ELSEVIER

European Journal of Pharmacology 378 (1999) 299–310

ejp

www.elsevier.nl/locate/ejphar

FR145715, a novel histamine H_2 receptor antagonist, with specific anti-*Helicobacter pylori* activities

Hirohumi Ishikawa [a,*], Harunobu Ito [a], Masahide Higaki [a], Mika Higaki [a], Yoshimi Matsumoto [a], Toshiaki Kamimura [a], Yousuke Katsura [b], Tetsuo Tomishi [b], Yoshikazu Inoue [b], Hisashi Takasugi [b], Masaaki Tomoi [c], Steven Krakowka [d], Keizo Yoshida [e]

[a] *Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical, 1-6, 2-Chome, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[b] *Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical, 1-6, 2-Chome, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[c] *Project Accelerating Staff, Fujisawa Pharmaceutical, 1-6, 2-Chome, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[d] *Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 USA*
[e] *Exploratory Clinical Research, Fujisawa Pharmaceutical, 1-6, 2-Chome, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*

Received 17 June 1999; accepted 25 June 1999

Abstract

The pharmacological profile of *N*-[3-[2-[*N*′-(2-methoxyethyl)guanidino]thiazol-4yl]benzyl-acetamide (FR145715), a novel histamine H_2 receptor antagonist, was examined in both in vitro and in vivo models using experimental animals in comparison with ranitidine. In isolated guinea-pig atria, FR145715 antagonized the effect of histamine on heart rate with approximately three times more potent activity than ranitidine. In in vivo experiments, intraduodenal FR145715 dose-dependently inhibited spontaneous gastric acid secretion in rats (Shay's rats), with a ED_{50} value of 18.4 mg/kg, which was comparable to that of ranitidine (30.5 mg/kg). FR145715 also inhibited histamine-stimulated acid secretion in stomach-perfused anaesthetized rats (Schild's rats), when given intravenously and intraduodenally with ED_{50} values of 0.59 and 2.72 mg/kg, respectively. Ranitidine displayed more potent activity having respective ED_{50} values of 0.10 and 0.17 mg/kg. In Heidenhain pouch dogs, intravenous and oral FR145715 dose-dependently inhibited gastrin-stimulated acid secretion with respective ED_{50} values of 0.12 and 0.32 mg/kg, which were similar to those of ranitidine (0.09 and 0.33 mg/kg). In gastric ulcer models, FR145715 dose-dependently inhibited water immersion restraint stress- and acidified aspirin-induced gastric lesions with ED_{50} values of 3.2 and 15.1 mg/kg (po), respectively. The comparative compound, ranitidine, also showed beneficial effects on stress-induced gastric ulcers with an ED_{50} value of 1.5 mg/kg (po). However, it failed to inhibit acidified aspirin-induced gastric ulcers. FR145715 inhibited HCl-induced gastric lesions in rats, while pre-treatment with indomethacin abolished its beneficial effects, suggesting that FR145715 has a so-called cytoprotective effect which is dependent on endogenous prostaglandin production. In addition to its atypical profile as a histamine H_2 receptor antagonist, FR145715 exhibited strong anti-microbial activities against strains of *Helicobacter pylori* (*H. pylori*) with a mean minimal inhibitory concentration value of 0.32 μg/ml. Moreover, FR145715 showed no anti-microbial effects on 25 other bacteria examined. In addition, in vivo experiments using gnotobiotic piglets infected with *H. pylori*, FR145715 (16 mg/kg, t.i.d.) completely eliminated the organism with reduced intensity of inflammation, when treated orally for 10 days. These data demonstrate that FR145715 is a novel histamine H_2 receptor antagonist having potent and selective anti-*H. pylori* activities as well as cytoprotective properties. The present data suggest that FR145715 might be useful for the patients suffering from ulcer relapse, since the drug might be able to eradicate *H. pylori* in the stomach, which is considered a key factor to cause ulcer recurrence in humans.

Keywords: FR145715; Histamine H_2 receptor antagonist; Ulcer; *Helicobacter pylori*

1. Introduction

Since Black et al. first defined the histamine H_2 receptor and its involvement in gastric acid secretion, histamine H_2 receptor antagonists, for example, cimetidine, ranitidine, famotidine, roxatidine and nizatidine have been developed and used clinically as anti-acid secretagogues (Black et al., 1972; Brimblecombe et al., 1975). These histamine H_2 receptor antagonists have revolutionized the treatment of peptic ulcers with their prominent therapeutic effects.

* Corresponding author. Tel.: +81-06-6390-1152; fax: +81-06-6304-5367

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00466-5

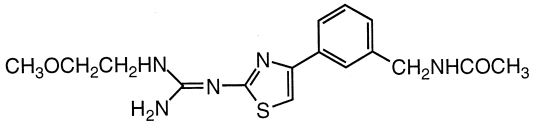

Fig. 1. Chemical structure of FR145715.

However, despite remarkable improvement in the treatment of peptic ulcers, it is still difficult to alter the natural history of peptic ulcers, i.e., recurrence of ulcer is still a problem.

Since the discovery of *Helicobacter pylori* (*H. pylori*) (Warren and Marshall, 1983), extensive studies with this microorganism have led it to be recognized as one of the major causes of chronic gastritis, and of the development of peptic ulcer (Valle et al., 1991; Graham et al., 1992; Patchett et al., 1992; Marshall, 1994). In 1994, a Consensus Development Conference sponsored by the National Institute of Health concluded that ulcer patients with *H. pylori* infection require anti-microbial treatment in addition to antisecretory agents, both on first presentation with the illness and recurrence. Therapeutic regimens with regard to *H. pylori* eradication have been reported by many investigators. Monotherapies with antibiotics or anti-ulcer agents seem to have little or no effect on eradication of *H. pylori*. However, dual therapies or triple therapies with anti-ulcer and antibiotic agents have proven high eradication rates (Glupczynski and Burette, 1990; Chiba et al., 1992; Sugiyama et al., 1995; Axon, 1996).

In this paper, we report a novel histamine H_2 receptor antagonist, *N*-[3-[2-[*N′*-(2-methoxyethyl)guanidino]thiazol-4yl]benzyl-acetamide (FR145715), which has potent and selective anti-microbial activity against *H. pylori*. The chemical structure of FR145715 is shown in Fig. 1. Our data suggest that FR145715 may be useful for patients with peptic ulcer, especially those having *H. pylori* and high recurrence.

2. Materials and methods

2.1. Effect on isolated guinea-pig right atria

The right atria of male Hartley guinea pigs, weighing 480–660 g, were isolated and suspended in 50 ml Magnus chambers containing Tyrode solution of the following composition: 136.9 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4 and 5.6 mM glucose. The Tyrode solution was maintained at 30°C and bubbled with a 95% O_2 and 5% CO_2 gas mixture. The preparation was attached to a force displacement transducer, connected to a polygraph through an amplifier and a cardiotachometer. The amplitude of contraction was measured with a force displacement transducer, and the beating rate was determined by a cardiotachometer triggered by contractile wave signals. Histamine (5.4 mM) was added to the Magnus chamber and the increase in beating rate after dosing was measured for about 30 min. After washing out histamine by changing the Tyrode solution, a test compound was added. At 30 min later, histamine was added and the increase in beating rate was measured. The effect of drugs was estimated by the difference in histamine-induced increases in beating rate between pre- and post-drug treatment.

2.2. Spontaneous gastric acid secretion in Shay's rats

Male Sprague–Dawley rats (Nihon Charles-River, Nihon SLC), weighing 92–122 g, were deprived of food for 24 h before the experiment, with free access to water. Under ether anesthesia, the abdomen was incised and the pylorus ligated. The animals were sacrificed 4 h after the pylorus ligation and the gastric contents were collected and analysed for volume and acidity. The acidity was determined by automatic titration of the gastric juice with 0.1 N NaOH to pH 7.0 (Comtite-8, Hiranuma). Gastric acid output was expressed as μeq/4 h per 100 g body weight. Each drug or vehicle was administered intraduodenally immediately after pylorus ligation.

2.3. Histamine-stimulated gastric acid secretion in Schild's rats

Male Sprague–Dawley rats (Nihon Charles-River), weighing 208–311 g, were deprived of food for 24 h before the experiment, with free access to water. Animals were anaesthetized with intraperitoneal urethane at 1.25 g/kg. The abdomen was incised, and the stomach and duodenum exposed. A polyethylene cannula (Hibiki Fr No.9 outer diameter: 3 mm) was introduced into the stomach through the forestomach and secured tightly with suture. Another polyethylene cannula was introduced through a cut at the duodenum into the stomach. The duodenum was ligated together with the cannula with suture. The stomach was perfused with saline at a rate of 1 ml/min. The perfusate was titrated continuously with an automatic titrator (Radiometer Copenhagen) with 25 mM NaOH to estimate gastric acid output. Histamine was infused intravenously (3 mg kg^{-1} h^{-1}) at a rate of 1 ml kg^{-1} h^{-1} for the entire period of the experiment. Drugs were given intravenously or intraduodenally after the gastric acid secretion reached plateau.

2.4. Gastric acid secretion in Heidenhain's pouch dogs

Beagle dogs of both sexes, weighing 13.0–14.5 kg, with Heidenhain pouches were used. The animals were allowed to recover from surgery for at least one month before the experiments. Animals were used with more than 1 week intervals to allow animals to recover from the

previous experiment. Dogs were deprived of food for 18 h, with free access to water before the experiments. On the day of experiment, the brachial vein was cannulated for the continuous infusion of tetra-gastrin. Tetra-gastrin was infused intravenously (10 μg kg^{-1} h^{-1}) at a rate of 0.1 ml kg^{-1} min^{-1} for the entire period of the experiments. Drugs were given intravenously or orally after the gastric acid secretion reached plateau. The acidity of the gastric juice was measured according to the same procedure as described in the method for Shay's rats. Gastric acid output was expressed as μeq/15 min.

2.5. Water immersion restraint stress-induced gastric lesions

Male Sprague–Dawley rats (Nihon Charles-River), weighing 194–248 g, were deprived of food for 24 h before the experiment with free access to water. The animals were placed in restraint cages and then immersed vertically to the level of the xiphoid in a water bath (22°C). After 7 h, animals were sacrificed, and the stomach of each rat was removed and inflated by injection of 12.5 ml of 2% formalin solution to fix the inner layers of the gastric walls. The isolated stomachs were kept in 2% formalin solution for at least 10 min. The stomach was then incised along the greater curvature, and the area of the lesion was macroscopically measured. Ulcer index of the stomach was expressed as the summation of the area (mm^2) of the lesion. Drugs were given orally immediately before water immersion restraint stress subjection.

2.6. Acidified aspirin-induced gastric lesions

Male Sprague–Dawley rats (Nihon Charles-River), weighing 165–232 g, were deprived of food for 24 h before the experiment, with free access to water. Aspirin (200 mg/kg), suspended in 0.1% methylcellulose solution containing 0.2 N hydrochloric acid (HCl), was administered orally at a volume of 10 ml/kg. The animals were sacrificed 1 h later and the gastric lesion of each stomach was macroscopically measured. Ulcer index was expressed as the summation of the length (mm) of the lesions. Drugs were given orally 30 min before acidified aspirin administration.

2.7. HCl-induced gastric lesions

Male Sprague–Dawley rats (Nihon Charles-River), weighing 199–225 g, were deprived of food for 24 h with free access to water before experiments. FR145715 (32 mg/kg), misoprostol (10 μg/kg) or vehicle was given orally 30 min before intragastric 0.6 N HCl challenge. Indomethacin (5 mg/kg) was treated orally 30 min prior to FR145715 or misoprostol administration. Vehicle, instead of indomethacin, was treated to animals in control group and another FR145715-treated group. Animals were sacrificed 1 h after HCl challenge. The stomach was incised along the greater curvature, and the area of the lesion was macroscopically measured. Ulcer index was expressed as the summation of the length (mm) of the lesion.

2.8. Antibacterial activity

Anti-*H. pylori* activity was determined in vitro by the agar dilution method. Test strain was precultured at 37°C for 3 days in Brucella agar containing 7% horse serum and 2% starch, and suspended in buffered saline to give the turbidity equivalent to McFarland No.1. The bacterial suspensions diluted by 100-fold were inoculated with a multipoint replicator onto a Brucella agar plus 7% lysed horse blood plate containing serial twofold dilutions of each drug at 37°C for 3 days. Incubation was carried out in an atmosphere of 10% CO_2. Minimum inhibitory concentration (MIC) was identified as the lowest drug concentration that inhibited macroscopic colonial growth after incubation. Antibacterial activity against other bacteria was also determined by the same agar dilution method, and test strains were precultured in Brucella agar containing 7% horse serum at 37°C for 3 days (*C. jejuni*, *C. coli* and *C. fetus*), Mueller–Hinton agar containing 5% horse serum (chocolate-agar) at 37°C for 18 h (*H. influenzae*), Gam-agar at 37°C for 18 h (*C. difficile*, *C. perfringens* and *B. fragilis*) and Mueller–Hinton agar at 37°C for 18 h (other bacteria).

2.9. Eradication experiments using gnotobiotic piglets infected with H. pylori

One litter ($n = 6$) of gnotobiotic piglets were derived by routine caesarian section and divided into two groups as follows: Group A was infected and treated with 0.5% methyl cellulose alone; and Group B was infected and treated with FR145715, 16.0 mg/kg, t.i.d. in distilled water. All piglets in both groups were orally infected with approximately 109 colony forming units (cfu) *H. pylori*, 26695 at 3 days of age after pretreatment with cimetidine. At 10 days after infection, treatment was initiated for all piglets of both groups and continued three times per day (0730, 1200 and 1600 h) for 10 days. The morning after the last treatment, piglets were sedated with ketamine and removed from the isolation units. A terminal serum sample was collected and the piglets were euthanatized with intravenous Euthol. The stomach was exteriorized, ligated at the esophagus, transected and removed. The stomach was opened longitudinally along the greater and lesser curvatures, and gross observations recorded. One-half of the stomach was placed in a sterile petri dish and the gastric mucosa was carefully dissected free of the underlying muscularis. After weighing, the mucosa was ground in 10 volumes of sterile Brucella broth in a glass ten Brock

Table 1
Effect of FR145715 and ranitidine on histamine-induced increase in heart rate of isolated guinea-pig atrium
IC_{50} values were estimated from the concentration response studies in the presence of 5.4 μM histamine.

Drugs	*N*	IC_{50} (μM)
FR145715	3	1.3±0.5
Ranitidine	3	3.3±0.8

grinder and duplicate 10-fold dilutions of supernatant was plated onto modified TVAP agar plates for enumeration and quantitative determination of bacterial cfu per gram of gastric mucosa. All plates were incubated for 4–5 days at 37°C, 10% CO_2. Recovered organisms were identified by colonial morphology, gram stain and standard biochemical tests. Plates scored as negative were swabbed and re-streaked onto blood agar plates without antibiotics and incubated for 4–5 days at 37°C to confirm the culture results. For histopathologic evaluation, formalin-fixed one-half stomachs or biopsy sites from each anatomic region from above were removed from formalin, trimmed, embedded in paraffin, sectioned at 5–6 and stained with hematoxylin and eosin and Warthin–Starry (W/S) stains for histologic evaluation and demonstration of organisms, respectively.

2.10. Drugs

FR145715 (*N*-[3-[2-[*N*′-(2-methoxyethyl)guanidino]-thiazol-4yl]benzyl-acetamide), cimetidine, roxatidine acetate hydrochloride and famotidine were synthesized at the Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical. Ranitidine hydrochloride (Sigma), histamine dihydrochloride (Nacalai Tesque), tetragastrin (San-a Pharmaceutical), aspirin (Wako) and indomethacin (Yashiro Pharmaceutical) were obtained commercially. In in vitro experiments, FR145715 was dissolved in dimethylsulfoxide (DMSO), and ranitidine was dissolved in distilled water, while the other drugs were dissolved in water containing HCl and then adjusted to a final pH of 7.0 with NaOH. To examine anti-bacterial activity, FR145715, ranitidine, cimetidine, famotidine, roxatidine, omeprazole and lansoprazole were dissolved in DMSO, and peptobismol and plaunotol were suspended in distilled water. Amoxicillin, clarithromycin and metronidazole were dissolved in NaHCO3, methanol and distilled water, respectively. In in vivo experiments, FR145715 was suspended in 0.1% methylcellulose solution for oral and intraduodenal administration. Indomethacin and misoprostol were also suspended in 0.1% methylcellulose solution. For intravenous administration, FR145715 was dissolved in saline. In Schild's rats, FR145715 was suspended in 0.1% methylcellulose containing 0.9% NaCl for intravenous administration. Ranitidine was dissolved in saline for intravenous injection. For oral or intraduodenal administration, ranitidine was dissolved in either distilled water, saline or 0.1% methylcellulose solution.

2.11. Statistical analysis and miscellaneous

Results are expressed as the mean ± S.E., and statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison's test when comparing unpaired groups. When statistical comparisons were made between pre- and post-drug treatment, paired Student's *t*-test was used. ED_{50} values for drugs in Schild's rats and Heidenhain's pouch dogs were estimated using a non-linear regression analysis from max-

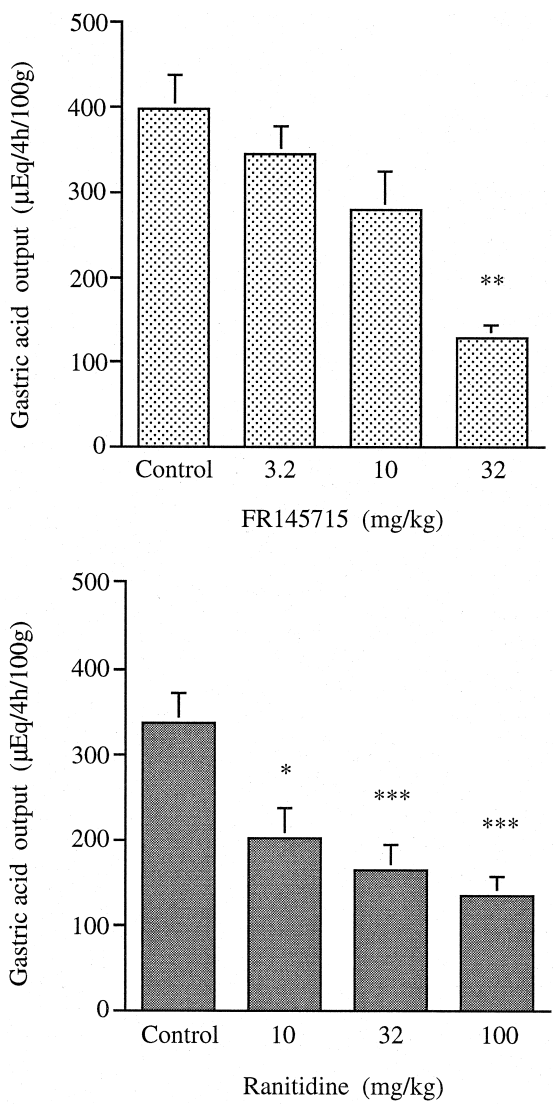

Fig. 2. Effect of FR145715 and ranitidine on spontaneous gastric acid secretion in pylorus ligated rats. FR145715 or ranitidine was administered intraduodenally immediately after pylorus ligation. Gastric juice secreted over a 4 h period was titrated with NaOH to estimate gastric acid output. Gastric acid output was expressed as μeq/4 h per 100 g body weight. Each column represents the mean±S.E.M. of 9–11 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. control, ANOVA followed by Dunnett's test).

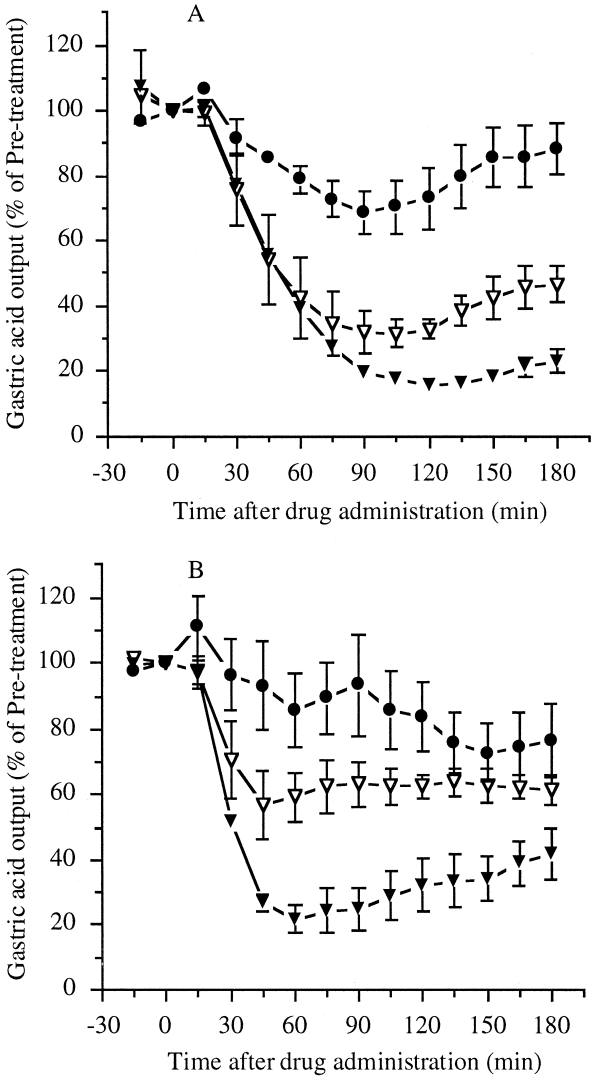

Fig. 3. Effect of FR145715 and ranitidine on histamine-stimulated gastric acid secretion in Schild's rats. Histamine (3 mg kg^{-1} h^{-1}) was given intravenously at a rate of 1 ml kg^{-1} h^{-1} for the entire period of the experiment. FR145715 (A: (●) 0.32, (▽) 1.0, (▼) 3.2 mg/kg) and ranitidine (B: (●) 0.01, (▽) 0.1, (▼) 1.0 mg/kg) were given intravenously after the gastric acid secretion reached plateau. The perfusate for each 15 min was titrated with NaOH to estimate gastric acid output. Each value represents the mean ± S.E.M. of three to four experiments. Gastric acid output immediately before drug administration (pre-treatment) was in the range of 18.3–25.8 μeq/15 min. $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. pre-treatment, paired *t*-test).

imal inhibitory effects (%) obtained in dose-response studies.

3. Results

3.1. Effect on isolated guinea-pig right atria

To examine the antagonistic activity against histamine H_2 receptors, we used isolated guinea-pig atrium whose beating rate is regulated by the histamine H_2 receptor. Histamine (5.4 μM) stimulated the beating rate of the atria from 58 ± 4 to 70 ± 4 beats/min. When FR145715 was added, the histamine-stimulated beating rate was inhibited in a concentration-dependent manner (Table 1). IC_{50} value was estimated at 1.1 μM. Ranitidine showed similar effects with an IC_{50} value of 3.4 μM.

3.2. Effect on spontaneous gastric acid secretion in Shay's rats

Ligation of the pylorus for 4 h resulted in an accumulation of gastric juice in the stomach. The mean total acid

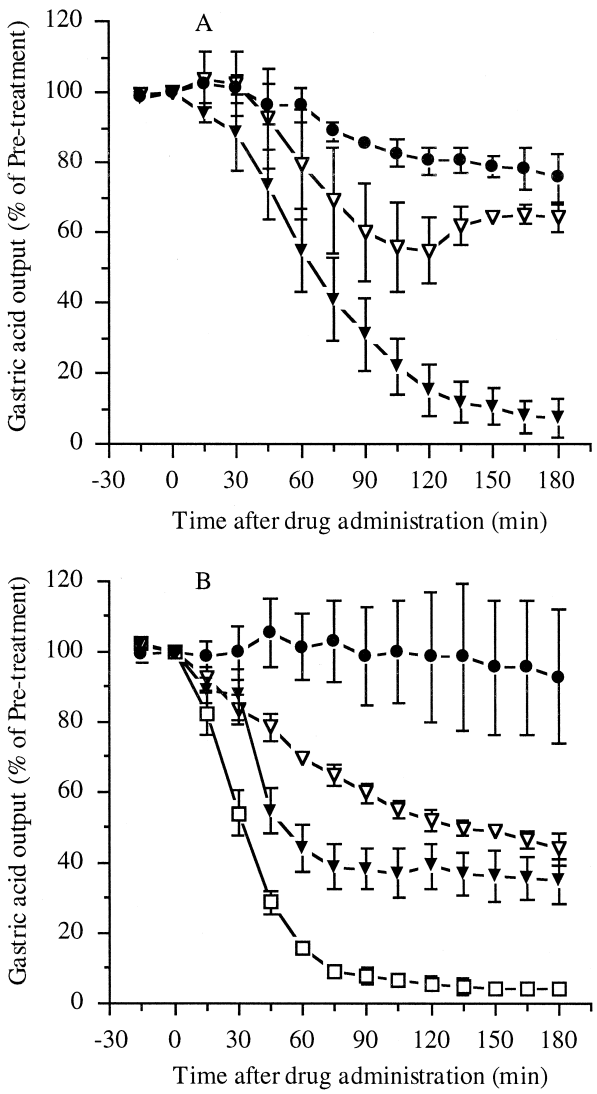

Fig. 4. Effect of FR145715 and ranitidine on histamine-stimulated gastric acid secretion in Schild's rats. Histamine (3 mg kg^{-1} h^{-1}) was given intravenously at a rate of 1 ml kg^{-1} h^{-1} for the entire period of the experiment. FR145715 (A: (♦) 1.0, (▽) 3.2, (▼) 10 mg/kg) and ranitidine (B: (●) 0.01, (▽) 0.1, (▼) 1.0, (□) 10 mg/kg) were given intraduodenally after the gastric acid secretion reached plateau. The perfusate for each 15 min was titrated with NaOH to estimate gastric acid output. Each value represents the mean ± S.E.M. of three to four experiments. Gastric acid output immediately before drug administration (pre-treatment) was in the range of 15.5–26.1 μeq/15 min. $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. pre-treatment, paired *t*-test).

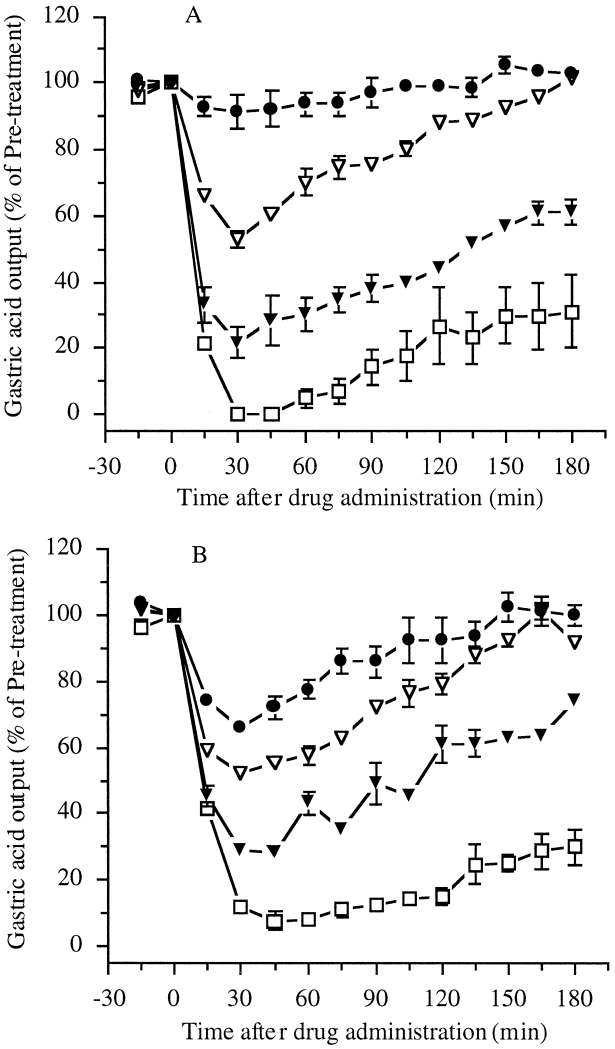

Fig. 5. Effect of FR145715 and ranitidine on tetragastrin-stimulated gastric acid secretion in Heidenhain's pouch dogs. Tetragastrin (10 μg kg^{-1} h^{-1}) was given intravenously at a rate of 0.1 ml/min for the entire period of the experiment. FR145715 (A) and ranitidine (B) were given intravenously after the gastric acid secretion reached plateau. Gastric juice secreted during each 15 min period was collected, and titrated with NaOH to estimate gastric acid output. Each data point represents the mean of four experiments. Gastric acid output immediately before drug administration (pre-treatment) was in the range of 325–443 μeq/15 min. (●) 0.032, (▽) 0.1, (▼) 0.32, (□) 1.0 mg/kg; $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. pre-treatment, paired *t*-test).

output of the control group was in the range of 338–398 μeq/4 h per 100 g body weight. Intraduodenal administration of FR145715 (3.2–32 mg/kg) or ranitidine (10–100 mg/kg) dose-dependently inhibited acid output, with ED_{50} values of 18.4 and 30.5 mg/kg, respectively (Fig. 2).

3.3. Effect on histamine-stimulated gastric acid secretion in Schild's rats

Intravenous infusion of histamine stimulated gastric acid secretion up to levels of 15.5–26.1 μeq/15 min compared with the basal secretion of 3–4 μeq/15 min. Intravenous injection of FR145715 (0.32–3.2 mg/kg) dose-dependently inhibited gastric acid output, with an ED_{50} value of 0.59 mg/kg (Fig. 3A). Ranitidine also inhibited histamine-stimulated gastric acid secretion, giving an ED_{50} value of 0.10 mg/kg (Fig. 3B). When drugs were given intraduodenally, both drugs inhibited acid output with a slow onset as expected (Fig. 4A,B). ED_{50} values were estimated at 2.72 and 0.17 mg/kg for FR145715 and ranitidine, respectively.

3.4. Effect on gastrin-induced gastric acid secretion in Heidenhain's pouch dogs

In our preliminary experiment, tetra-gastrin stimulated gastric acid secretion at doses of 1–100 μg kg^{-1} h^{-1},

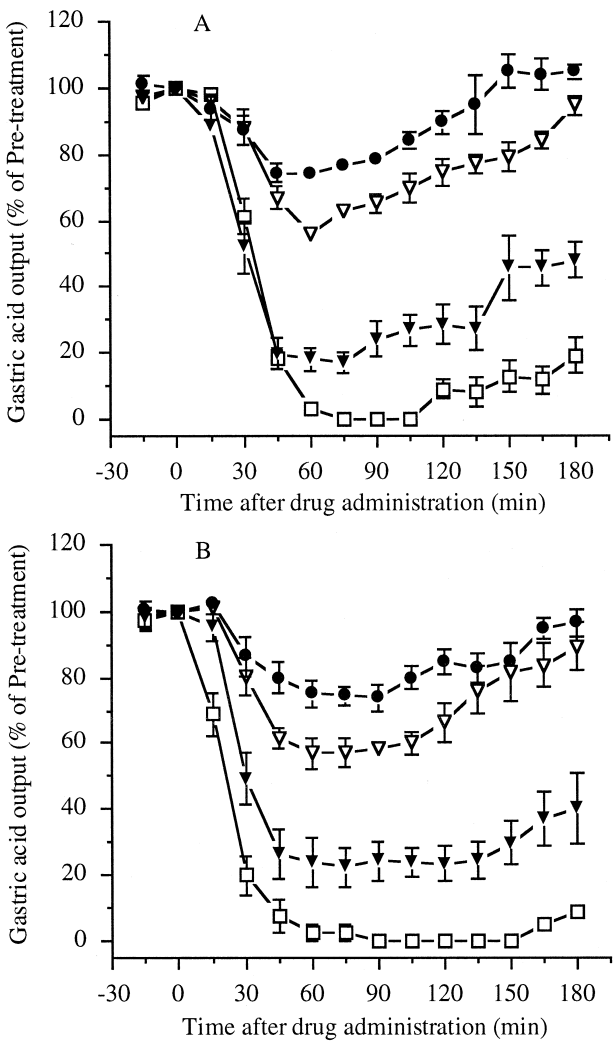

Fig. 6. Effect of FR145715 and ranitidine on tetragastrin-stimulated gastric acid secretion in Heidenhain's pouch dogs. Tetragastrin (10 μg kg^{-1} h^{-1}) was given intravenously at a rate of 0.1 ml/min for the entire period of the experiment. FR145715 (A) and ranitidine (B) were given orally after the gastric acid secretion reached plateau. Gastric juice secreted during each 15 min period was collected, and titrated with NaOH to estimate gastric acid output. Each data point represents the mean of four experiments. Gastric acid output immediately before drug administration (pre-treatment) was in the range of 312–410 μeq/15 min. (●) 0.1, (▽) 0.32, (▼) 1.0, (□) 3.2 mg/kg; $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. pre-treatment, paired *t*-test).

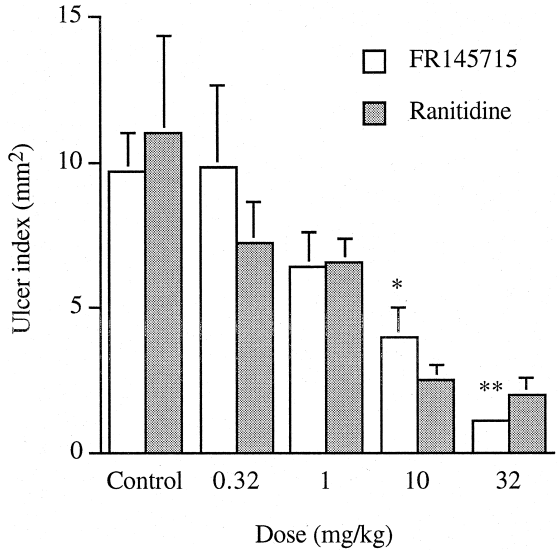

Fig. 7. Effect of FR145715 and ranitidine on water immersion restraint stress-induced gastric lesion in rats. Animals were subjected to water immersion restraint stress at 23°C for 7 h. FR145715 (open column) and ranitidine (shaded column) were given orally 30 min before initiation of the stress. The gastric lesions were macroscopically scored and expressed as area (mm^2). Each column represents the mean ± S.E.M. of 4–10 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. control, ANOVA followed by Dunnett's test).

revealing 10 μg kg^{-1} h^{-1} to be a sub-maximal dose for stimulation of acid secretion in Heidenhain pouch dogs. At this dose, tetra-gastrin stimulated gastric acid secretion up

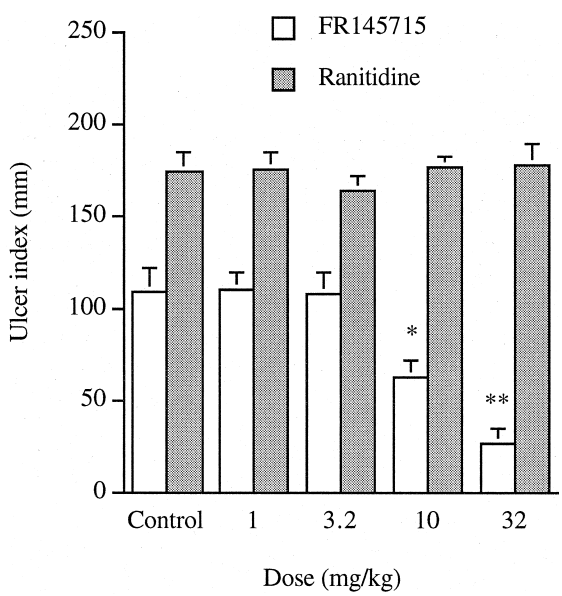

Fig. 8. Effect of FR145715 and ranitidine on acidified aspirin-induced gastric lesion in rats. FR145715 (open column) or ranitidine (shadowed column) was administered orally 30 min before acidified aspirin administration. Animals were sacrificed 1 h after acidified aspirin administration. The gastric lesions were macroscopically scored and expressed as length (mm). Each column represents the mean ± S.E.M. of 9–10 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. control, ANOVA followed by Dunnett's test).

to levels of 312–443 μeq/15 min compared with negligible levels of basal acid secretion. Intravenous injection of FR145715 at doses of 0.032–1.0 mg/kg dose-dependently inhibited acid output (Fig. 5A). Ranitidine also inhibited tetragastrin-stimulated acid secretion in a similar manner (Fig. 5B). Maximal inhibition exhibited by these drugs was observed at 30–45 min after drug administration and then gradually recovered. ED_{50} values were estimated at 0.12 and 0.09 mg/kg for FR145715 and ranitidine, respectively. Oral administration of these drugs also inhibited acid output dose dependently as shown in Fig. 6. ED_{50} values for FR145715 and ranitidine were 0.32 and 0.33 mg/kg, respectively.

3.5. Effect on water immersion restraint stress-induced gastric lesions in rats

Water immersion restraint stress for 7 h induced multiple mucosal lesions, mainly in the corpus area. In the control group, the mean ulcer index was in the range of

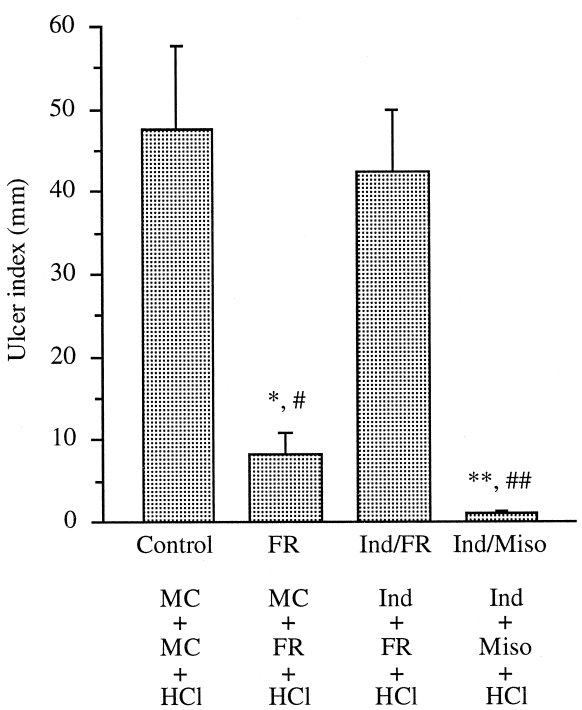

Fig. 9. Effect of indomethacin treatment on beneficial effects of FR145715 and misoprostol on HCl-induced gastric lesions in rats. Rats were deprived of food for 24 h with free access to water before experiments. FR145715 (32 mg/kg), misoprostol (10 μg/kg) or vehicle (control) was given orally 30 min before intragastric 0.6 N HCl challenge. Indomethacin (5 mg/kg) was treated orally 30 min prior to FR145715 (Ind/FR) or misoprostol (Ind/Miso) administration. Vehicle instead of indomethacin was treated to animals in another FR145715-treated group (FR). Animals were sacrificed 1 h after HCl challenge. Severity of the gastric lesion was estimated by the total length of the lesion (mm). Each column represents the mean ± S.E.M. of seven animals. $^{*}P < 0.05$, $^{**}P < 0.01$ (vs. control group, ANOVA followed by Dunnett's test); $\#P < 0.05$, $\#\#P < 0.01$ (vs. Ind/FR group, ANOVA followed by Dunnett's test).

Table 2
Anti-*H. pylori* activity of histamine H_2 receptor antagonists, proton pump inhibitors, other anti-ulcer agents and antibiotic agents
MIC was determined by the lowest drug concentration that inhibited macroscopic colonial growth after incubation. Mean MIC and the range of MIC values were estimated using 10 strains of *H. pylori*.

Drugs	Mean MIC (μg/ml; 10 strains)	Range of MIC (μg/ml; 10 strains)
FR145715	0.32	0.2–0.39
Ranitidine	> 1600	> 1600
Cimetidine	984	400–1600
Famotidine	1492	800–1600
Roxatidine	> 1600	> 1600
Omeprazole	22	12.5–25
Lansoprazole	2.9	1.56–6.25
Peptobismol	4.4	3.13–6.25
Plaunotol	2.3	1.56–3.13
Amoxicillin	0.0185	< 0.1
Clarithromycin	0.057	0.025–0.1
Metronidazole	5.4	1.56–25

9.7–11.0 mm^2. Oral administration of FR145715 dose-dependently inhibited stress-induced mucosal lesions (Fig. 7). At the highest dose examined (32 mg/kg), the gastric mucosal lesion was inhibited by 88.7%. Ranitidine also suppressed gastric lesions to a similar extent (Fig. 7). ED_{50} values were 3.2 and 1.5 mg/kg for FR145715 and ranitidine, respectively.

3.6. Effect on acidified aspirin-induced gastric lesions in rats

Oral administration of acidified aspirin induced multiple linear lesions in the gastric mucosa. In the control group, the mean ulcer index was in the range of 109–175 mm. Oral administration of FR145715 at doses of 10 and 32 mg/kg significantly inhibited gastric mucosal lesions, with an ED_{50} value of 15.1 mg/kg (Fig. 8). However, ranitidine failed to inhibit the lesions even at a dose of 32 mg/kg.

3.7. HCl-induced gastric lesions

Oral FR145715 at 32 mg/kg inhibited gastric lesions induced by 0.6 N HCl in rats. Indomethacin, when given orally at a dose of 5 mg/kg 30 min prior to FR145715 administration, completely abolished beneficial effect of FR145715 (Fig. 9). However, pre-treatment with indomethacin did not affect beneficial effect of misoprostol.

3.8. Antibacterial activity

Anti-*H. pylori* activities were evaluated for FR145715, four other histamine H_2 receptor antagonists, i.e., raniti-

Table 3
Antibacterial activity against various bacteria of histamine H_2 receptor antagonists, proton pump inhibitors and other anti-ulcer agents
No.: number of strains, FR: FR145715, Omep: omeprazole, Lanso: lansoprazole, Pepto: peptobismol, Plau: plaunotol, Amoxi: amoxicillin, Metro: metronidazole. Values are the mean MICs (μg/ml). N.T.: not tested.

Organism	No.	FR	Omep	Lanso	Pepto	Plau	Amoxi	Metro
C. jejuni	(8)	> 100	> 100	> 100	7.4	> 100	0.78–3.13	0.78–> 100[a]
C. coli	(1)	> 100	> 100	> 100	6.25	> 100	6.25	0.78
C. fetus	(1)	> 100	> 100	> 100	12.5	> 100	0.39	12.5
Staphylococcus aureus	(9)	> 100	> 100	> 100	> 100	> 100	0.39	> 100
S. epidermidis	(1)	> 100	> 100	> 100	> 100	> 100	0.78	> 100
E. faecalis	(9)	> 100	> 100	> 100	> 100	> 100	0.78	> 100
Bacillus subtilis	(1)	> 100	> 100	> 100	> 100	25	≤ 0.025	> 100
B. catarrhalis	(9)	> 100	N.T.	N.T.	N.T.	12.5	0.148	N.T.
H. influenzae	(20)	> 100	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Escherichia coli	(9)	> 100	> 100	> 100	> 100	> 100	0.39–> 100[a]	> 100
K. pneumoniae	(9)	> 100	> 100	> 100	> 100	> 100	> 100	> 100
K. oxytoca	(1)	> 100	> 100	> 100	> 100	> 100	100	> 100
P. mirabilis	(3)	> 100	> 100	> 100	> 100	> 100	0.98	> 100
Proteus vulgaris	(2)	> 100	> 100	> 100	> 100	> 100	71	> 100
Serratia marcescens	(1)	> 100	> 100	> 100	> 100	> 100	> 100	> 100
C. freundii	(1)	> 100	> 100	> 100	> 100	> 100	> 100	> 100
E. cloacae	(1)	> 100	> 100	> 100	> 100	> 100	100	> 100
E. aerogenes	(1)	> 100	> 100	> 100	> 100	> 100	> 100	> 100
P. aeruginosa	(4)	> 100	> 100	> 100	> 100	> 100	> 100	> 100
C. albicans	(3)	> 100	> 100	> 100	> 100	> 100	> 100	N.T.
C. tropicalis	(2)	> 100	> 100	> 100	> 100	> 100	> 100	N.T.
T. beigelli	(1)	> 100	> 100	> 100	> 100	> 100	> 100	N.T.
C. difficile	(4)	> 100	> 100	> 100	42	42	0.28	0.2
C. perfringens	(6)	> 100	> 100	> 100	> 100	45	0.027	1.75
B. fragilis	(10)	> 100	> 100	> 100	54	44	2.1	0.5

[a]Resistant strain gave mean minimum inhibitory concentration greater than 100 μg/ml.

Table 4
Gross observations in gnotobiotic piglets infected with *H. pylori*
Visually scored as: 0 = absent or no change from normal; 1 = minimal change from normal; 2 = moderate change from normal; 3 = severe change from normal; and 4 = very severe change from normal.

Drug mg/kg (t.i.d.)	Piglet no.	Excess luminal mucus	Lymphoid follicle	Submucosal edema	Ulcers or erosions
Control	1	2	2	1	0
	2	2	3	1	0
	3	1	2	0	0
FR145715	1	3	2	0	0
16	2	3	2	0	0
	3	1	1	0	0

dine, cimetidine, famotidine and roxatidine, the gastric proton pump inhibitors, omeprazole and lansoprazole, and the anti-ulcer agents plaunotol and peptobismol, in addition to antibiotic agents amoxicillin, clarithromycin and metronidazole. Results were shown in Table 2 with minimum inhibitory concentrations and the ranges of minimum inhibitory concentrations of drugs. FR145715 showed potent activity against 10 strains of *H. pylori*, with a mean minimum inhibitory concentration of 0.32 μg/ml. However, other histamine H_2 receptor antagonists, ranitidine, cimetidine, famotidine and roxatidine were essentially inactive, and the minimum inhibitory concentrations of these drugs were far greater than FR145715. Proton pump inhibitors, omeprazole and lansoprazole showed anti-*H. pylori* activities with mean minimum inhibitory concentrations of 22 and 2.9 μg/ml, respectively. The anti-ulcer agents, peptobismol and plaunotol also showed anti-*H. pylori* activities with mean minimum inhibitory concentrations of 4.4 and 2.3 μg/ml, respectively. As expected, comparative antibiotic agents, amoxicillin, clarithromycin and metronidazole, showed potent activities against *H. pylori* with a minimum inhibitory concentration of 0.0185, 0.057 and 5.4 μg/ml, respectively (Table 2). Unlike antibiotic agents, FR45715 had no effect on 25 other bacteria even at 100 μg/ml (Table 3).

3.9. Eradication experiment using gnotobiotic piglets infected with H. pylori

In gross observations, toxicity was not observed as the result of treatment with either drug nor with vehicle alone. Infected piglets remained asymptomatic throughout the observation period. Table 4 summarizes the gross findings in the stomachs of piglets from this study. All piglets from both groups exhibited submucosal lymphoid follicles, which were most prominent along the lesser curvature. Mild submucosal fundic edema was noted in several infected piglets in control group. Gastric ulcers and erosions were not observed. Table 5 summarizes the histopathologic findings in this study. Inflammatory lesions associated with *H. pylori* gastritis consisted of focal and diffuse lymphoplasmacytic cellular infiltrates in the lamina propria of the gastric mucosa. Cellular aggregates ranged from small focal collections of leukocytes to large, prominent well-organized lymphoid follicles. Occasional neutrophils and eosinophils were noted in the gastric mucosa as well. Tissue samples from drug-treated group in general exhibited a diminished inflammatory response manifested chiefly as reduced size and frequency of lymphoid follicles. In this group, the inflammatory lesions appeared to regress vs. the control group. Focal or diffuse submucosal edema was also noted in some of the sections examined. All infected piglets regardless of treatment exhibited histologic evidence of gastritis which was especially prominent in the cardia and antral regions previously established as predilection sites for infection. Organisms were recovered from three of three piglets in control group and colony counts in these positive animals ranged from $0.66–1.61 \times 10^6$ cfu/g (Table 6). The W/S data was consistent with *H. pylori* culture and reisolation results.

In contrast, *H. pylori* was not recovered from any piglet treated with 16.0 mg/kg FR145715. Re-streaks from each were also culture-negative. The W/S data was compatible with culture results. No other microbes besides

Table 5
Histopathologic findings in gnotobiotic piglets infected with *H. pylori*
Visually scored as 0 = no change from normal; 1 = minimal change from normal (scattered foci of cells with rare small follicles); 2 = moderate change from normal (confluent foci of cells, occasional lymphoid follicles); 3 = severe change from normal (diffuse inflammatory cell infiltrates and/or frequent lymphoid follicles).

Drug mg/kg (t.i.d.)	Piglet no.	Anatomical region of the stomach			
		Cardia	Fundus	Antrum	Pylorus
Control	1	3[a,b]	3	3	1
	2	3[b]	2	2	1
	3	3	2	3	1
FR145715	1	2	0	1	0
16	2	2	1	1	0
	3	3	1	2	0

[a] Lymphoplasmacytic cellular infiltrates in the lamina propria.
[b] Moderate submucosal edema.

Table 6
Eradication experiments using gnotobiotic piglets infected with *H. pylori*; CFU: colony forming units.

Drug mg/kg (t.i.d.)	Piglet no.	CFU/g gastric mucosa ($\times 10^6$)	W/S stain sections[a]			
			Cardia	Fundus	Antrum	Pylorus
Control	1	1.61	+	+	+	+
	2	0.89	+	+	+	−
	3	0.66	+	−	+	−
FR145715	1	0.0[b]	−	−	−	−
16	2	0.0	−	−	−	−
	3	0.0	−	−	−	−

[a] Visually scored as: (+) = structure compatible with *H. pylori* detected in mucus are attached to gastric epithelia or (−) = no *H. pylori* seen.
[b] Culture negative-swabs from TVAP plates were re-streaked onto blood agar. No growth was detected.

H. pylori were recovered from the isolation units housing the gnotobiotic piglets.

4. Discussion

FR145715 is a novel histamine H_2 receptor antagonist possessing a guanidinothiazole ring structure. In the present study, we examined the pharmacological profile of FR145715 as an anti-ulcer agent in comparison with ranitidine, a potent histamine H_2 receptor antagonist. In isolated guinea-pig atrium studies, FR145715 antagonized histamine-induced positive chronotropic response with approximately three times more potent action than ranitidine, indicating that FR145715 is a potent histamine H_2 receptor antagonist. In Shay's rats, intraduodenal FR145715 inhibited spontaneous acid secretion, with slightly greater potency than ranitidine. However, in histamine-stimulated acid secretion in Schild's rats, FR145715 was approximately 6 and 16 times less potent than ranitidine when administered intravenously and intraduodenally, respectively. These results are incompatible with those obtained in Shay's rats study. We have no clear explanation for the discrepancy. However, it might be due to the difference in pharmacokinetics of these two drugs between concious (Shay's rats) and anaesthetized (Schild's rats) condition, although we have not examined pharmacokinetics of these two drugs under the identical condition. In Heidenhain pouch dogs, FR145715 inhibited acid secretion to a similar extent as ranitidine, despite the fact that FR145715 is three times more potent than ranitidine in in vitro atria studies. These incompatible observations might be attributed to the different pharmacokinetics between FR145715 and ranitidine in Heidenhain pouch dogs, i.e., half-life for FR145715 in dogs was 1.84 h (oral bio-availability: 73%–74%) (data not shown), whereas that for ranitidine was 3.9 h (oral bio-availability: 73%) (Eddershaw et al., 1996), although the exact reason is not clear at present.

In experimental ulcer models, water immersion restraint stress-induced gastric lesions are considered to be causally related to increased gastric secretion (Kitagawa et al., 1979). In this model, the inhibitory effect of FR145715 was approximately one-half as potent as ranitidine. These observations are in agreement with in vivo studies of gastric acid secretion, although gastric secretory inhibition could not be attributed 100% to the beneficial effect on this stress-induced ulcer model.

In our present studies, FR145715 significantly inhibited gastric lesions induced by aspirin in the presence of 200 mM HCl at doses of 10 and 32 mg/kg, while ranitidine failed to prevent it at the identical doses. Other conventional histamine H_2 receptor antagonists, famotidine and cimetidine also failed to prevent acidified aspirin-induced gastric lesions, whereas misoprostol, a synthetic prostaglandin E_1 analogue, markedly inhibited these gastric lesions (data not shown). It was reported that both ranitidine and cimetidine protected against aspirin-induced gastric lesions in rats in the presence of exogenous 160 mM HCl (Bunce et al., 1981). In contrast, it has been reported that the presence of 160 mM HCl in the stomach throughout the experiment reversed the inhibitory effect of cimetidine on aspirin-induced gastric lesions in rats, while prostaglandin E_2 inhibited gastric lesions caused by acidified aspirin (Carmichael et al., 1978). Our results agree with the latter observation while the former findings are incompatible with our present results. We have no clear explanation about these discrepancies. However, our results may at least suggest that FR145715 may have a so called cytoprotective effect which is considered to be independent on anti-secretory action. Cytoprotective effect of FR145715 was also suggested by an another ulcer model induced by 0.6 N HCl in rats, where FR145715 significantly inhibited ulcer formation. A possible mechanism responsible for cytoprotective properties of FR145715 could be a stimulatory effect on prostaglandin synthesis, since beneficial effect of FR145715 on HCl-induced gastric lesion was completely abolished by indomethacin pretreatment, while misoprostol still exerted complete inhibition under the same condition (Fig. 9).

FR145715 showed potent antibacterial activity against *H. pylori*. In contrast, conventional histamine H_2 receptor antagonists, ranitidine, cimetidine, famotidine and roxatidine were essentially inactive, and these results are in agreement with those previously reported (Goodwin et al.,

1986; Shungu et al., 1987; Iwahi et al., 1991). Other anti-ulcer agents, omeprazole, lansoprazole, peptobismol and plaunotol showed notable antibacterial activity against *H. pylori*. These observations are consistent with the findings reported by other researchers (Iwahi et al., 1991; Haas et al., 1990; Koga et al., 1996). FR145715 exhibited the strongest anti-*H. pylori* activities amongst the anti-ulcer agents that we tested. Antibiotic agents, amoxicillin and clarithromycin, as expected, showed extremely potent antibacterial activities against *H. pylori*. Metronidazole showed moderate activity with minimum inhibitory concentrations of 1.56–25 μg/ml. These results are consistent with the previous reports (Haas et al., 1990; Flamm et al., 1996) in which the authors described minimum inhibitory concentrations of 1–32 μg/ml for metronidazole. Although the anti-*H. pylori* activity of FR145715 (mean minimum inhibitory concentration: 0.32 μg/ml) was 6–17 times less potent than amoxicillin and clarithromycin, FR145715 showed more potent activity than metronidazole, which is being prescribed clinically for eradication of *H. pylori*.

Gnotobiotic piglets infected with *H. pylori* have been shown to be useful as an in vivo method for preclinical evaluations of anti-microbial therapy for *H. pylori* associated gastroduodenal diseases (Eaton et al., 1989; Eaton et al., 1992; Krakowka et al., 1998). In this model, we were able to show that FR145715 completely eliminated culturable *H. pylori* from infected piglets, with reduced intensity of inflammation. These results suggest that FR145715 may be beneficial for patients having gastric ulcer or gastritis with *H. pylori* infection if this agent could eradicate *H. pylori* from the stomach.

It has been reported that ebrotidine, a recently developed histamine H_2 receptor antagonist, has anti-microbial activities against *H. pylori* with a mean minimum inhibitory concentration of 75 μg/ml. In addition, ebrotidine at 100 μg/ml enhanced anti-microbial activity of antibiotics such as erythromycin, amoxicillin, metronidazol and clarithromycin, while ranitidine had no such effects (Palacin et al., 1997). It has also been demonstrated that ebrotidine inhibits *H. pylori* urease, protease, lipase and phospholipase A_2 activities (Piotrowski et al., 1995; Slomiany et al., 1997). Among these enzymes, it has been shown that *H. pylori* urease is important in the pathogenesis of gastritis induced by *H. pylori* in piglets (Eaton et al., 1991). Therefore, anti-microbial activity of ebrotidine might be attributed to its inhibitory action against *H. pylori* urease. It is also possible that anti-microbial activity of FR145715 could be due to its inhibitory action against urease activity. However, FR145715 had no effect on *H. pylori* urease even at a concentration of 10 μg/ml (data not shown). Although FR145715 shows bactericidal effect on *H. pylori*, the site of action through which FR145715 exerts its specific anti-microbial activity remains to be elucidated.

It has been shown that both ebrotidine and ranitidine are equally effective, when treated with amoxicillin and metronidazole, in controlling the symptoms and eradicating *H. pylori* in patients with duodenal ulcer. As we have shown, FR145715 has a more potent anti-microbial activity against *H. pylori*, and shows eradicating effect on gnotobiotic piglets infected with *H. pylori*. Therefore, it is conceivable that FR145715 might exert stronger effects than ebrotidine in eradicating *H. pylori* in humans. Moreover, FR145715 had selective antibacterial activities against *H. pylori* having no effect against various gram-negative, gram-positive bacteria, anerobic bacteria, campylobacter genus and fungi. These results suggest that FR145715 may not disturb the balance of microorganisms in the gut.

In conclusion, FR145715 is a novel histamine H_2 receptor antagonist possessing potent, specific anti-*H. pylori* activity as well as cytoprotective properties, and may prove to be a useful agent in the clinical treatment of *H. pylori* associated gastroduodenal diseases.

Acknowledgements

We thank Dr. David Barrett at Medicinal Chemistry Research Laboratories for critically reading the manuscript.

References

Axon, A.T.R., 1996. Eradication of *Helicobacter pylori*. Scand. J. Gastroenterol. 31 (Suppl. 214), 47–53.

Black, J.W., Duncan, W.A.M., Durant, C.J., Ganellin, C.R., Parsons, E.M., 1972. Definition and antagonism of histamine H_2-receptors. Nature 236, 385–390.

Brimblecombe, R.W., Duncan, W.A.M., Durant, G.J., Emmett, J.C., Ganellin, C.R., Parsons, M.E., 1975. Cimetidine a non-thiourea H_2-receptor antagonist. J. Int. Med. Res. 3, 86–92.

Bunce, K.T., Daly, M.J., Humphray, J.M., Stables, R., 1981. H_2-receptor antagonists protect against aspirin-induced gastric lesions in the rat. Agents Actions 11, 167–170.

Carmichael, H.A., Nelson, L.M., Russell, R.I., 1978. Cimetidine and prostaglandin: evidence for different modes of action on the rat gastric mucosa. Gastroenterology 74, 1229–1232.

Chiba, N., Rao, B.V., Rademaker, J.W., Hunt, R.H., 1992. Meta-analysis of the efficacy of antibiotic therapy in eradicating *Helicobacter pylori*. Am. J. Gastroenterol. 87, 1716–1727.

Eaton, K.A., Brooks, C.L., Morgan, D.R., Krakowka, S., 1989. Campylobacter pylori virulence factors in gnotobiotic piglets. Infect. Immun. 57, 1119–1125.

Eaton, K.A., Morgan, D.R., Krakowka, S., 1991. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. Infect. Immun. 59, 2470–2475.

Eaton, K.A., Morgan, D.R., Krakowka, S., 1992. Motility as a factor in the colonization of gnotobiotic piglets by *Helicobacter pylori*. J. Med. Microbiol. 37, 123–127.

Eddershaw, P.J., Chadwick, A.P., Higton, D.M., Fenwick, S.H., Linacre, P., Jenner, W.N., Bell, J.A., Manchee, G.R., 1996. Absorption and disposition of ranitidine hydrochloride in rat and dog. Xenobiotica 26, 947–956.

Flamm, R.K., Beyer, J., Tanaka, S.K., Clement, J., 1996. Kill kinetics of antimicrobial agents against *Helicobacter pylori*. J. Antimicrob. Chemother. 38, 719–725.

Glupczynski, Y., Burette, A., 1990. Drug therapy for *Helicobacter pylori* infection: problems and pitfalls. Am. J. Gastroenterol. 85, 1545–1551.

Goodwin, C.S., Blake, P., Blincow, E., 1986. The minimum inhibitory and bactericidal concentrations of antibiotics and anti-ulcer agents against *Campylobacter pyloridis*. J. Antimicrob. Chemother. 17, 309–314.

Graham, D.Y., Lew, G.M., Klein, P.D., Evans, D.G., Evans, D.J., Saeed, Z.A., Malaty, H.M., 1992. Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer. A randomized, controlled study. Ann. Intern. Med. 116, 705–708.

Haas, C.E., Nix, D.E., Schentag, J.J., 1990. In vitro selection of resistant *Helicobacter pylori*. Antimicrob. Agents Chemother. 34, 1637–1641.

Iwahi, T., Satoh, H., Nakao, M., Iwasaki, T., Yamazaki, T., Kubo, K., Tamura, T., Imada, A., 1991. Lansoprazole, a novel benzimidazole proton pump inhibitor, and its related compounds have selective activity against *Helicobacter pylori*. Antimicrob. Agents Chemother. 35, 490–496.

Kitagawa, H., Fujiwara, M., Osumi, Y., 1979. Effects of water-immersion stress on gastric secretion and mucosal blood flow in rats. Gastroenterology 77, 298–302.

Koga, T., Kawada, H., Utsui, Y., Domon, H., Ishii, C., Yasuda, H., 1996. In-vitro and antibacterial activity of plaunotol, a cytoprotective antiulcer agent, against *Helicobacter pylori*. J. Antimicrob. Chemother. 37, 919–929.

Krakowka, S., Eaton, K.A., Leunk, R.D., 1998. Antimicrobial therapies for *Helicobacter pylori* infection in gnotobiotic piglets. Antimicrob. Agents Chemother. 42, 1549–1554.

Marshall, B.J., 1994. *Helicobacter pylori*. Am. J. Gastroenterol. 89, S116–S128.

Palacin, C., Tarrago, C., Sacristan, A., Qrtiz, J.A., 1997. In vitro anti-*Helicobacter pylori* activity of ebrotidine. Arzneim.-Forsch./Drug Res. 47 (I), 471–474.

Patchett, S., Beattie, S., Leen, E., Keane, C., O'Morain, C., 1992. *Helicobacter pylori* and duodenal ulcer recurrence. Am. J. Gastroenterol. 87, 24–27.

Piotrowski, J., Slomiany, A., Slomiany, B.L., 1995. Inhibition of *Helicobacter pylori* urease activity by ebrotidine. Biochem. Mol. Int. 37, 247–253.

Shungu, D.L., Nalin, D.R., Gilman, R.H., Gadebusch, H.H., Cerami, A.T., Gill, C., Weissberger, B., 1987. Comparative susceptibilities of *Campylobacter pylori* to norfloxacin and other agents. Antimicrob. Agents Chemother. 31, 949–950.

Slomiany, B.L., Piotrowski, J., Slomiany, A., 1997. Anti-*Helicobacter pylori* activities of ebrotidine. Arzneim.-Forsch./Drug Res. 47 (1), 475–482.

Sugiyama, T., Hisano, K., Ochiai, T., Fujita, N., Kobayashi, T., Yabana, T., Kurokawa, I., Yachi, A., 1995. Lansoprazole versus lansoprazole plus amoxicillin treatment for eradication of *Helicobacter pylori* in patients with gastric ulcer. J. Clin. Gastroenterol. 20 (Suppl.2), S104–S106.

Valle, J., Seppälä, K., Sipponen, P., Kosunen, T., 1991. Disappearance of gastritis after eradication of *Helicobacter pylori*. A morphometric study. Scand. J. Gastroenterol. 26, 1057–1065.

Warren, J.R., Marshall, B., 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1, 1273–1275.