ED50 value (95% confidence limits) was 22·9 (6·2–79·3) mg kg$^{-1}$ (Table 1). Omeprazole given intraduodenally inhibited basal gastric acid secretion with an ED50 of 2·4 (0·8–7·2) mg kg$^{-1}$.

*Effects on water-immersion stress-induced gastric lesions*

Water-immersion stress for 7 h caused several linear and dotted erosions in the glandular stomach with a mean lesion index in vehicle-treated rats of $43.9 \pm 4.2$ mm (n = 8) in the YJA20379-6 experiment. YJA20379-6 administered orally at doses of 3, 10 and 30 mg kg$^{-1}$ dose-dependently inhibited formation of these lesions; the percentage inhibition of the lesion index at each dose was 26·5, 45·3 and 56·7%, respectively. The ED50 value of YJA20379-6 (95% confidence limits) was 16·8 (6·2–45·4) mg kg$^{-1}$ (Table 2). Omeprazole also significantly inhibited lesion formation; the percentage inhibition of the lesion index at each dose was 19·9, 69·7 and 95·7%, respectively. The ED50 of omeprazole was 6·4 (3·8–10·8) mg kg$^{-1}$. YJA20379-6 was less potent than omeprazole (Table 2).

*Effects on indomethacin-induced gastric lesions*

Indomethacin caused multiple lesions in the glandular stomach 7 h after treatment; the mean lesion index in vehicle-treated rats was $26.7 \pm 4.9$ mm (n = 6) in the YJA20379-6 experiment. YJA20379-6 administered orally at doses of 1, 3 and 10 mg kg$^{-1}$ dose-dependently inhibited formation of these lesions; the percentage inhibition of the lesion index at each dose was 49·4, 73·8 and 80·8%, respectively. The ED50 of YJA20379-6 was 0·8 (0·2–4·6) mg kg$^{-1}$ (Table 3). Omeprazole also significantly inhibited the development of lesions; the percentage inhibition of the lesion index at each dose was 43·5, 80·6 and 97·7%, respectively. The ED50 value of omeprazole was

Table 1. Inhibitory effects of YJA20379-6 and omeprazole on gastric acid secretion in pylorus-ligated rats.

| Treatment | Gastric acid secretion | | Inhibition (%) | ED50 (mg kg$^{-1}$) (95% confidence limits) |
|---|---|---|---|---|
| | Volume (mL/4 h) | Total acid output (mEq kg$^{-1}$/4 h) | | |
| Vehicle | 6·08 ± 0·75 | 3·27 ± 0·81 | – | |
| YJA20379-6 (3 mg kg$^{-1}$) | 6·07 ± 0·98 | 3·56 ± 0·80 | −8·9 | 22·9 |
| YJA20379-6 (10 mg kg$^{-1}$) | 4·13 ± 0·40 | 2·23 ± 0·54 | 31·8 | (6·2–79·3) |
| YJA20379-6 (30 mg kg$^{-1}$) | 3·10 ± 0·46 | 1·45 + 0·33* | 55·7 | |
| Vehicle | 5·58 ± 0·58 | 3·42 ± 0·60 | – | |
| Omeprazole (3 mg kg$^{-1}$) | 3·37 ± 0·50 | 1·67 ± 0·23** | 51·2 | 2·4 |
| Omeprazole (6 mg kg$^{-1}$) | 3·12 ± 0·49 | 0·76 ± 0·37** | 77·8 | (0·8–7·2) |
| Omeprazole (12 mg kg$^{-1}$) | 2·78 ± 0·20 | 0·69 + 0·19** | 79·8 | |

The test compounds were administered intraduodenally immediately after pylorus ligation. Gastric contents were collected 4 h after ligation and analysed for acid output. Values are means ± s.e.m. of results from three experiments (n = 7). $^{*}P < 0.05$, $^{**}P < 0.01$ significantly different from the vehicle-treated control group.

Table 2. Effects of YJA20379-6 and omeprazole on water-immersion stress-induced gastric lesions in rats.

| Treatment | Lesion index (mm) | Inhibition (%) | ED50 (mg kg$^{-1}$) (95% confidence limits) |
|---|---|---|---|
| Vehicle | 43·91 ± 4·22 | – | |
| YJA20379-6 (3 mg kg$^{-1}$) | 32·29 ± 4·29 | 26·5 | 16·8 |
| YJA20379-6 (10 mg kg$^{-1}$) | 24·00 ± 2·76** | 45·3 | (6·2–45·4) |
| YJA20379-6 (30 mg kg$^{-1}$1) | 19·00 ± 3·64** | 56·7 | |
| Vehicle | 43·91 ± 3·17 | – | |
| Omeprazole (3 mg kg$^{-1}$) | 35·17 ± 2·18 | 19·9 | 6·4 |
| Omeprazole (10 mg kg$^{-1}$) | 13·29 ± 1·90** | 69·7 | (3·8–10·8) |
| Omeprazole (30 mg kg$^{-1}$) | 1·88 ± 0·83** | 95·7 | |

Drug or vehicle was given orally 30 min before the start of water immersion. Rats (n = 8) were killed 7 h after immersion and the lesion index measured. Values are means ± s.e.m. of results from three experiments. $^{**}P < 0.01$ significantly different from vehicle-treated control group.

Table 3. Effects of YJA20379-6 and omeprazole on indomethacin-induced gastric lesions in rats.

| Treatment | Lesion index (mm) | Inhibition (%) | ED50 (mg kg$^{-1}$) (95% confidence limits) |
|---|---|---|---|
| Vehicle | 26·67 ± 4·94 | – | |
| YJA20379-6 (1 mg kg$^{-1}$) | 13·50 ± 2·16* | 49·4 | 0·8 |
| YJA20379-6 (3 mg kg$^{-1}$) | 7·00 ± 2·27** | 73·8 | (0·2–4·6) |
| YJA20379-6 (10 mg kg$^{-1}$) | 5·13 ± 1·98** | 80·8 | |
| Vehicle | 25·59 ± 3·34 | – | |
| Omeprazole (1 mg kg$^{-1}$) | 14·46 ± 1·13** | 43·5 | 1·2 |
| Omeprazole (3 mg kg$^{-1}$) | 4·96 ± 0·87** | 80·6 | (0·5–2·7) |
| Omeprazole (10 mg kg$^{-1}$) | 0·59 ± 0·30** | 97·7 | |

Drug or vehicle was given orally 30 min before subcutaneous administration of indomethacin (35 mg kg$^{-1}$). Rats (n = 7) were killed 7 h after indomethacin and the lesion index was measured. Each value is the mean lesion index ± s.e.m. of results from three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly different from vehicle-treated control group.

Table 4. Effects of YJA20379-6 and omeprazole on absolute ethanol-induced gastric lesions in rats.

| Treatment | Lesion index (mm) | Inhibition (%) | ED50 (mg kg$^{-1}$) (95% confidence limits) |
|---|---|---|---|
| Vehicle | 82·67 + 16·59 | – | |
| YJA20379-6 (3 mg kg$^{-1}$) | 65·33 ± 9·83* | 21·0 | 13·3 |
| YJA20379-6 (10 mg kg$^{-1}$) | 51·00 ± 10·63** | 38·3 | (6·1–29·4) |
| YJA20379-6 (30 mg kg$^{-1}$) | 24·40 ± 10·63** | 70·5 | |
| Vehicle | 84·40 ± 9·16 | – | |
| Omeprazole (3 mg kg$^{-1}$) | 83·97 ± 1·10 | 0·5 | 17·1 |
| Omeprazole (10 mg kg$^{-1}$) | 55·50 ± 3·50** | 34·2 | (9·6–30·3) |
| Omeprazole (30 mg kg$^{-1}$) | 23·00 ± 3·62** | 72·7 | |

Drug or vehicle was given orally 30 min before the administration of absolute ethanol. Rats (n = 7) were killed 1·5 h after ethanol and the lesion index was measured. Each value is the mean lesion index ± s.e.m. of results from three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly different from vehicle-treated control group.

1·2 (0·5–2·7) mg kg$^{-1}$ (Table 3). The effect of YJA20379-6 was similar to that of omeprazole.

*Effects on ethanol-induced gastric lesions*

When absolute ethanol was given to the control animals haemorrhagic band-like lesions developed in the glandular portion of the stomach; the mean lesion index in vehicle-treated animals was 82·7 ± 16·6 mm (n = 7). YJA20379-6 administered orally at doses of 3, 10 and 30 mg kg$^{-1}$ dose-dependently inhibited lesion formation; the percentage inhibition of the lesion index at each dose

Table 5. Effects of YJA20379-6 and omeprazole on mepirizole-induced duodenal ulcers in rats.

| Treatment | Ulcer index ($mm^2$) | Inhibition (%) | ED50 ($mg\,kg^{-1}$) (95% confidence limits) |
|---|---|---|---|
| Vehicle | 14·00 ± 3·10 | – | |
| YJA20379-6 (3 $mg\,kg^{-1}$) | 13·00 ± 1·58 | 7·1 | 20·2 |
| YJA20379-6 (10 $mg\,kg^{-1}$) | 9·40 ± 2·19* | 32·9 | (10·1–40·5) |
| YJA20379-6 (30 $mg\,kg^{-1}$) | 5·60 ± 2·10** | 60·0 | |
| Vehicle | 13·38 ± 2·10 | – | |
| Omeprazole (1 $mg\,kg^{-1}$) | 10·29 ± 2·30 | 23·1 | 2·8 |
| Omeprazole (3 $mg\,kg^{-1}$) | 6·57 ± 2·30* | 50·9 | (1·2–6·3) |
| Omeprazole (10 $mg\,kg^{-1}$) | 2·29 ± 1·38** | 82·9 | |

Drug or vehicle was given orally 30 min before administration of mepirizole. Rats (n = 8) were killed 24 h after mepirizole and the duodenal ulcer index was measured. Each value represents the mean duodenal ulcer index ± s.e.m. of results from three experiments. *$P < 0.05$, **$P < 0.01$ significantly different from vehicle-treated control group.

was 21·0, 38·3 and 70·5%, respectively. The ED50 value of YJA20379-6 was 13·3 (6·1–29·4) $mg\,kg^{-1}$ (Table 4). Omeprazole also significantly inhibited formation of 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 (9·6–30·3) $mg\,kg^{-1}$ (Table 4). The effect of YJA20379-6 was similar to that of omeprazole.

*Effects on mepirizole-induced duodenal ulcers*

Mepirizole resulted in the development of one or two penetrating ulcers in the proximal duodenum; the mean ulcer index in the control animals was 14·0 ± 3·1 mm (n = 8). YJA20379-6, administered orally at doses of 3, 10 and 30 $mg\,kg^{-1}$, dose-dependently inhibited formation of these ulcers; the percentage inhibition of the ulcer index at each dose was 7·1, 32·9 and 60·0%, respectively. The ED50 value of YJA20379-6 was 20·2 (10·1–40·5) $mg\,kg^{-1}$ (Table 5). Omeprazole also significantly inhibited ulcer formation; the inhibition of the ulcer index at each dose was 23·1, 50·9 and 82·9%, respectively. The ED50 of omeprazole was 2·8 (1·2–6·3) $mg\,kg^{-1}$ (Table 5). YJA20379-6 was approximately 10-times less potent than omeprazole.

*Effects on acetic acid-induced gastric ulcers*

Submucosal injection of 30% acetic acid (0·02 mL) induced the development of visible and consistent ulcers within the stomach; the mean ulcer index in vehicle-treated rats was 21·8 ± 2·2 $mm^2$ (n = 10) in the YJA20379-6 experiment. YJA20379-6 given orally twice daily for 8 days dose-dependently accelerated the healing of the ulcers, the inhibition of the ulcer index at 20, 60 and 200 $mg\,kg^{-1}\,day^{-1}$ was 6·5, 30·0 and 36·6%, respectively. Omeprazole also dose-dependently accelerated the healing of the ulcers; the inhibition of the ulcer index at the same doses was 24·5, 27·1 and 44·0%, respectively (Table 6). The healing effect of YJA20379-6 was similar to that of omeprazole.

Table 6. Effects of YJA20379-6 and omeprazole on the healing of acetic acid-induced gastric ulcers in rats.

| Treatment | Ulcer index ($mm^2$) | Inhibition (%) |
|---|---|---|
| Vehicle | 21·75 ± 2·23 | – |
| YJA20379-6 (10 $mg\,kg^{-1}$) | 20·34 ± 3·00 | 6·5 |
| YJA20379-6 (30 $mg\,kg^{-1}$) | 15·23 ± 2·55* | 30·0 |
| YJA20379-6 (100 $mg\,kg^{-1}$) | 13·80 ± 1·70** | 36·6 |
| Vehicle | 21·75 ± 1·30 | – |
| Omeprazole (10 $mg\,kg^{-1}$) | 16·42 ± 2·08 | 24·5 |
| Omeprazole (30 $mg\,kg^{-1}$) | 15·86 ± 3·00 | 27·1 |
| Omeprazole (100 $mg\,kg^{-1}$) | 12·18 ± 2·62** | 44·0 |

Drug or vehicle was given orally twice daily for 8 days after ulceration. The rats (n = 10) were killed 16 h after the last administration of the drug or vehicle and the gastric ulcer index was measured. Each value represents the mean gastric ulcer index ± s.e.m. of results from three experiments. *$P < 0.05$, **$P < 0.01$ significantly different from vehicle-treated control group.

## Discussion

YJA20379-6, a new proton pump inhibitor, has potent anti-secretory effect, anti-gastric and anti-duodenal mucosal lesion activity and potent healing effect on acetic acid-induced ulcers. No mortality resulted from intraperitoneal administration of 2000 mg $kg^{-1}$ YJA20379-6 to male ICR mice ($n = 10$) in an acute toxicity test. It is suggested that the intraperitoneal LD50 of YJA20379-6 might be more than 2000 mg $kg^{-1}$ and that it is a safe compound.

Omeprazole, used world-wide as an anti-ulcer drug, was first described in 1983 (Wallmark et al 1983). Under acid conditions it binds irreversibly to the SH group of $H^+/K^+$-ATPase in the parietal cells and markedly inhibits gastric acid secretion in rats and dogs for a prolonged period. Continuous long-term administration of omeprazole has been shown to induce the development of gastric carcinoma in experimental animals (Ekman et al 1985; Tielemans et al 1989). This unwanted side effect is now thought to be because pharmacological blockade of acid secretion results in long-lasting hypergastrinaemia, which in turn gives rise to hyperplasia of certain endocrine cells, the so-called enterochromaffin-like cells (Larsson et al 1986). An attempt was, therefore, made to discover a new drug such as YJA20379-6 which would have potent anti-secretory activity but without effect on endogenous hormones. The current studies were designed to determine whether YJA20379-6 met these criteria.

The inhibitory activity of YJA20379-6 on rabbit gastric $H^+/K^+$-ATPase was about 14 times more potent than that of omeprazole, and in-vivo YJA20379-6 significantly suppressed basal gastric acid secretion. In pylorus-ligated rats the effect of YJA20379-6 was about 10-times less potent than that of omeprazole administered intraduodenally. YJA20379-6 has the characteristic of potent inhibitory activity on in-vitro gastric $H^+/K^+$ATPase and mild suppression of in-vivo gastric acid secretion, which implies that inhibition of gastric secretion by YJA20379-6 might be reversible, in contrast with the behaviour of omeprazole. It is possible, therefore, that this drug markedly suppresses gastric acid secretion but only in the short-term. It is not clear, however, whether the two are functioning by the same mechanism.

As expected from its anti-secretory effect, YJA20379-6 had a potent protective effect on various types of acute gastric mucosal lesion, i.e. indomethacin-, absolute ethanol- and water-immersion stress-induced gastric lesions. YJA20379-6 also prevented mepirizole-induced acute duodenal ulcers, which are thought to be induced by inflow of accumulated gastric juice into the proximal duodenum with consequential attenuation of the defensive mechanism (Okabe et al 1982; Tanaka et al 1989).

The pathogenesis of these lesions is related to gastric acid secretion, and anti-ulcer activity depends closely on anti-secretory potency. The anti-lesion effects of YJA20379-6 were 5–10-times less potent than those of omeprazole. The ED50 values of YJA20379-6 on these experimental lesions were lower than for suppression of acid output in pylorus-ligated rats. These results confirm previous findings that anti-secretory doses of omeprazole inhibit indomethacin-, water-immersion- and mepirizole-induced lesions and that gastric acid secretion is involved in the pathogenesis of the ulcers (Kitagawa et al 1979; Kolfshoten et al 1983). In particular, the anti-ulcer effect of YJA20379-6 on indomethacin-induced gastric lesions was similar to that of omeprazole. Because the anti-secretory effect of YJA20379-6 is relatively weak, the anti-ulcer effect seen on indomethacin-induced gastric lesions was especially noteworthy and implies that YJA20379-6 prevents the formation of lesions via an anti-secretory mechanism and action on mucosal prostaglandin production, because the pathogenesis of indomethacin-induced gastric lesions involves increases in gastric acid and gastric motility and the depletion of mucosal prostaglandins (Lippman 1974; Takeuchi et al 1986).

Oral administration of YJA20379-6 also prevented mucosal lesions caused by absolute ethanol. Necrotizing agent (e.g. hydrochloric acid, ethanol)-induced gastric lesions are not inhibited by suppression of gastric acid secretion, but rather by cytoprotective effects. It was shown that omeprazole protected gastric mucosa against absolute ethanol by the same route as YJA20379-6, but when administered by intravenous injection it was not protective (Mattsson et al 1983). The inhibitory mechanisms of omeprazole in this lesion model are related to the activation of defensive mechanisms such as an increase in mucosal blood flow and glycoprotein content (Holm 1988; Ichikawa et al 1991). YJA20379-6 also had a cytoprotective effect, but the dosage required in this lesion was similar to that required against acid secretion of pylorus-ligated rats. It is therefore likely that YJA20379-6 exerts a cytoprotective effect via a mechanism different from that of omeprazole. Further experiments are required to determine the precise mechanisms of action of this drug. YJA20379-6 (60 mg $kg^{-1}$ $day^{-1}$) 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 YJA20379-6. Yamamoto et al (1984) also reported that omeprazole (100 mg $kg^{-1}$, twice-a-day) accelerated the healing. It is postulated that this acceleration of the healing of ulcers is a result of biosynthesis of gastric mucus glycoprotein and the anti-secretory action of omeprazole (Ichikawa et al 1991). Although the results suggest that the therapeutic effects of YJA20379-6 might be similar to those of omeprazole, more detailed experiments are needed to confirm the mechanisms.

In conclusion, the new proton pump inhibitor YJA20379-6 has, in an animal model, excellent prophylactic and therapeutic effects on gastric and duodenal ulcers, mainly as a result of suppression of acid secretion through inhibition of $H^+/K^+$-ATPase and partly by protection of the gastroduodenal mucosa. It is expected, therefore, that YJA20379-6 will prove to be beneficial in man for the treatment of peptic ulcers and other diseases involving acid-induced damage.

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