JB-9322, a new selective histamine H₂-receptor antagonist with potent gastric mucosal protective properties

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- 1 JB-9322 is a selective histamine H₂-receptor antagonist with gastric antisecretory activity and mucosal protective properties.
- 2 The affinity of JB-9322 for the guinea-pig atria histamine H₂-receptor was approximately 2 times greater than that of ranitidine.
- 3 *In vivo*, the ID₅₀ value for the inhibition of gastric acid secretion in pylorus-ligated rats was 5.28 mg kg⁻¹ intraperitoneally. JB-9322 also dose-dependently inhibited gastric juice volume and pepsin secretion. In gastric lumen-perfused rats, intravenous injection of JB-9322 dose-dependently reduced histamine-, pentagastrin- and carbachol-stimulated gastric acid secretion.
- 4 JB-9322 showed antiulcer activity against aspirin and indomethacin-induced gastric lesions and was more potent than ranitidine.
- 5 JB-9322 effectively inhibited macroscopic gastric haemorrhagic lesions induced by ethanol. Intraperitoneal injection was effective in preventing the lesions as well as oral treatment. The oral ID₅₀ value for these lesions was 1.33 mg kg⁻¹. By contrast, ranitidine (50 mg kg⁻¹) failed to reduce these lesions. In addition, the protective effect of JB-9322 was independent of prostaglandin synthesis.
- 6 These results indicate that JB-9322 is a new antiulcer drug that exerts a potent cytoprotective effect in addition to its gastric antisecretory activity.

Keywords: JB-9322; ranitidine; histamine H₂-receptor antagonist; gastric acid secretion; mucosal protection; cytoprotection

Introduction

Since Black *et al.* (1972) first defined the H₂-receptor and characterized a number of H₂-antagonists, various types of these compounds have been developed and used clinically. The introduction of H₂-antagonists has revolutionized the treatment of peptic ulcers and it has been proved that ulcer healing effects of these drugs are due mainly to the inhibition of gastric acid secretion through blockade of histamine H₂-receptors by competitive antagonism at the receptor (Angus *et al.*, 1980; Takana *et al.*, 1987). However, it has been demonstrated that the recurrence ratio of peptic ulcer is relatively high after healing by long-term H₂-antagonist therapy (Grant *et al.*, 1989; Debongnie, 1992; Shamburek & Schubert, 1993). In the clinical stage, patients are treated with H₂-antagonists in combination with defensive factors potentiating-agents to prevent the ulcer relapse. On the other hand, interest has grown in discovering therapeutic agents that prevent ulcer formation or relapse by increasing defensive factors in the gut. The property of a drug that protects the gastric mucosa against necrotizing agents such as acid and ethanol has been coined cytoprotection (Robert *et al.*, 1979). Although these cytoprotective mechanisms are unknown, the protective effect by an agent is mainly considered to be due to the increase of gastric mucosal defensive integrity.

From this point of view, in our search for an H₂-receptor antagonist which exerts antisecretory and cytoprotective activities, we have found JB-9322 (Figure 1). In the present study we describe the pharmacological profile of JB-9322, a potent antisecretory H₂-antagonist which has inherent mucosal protective properties that are independent of endogenous prostaglandin synthesis, at doses similar to its antisecretory ID₅₀ value. Ranitidine has been utilized as reference H₂-receptor antagonist (Bradshaw *et al.*, 1979).

Methods

In vitro assay techniques

H₂-receptor antagonism in guinea-pig isolated atria Antagonism at the histamine H₂-receptor was determined in guinea-pig isolated right atria. Male Dunkin-Hartley guinea pigs weighing 350–600 g were killed by cervical dislocation and exsanguination. The hearts were excised from animals and placed in a Petri dish containing oxygenated (95% O₂ + 5% CO₂) Krebs-Henseleit solution (composition, mM: NaCl 118.0, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.3, MgSO₄·7H₂O 1.2, NaHCO₃ 30.0 and glucose 12.0). The right spontaneously beating atrium was dissected free carefully and suspended in 10-ml tissue bath with oxygenated Krebs-Henseleit solution and kept at 37°C. The tissue was attached with an initial load of 1 g to an isometric force-displacement transducer and recorded on a polygraph. After a 30-min stabilization period, three histamine concentration-response curves were established at 20-min intervals by measuring the chronotropic effect of increasing concentrations of histamine given in a cumulative fashion until the maximal response was consistent. The histamine-induced increase in atrial rate was allowed to plateau before the next successive concentration was added. Between each curve the tissues were washed out several times and the heart rate allowed to return to basal level. The second curve served as control and the third was

Figure 1 Chemical structure of JB-9322 (N-[3-[3-(piperidin-1-ylmethyl)phenoxy]propyl]-N'-cyclopropylmethyl-2-nitro-1,1-ethenediamine).

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repeated either 5 or 30 min after the tissue was incubated with various concentrations of JB-9322 or reference H_2 -antagonist, ranitidine, adding higher concentrations of histamine as needed.

H₁-receptor antagonism in guinea-pig isolated ileum A 2- to 3-cm piece of ileum was removed from the animal and suspended in 20 ml tissue muscle bath containing atropinized Tyrode solution of the following composition (mM): NaCl 136.9, KCl 2.7, $MgSO_4 \cdot 7H_2O$ 1.0, NaH_2PO_4 0.3, glucose 5.5, $CaCl_2$ 1.8, $NaHCO_3$ 11.9 and atropine 2.0×10^{-4} . The solution was maintained at 37°C and bubbled with air. The recordings were made as above. The tissue was attached to an isometric force-displacement transducer with an initial load of 1 g, and it was allowed to stabilize for 30 min. After the stabilization period, cumulative concentration-response curves to histamine were established in 15-min periods until obtaining a reproducible contraction. After 5-min incubation with 10^{-6} M of JB-9322 or ranitidine, the histamine concentration-response curve was repeated.

Muscarinic-receptor antagonism in rat isolated duodenum Male Wistar rats weighing 250 to 300 g fed *ad libitum* were used. Immediately after the rats had been killed by decapitation, the duodenum was surgically removed. A 2-cm length of duodenum was suspended in 10-ml tissue bath filled with Tyrode solution with the composition given above. The solution was maintained at 35°C and bubbled continuously with air. The tissue was attached to an isometric force-displacement transducer with an initial load of 1 g. After a 30-min stabilization period, concentration-response curves to acetylcholine in 10-min periods were carried out in a cumulative form until the maximal response was consistent. When a reproducible contraction was obtained, a new concentration-response curve was repeated after 5-min incubation with 10^{-6} M of JB-9322 or ranitidine.

Calculation and statistical analysis

Data were expressed as a percentage of the maximal agonist response established in the absence of the antagonist for each preparation in the control curve. Concentration-response curves in the above *in vitro* preparations were obtained by plotting the percentage of the maximal control response against log concentration of histamine or acetylcholine. Each point shows the mean and s.e.mean of at least 5 experiments. The cumulative concentration-response curves, with and without the antagonists were analysed using the Tallarida & Murray computer programme (1984). This programme subjects to linear regression the data between 20% and 80% of the maximum control response to obtain the EC_{50} values and the extreme values are not used.

In the atrium assay, the pA_2 values and slopes of the Schild regression were calculated according to the method of Arunlakshana & Schild (1959). The dose-ratios for this analysis have been calculated for each curve pair. Statistical comparisons between groups were performed by Student's *t* test for paired data, and values of *P* of 0.05 or less were regarded as significant from controls.

In vivo assay techniques

Wistar rats kept in cages with raised mesh bottoms were deprived of solid diet 24 h before experiments began but received a nutritive solution of 8 g 100 ml⁻¹ sucrose in 0.2 g 100 ml⁻¹ NaCl to avoid excessive dehydration. This solution was removed 2 h before starting experiments.

Effects on gastric acid secretion

Measurement of acid secretion in the lumen-perfused stomach of the anaesthetized rat The procedure followed for the measurement of gastric acid secretion was described by

Ghosh & Schild (1958). Male Wistar rats weighing 200–250 g were used. In brief animals were anaesthetized with urethane (1.25 g kg⁻¹, i.p.) and the trachea intubated. A soft catheter was passed down the oesophagus through an incision in the cervical side so that the opening was just proximal to the cardiac sphincter and secured in this position by a ligature. After a laparotomy, a second polyethylene cannula was inserted into the stomach via an incision in the duodenum, held in place by two ligatures and let outside the abdominal wall for collection of gastric secretion. In studies in which secretion was stimulated, the jugular vein was cannulated for administration of the secretagogues. The penile vein was cannulated for i.v. administration of saline or drugs. At the beginning of the experiment, the stomach was flushed with 20–40 ml of saline at 37°C to remove any solid content.

Once the surgical preparation had been completed, the gastric lumen was perfused continuously with warm saline (NaCl 0.9% w/v, 37°C) at a rate of 1 ml min⁻¹ by the use of a peristaltic pump via the oesophageal cannula. After a period of 45 min for stabilization, the perfusate flowing from the stomach was collected at 15-min intervals and H^+ output determined by automatic titration (Crison titrator, micro TT 2050, Barcelona, Spain) of aliquots (10 ml) of the perfusate with 0.01 N NaOH to pH 7.0. Gastric acid secretion was stimulated by the i.v. infusion at the rate of 1 ml h⁻¹ of histamine (5 mg kg⁻¹ h⁻¹) or pentagastrin (10 µg kg⁻¹ h⁻¹) during a 4.5 h period or during a 5 h period for carbachol (10 µg kg⁻¹ h⁻¹), starting 45 min after determination of three basal values of acid secretion. In the case of the stimulation with histamine and pentagastrin, the acid secretion reached a steady state 60 min after the beginning of infusion of the secretagogues. To reach the steady state in the carbachol-induced gastric acid secretion at 90 min, carbachol (5 µg kg⁻¹) was injected as a bolus through the cannulated penile vein when the infusion started.

H_2 -antagonists or vehicle (0.9% saline) were administered i.v. in a volume of 1 ml kg⁻¹ 60 min (histamine- and pentagastrin-stimulation) or 90 min (carbachol-stimulation) after the start of the secretagogue stimulation. Data were expressed as a percentage of the value of acid secretion 60 min (histamine and pentagastrin stimulation) or 90 min (carbachol stimulation) after the initiation of secretagogue infusion. The dose-response relationship was established using the area under the dose-response curve from 60 to 270 min (histamine and pentagastrin) or from 90 to 300 min (carbachol) after the start of secretagogue infusion.

In the experiments to determine the influence of JB-9322 and ranitidine on basal acid secretion, after stabilization (45 min) drugs or saline were infused continuously via the penile vein at 5 mg kg⁻¹ h⁻¹, 1 ml h⁻¹ for 150 min. The concentration of H^+ was estimated every 10 min.

Gastric secretion in pylorus-ligated rat The pylorus-ligated rat model first described by Shay *et al.* (1945) was used. Male Wistar rats weighing 180–200 g were used: 2 h before and throughout the collection periods no access to the nutritive solution was allowed. The surgical manipulation was carried out under light ether anaesthesia. A small abdominal incision was made and the pylorus was ligated. Care was taken to avoid trauma to the surrounding vasculature. The incision was closed with silk sutures and test substances or vehicle (0.9% saline) were administered i.p. in a volume of 1 ml kg⁻¹ immediately after surgery. The animals were killed 4 h after ligation of the pylorus, the abdomen re-opened and the stomach was excised carefully keeping the oesophagus closed, opened along the greater curvature and the luminal contents were collected and centrifuged for 15 min at 4500 r.p.m. to remove residual debris. The volume was measured and aliquots were taken for determination of the acidity by automatic titration, using the titration system mentioned above, with 0.1 N NaOH to the endpoint of pH 7.0. Total acid output was expressed as microequivalents of H^+ per 4 h. Pepsin concentration was determined by a modification of

the colorimetric method of Anson (1938) involving haemoglobin digestion (2%, pH 2.0, 37°C, 15 min) followed by alkaline condensation with Folin-Ciocalteu reagent and spectrophotometric measurement of the absorption at 578 nm.

For assessment of duration of antisecretory action in the Shay rat, in a separate series of experiments compounds 30 mg kg⁻¹ or saline were administered i.p. 7 h before the pylorus was ligated.

Experimentally induced gastric lesion studies

Aspirin and indomethacin-induced gastric lesions Male Wistar rats weighing 170–190 g were used. The selection of the ulcerogenic agent was the result of preliminary studies. Gastric lesions were induced by administration of aspirin (200 mg kg⁻¹, orally, suspended in 1% carboxymethylcellulose in water) plus indomethacin (40 mg kg⁻¹, s.c., dissolved in 0.9% saline) at the same time. The indomethacin solution was freshly prepared and the aspirin suspension homogenized immediately before use. JB-9322, ranitidine or vehicle (0.9% saline) were given orally (10 ml kg⁻¹) 30 min before the ulcerogenic drugs. Five hours after the aspirin plus indomethacin administration, rats were decapitated, their stomachs removed, opened along the greater curvature, gently rinsed under tap water and pinned on a paraffin and plastic polymer plate. The stomachs were then photographed, the photograph amplified and the area of each macroscopic lesion (mm²) in the glandular portion was measured by computerized planimetry (Ibas Interactive Image Analysis System, Kontron). The total area of the lesions was regarded as the lesion index. The lesion index was expressed as the mean lesion area for each group of rats.

The possible inaccuracy inherent in this assessment was minimized by ensuring that the lesions were measured by the same person.

Gastric protective activity in rats: ethanol-induced lesions Gastric protection activity studies were conducted in female Wistar rats weighing 170–200 g using the method of Robert *et al.* (1979). Rats were pretreated with drugs or vehicle (0.9% saline), administered either orally (10 ml kg⁻¹) or intraperitoneally (2 ml kg⁻¹); 30 min later the rats were given 1 ml of absolute ethanol orally. After another 30 min the animals were killed and the stomachs excised. The remaining procedure was the same as described above for aspirin and indomethacin-induced gastric lesions.

In experiments designed to determine whether mucosal protection by JB-9322 is dependent on the synthesis of prostaglandins, rats were given indomethacin (10 mg kg⁻¹) subcutaneously 90 min before the oral drug or vehicle pretreatment. This dose of indomethacin has been shown previously to eliminate the production of prostaglandins in the gastric mucosa (Ligumsky *et al.*, 1982). Protection was then determined as described.

The results were expressed as the percentage of the total glandular mucosal area occupied by necrotic lesions.

Calculations and statistical analysis

Data are presented as the mean $\pm$ s.e.mean. The inhibitory ratio (%) was obtained by comparing the values in the treated animals with that of the control group. The doses causing 50% inhibition (ID₅₀) were calculated from the dose-inhibition relationships by least squares regression: 95% confidence limits of the ID₅₀ values were determined by Simfit programme (University of Manchester, U.K.). The significance of differences was assessed by Student's unpaired *t* test and a *P* value of less than 0.05 was considered to be significant.

Drugs

The following drugs were used: JB-9322 (synthesized in the Department of Organic Chemistry, Faculty of Chemistry,

University of Salamanca), ranitidine hydrochloride, acetylcholine chloride, carbachol and aspirin (Sigma), histamine dihydrochloride (Merck), carboxymethylcellulose (Panreac). Indomethacin (Inacid, Merck Sharp & Dohme) and pentagastrin (Peptavlon, ICI) were used as the preparations available for clinical use. The compounds were dissolved in isotonic saline immediately before use. Doses of JB-9322 and ranitidine are expressed as free base.

Results

In vitro studies

The histamine H₂-receptor antagonist properties of the test compounds were determined by their inhibition of the chronotropic effects of histamine on guinea-pig isolated atria (Black *et al.*, 1972). In our preliminary experiments, JB-9322 and ranitidine showed the same antagonism on the response to histamine in atria with 5 min and 30 min incubations. Thus, for the assessment of the inhibitory effect of these drugs a 5 min incubation was used. JB-9322 and ranitidine did not affect the resting atrial rate (201.7 $\pm$ 4.2 contractions min⁻¹) during the period of incubation. JB-9322 (10⁻⁸–3 $\times$ 10⁻⁷ M) and ranitidine (10⁻⁷–3 $\times$ 10⁻⁶ M) produced a concentration-related parallel shift of histamine concentration-response curve to the right without affecting (*P* > 0.05) the maximal response (297.7 $\pm$ 6.9 contractions min⁻¹) (Figure 2). The Schild regressions of log (dose ratio-1) vs log molar concentration of these compounds were linear with slopes and 95% confidence limits of 0.94 (0.85–1.02) and 0.76 (0.55–1.04) for JB-9322 and ranitidine, respectively which were not significantly different from unity (Figure 2). JB-9322 and ranitidine showed a competitive antagonistic activity and their pA₂ values and 95% confidence limits were 7.76 (7.70–7.83) and 7.36 (7.01–7.71), respectively. The dose-ratios for the Schild analysis have been calculated for each curve pair and due to differences in the location of the control curve, the mean concentration-effect curves in Figure 2 do not accurately represent the information generated from an individual tissue. For this reason, the Schild plots for each compound are shown. Thus, JB-9322 has an affinity for the histamine H₂-receptor which is approximately 2 times greater than that of ranitidine.

JB-9322 and ranitidine 10⁻⁶ M did not cause any significant displacement of the histamine or acetylcholine concentration-response curve in ileal and duodenal preparations, respectively.

In vivo studies

Effect on gastric acid secretion

Effect on acid secretion in the gastric lumen-perfused rats Basal acid secretion was 3.57 $\pm$ 0.20 (μ Eq H⁺ 15 min⁻¹) in the gastric lumen-perfused rats. A stable secretion (24.76 $\pm$ 1.25 μ Eq H⁺ 15 min⁻¹) was seen 60 min after the infusion of histamine (submaximal dose: 5 mg kg⁻¹ h⁻¹). The histamine-induced acid secretion decreased gradually, but significant secretion was observed for at least 4.5 h after the beginning of the infusion. JB-9322 and ranitidine administered i.v. inhibited the histamine-stimulated acid secretion with a clear dose-response relationship. As shown in Figure 3, the maximum inhibitory effect obtained with JB-9322 and ranitidine was observed 30 min after the i.v. administration in both cases. However, the recovery from the antisecretory activity of JB-9322 was appreciably faster than the recovery from this of ranitidine. The values of ID₅₀ at each time interval are listed in Table 1.

On the pentagastrin- and carbachol-stimulated acid secretion, JB-9322 and ranitidine also inhibited the secretion dose-dependently and the maximum inhibitory effect was observed between 30–60 min after the i.v. administration of com-

pounds in both cases. But as in the case of histamine-stimulated acid secretion, the recovery from the inhibitory activity was faster with JB-9322 (Figure 3). The values of ID_{50} for pentagastrin and carbachol at each time interval are listed in Tables 2 and 3, respectively.

JB-9322 ($5 \text{ mg kg}^{-1} \text{ h}^{-1}$, i.v.) decreased the acid concentration of the unstimulated stomach but was less potent than ranitidine at the same dose (Figure 4).

Gastric secretion in pylorus-ligated rats Gastric secretion was evaluated as gastric juice volume, acid output and pepsin output for 4 h after ligation of the pylorus. These values in control rats given vehicle were $5.13 \pm 0.54 \text{ ml } 4 \text{ h}^{-1}$, $383.70 \pm 57.51 \mu\text{Eq H}^+ 4 \text{ h}^{-1}$ and $26.75 \pm 3.86 \text{ mg } 4 \text{ h}^{-1}$, respectively. When administered immediately after ligation, JB-9322 (3, 10 and 30 mg kg^{-1} , i.p.) dose-dependently inhibited the gastric acid secretion, gastric juice volume as well as pepsin secretion. Ranitidine (1, 3, 10 and 30 mg kg^{-1} , i.p.)

also caused a marked decrease in each secretory parameter. The decrease of pepsin output was due largely to a reduction in gastric volume inasmuch as concentration was not greatly changed. However at higher doses of these compounds, the acid output was inhibited to a greater extent than the volume of secretion (Figure 5).

The intraperitoneal ID_{50} values and 95% confidence limits for reduction in gastric acid secretion for JB-9322 and ranitidine were 5.28 mg kg^{-1} (3.69–7.64) and 3.72 mg kg^{-1} (1.23–7.30), respectively. JB-9322 inhibited to a greater extent the gastric juice volume and pepsin secretion.

When injected i.p. 7 h before ligation, an equipotent dose in terms of the immediate effect (30 mg kg^{-1}) of JB-9322 and ranitidine, ranitidine significantly inhibited both gastric juice volume and acid output; however, this potency was weaker in comparison with that observed when ranitidine was administered immediately after ligation. The effect of JB-9322 on gastric juice volume and acid output was not significant (Figure 6).

Figure 2 Effect of a 5-min incubation with JB-9322 (a) and ranitidine (b) on the positive chronotropic concentration-response curve to histamine in guinea-pig isolated right atrium. Control (●); JB-9322: $3 \times 10^{-7} \text{ M}$ (Δ), 10^{-7} M (□), $3 \times 10^{-8} \text{ M}$ (■), 10^{-8} M (○); ranitidine: $3 \times 10^{-6} \text{ M}$ (Δ), 10^{-6} M (□), $3 \times 10^{-7} \text{ M}$ (■), 10^{-7} M (○). Each curve represents the mean $\pm$ s.e. mean response obtained in at least 5 tissues. Beside these curves, Schild plots for JB-9322 and ranitidine are shown. DR = histamine dose-ratio.

Figure 3 Antisecretory effect of JB-9322 (a) and ranitidine (b) on the histamine-, pentagastrin- and carbachol-stimulated gastric acid secretion in lumen-perfused rats. Data were expressed as a percentage of value of acid secretion 60 min (histamine- and pentagastrin-stimulation) or 90 min (carbachol-stimulation) after the initiation of secretagogue infusion. Each point indicates the mean $\pm$ s.e. mean obtained from 5 to 9 rats. Control (O); JB-9322 (mg kg⁻¹, i.v.): 0.3 (●), 0.6 (▲), 1 (□), 3 (Δ). Ranitidine (mg kg⁻¹, i.v.): 0.3 (●), 1 (□), 3 (Δ).

Table 1 ID₅₀ of JB-9322 and ranitidine for inhibition of histamine-stimulated gastric acid secretion in lumen perfused rats

Treatment (i.v.)	ID ₅₀ (mg kg ⁻¹)				
	Time after drug administration (min)				
	60–120	60–180	60–210	60–240	60–270
Ranitidine	0.34 (0.12–0.81)	0.65 (0.33–1.15)	0.77 (0.44–1.31)	0.96 (0.60–1.52)	1.20 (0.87–1.73)
JB-9322	0.89 (0.56–1.44)	1.31 (1.16–1.49)	1.79 (1.60–2.03)	2.56 (2.23–2.98)	4.25 (3.41–5.46)

Each value represents ID₅₀ with the 95% confidence limits in parentheses.

Table 2 ID₅₀ of JB-9322 and ranitidine for inhibition of pentagastrin-stimulated gastric acid secretion in lumen-perfused rats

Treatment (i.v.)	ID ₅₀ (mg kg ⁻¹)				
	Time after drug administration (min)				
	60–120	60–180	60–210	60–240	60–270
Ranitidine	1.24 (0.78–2.02)	0.73 (0.67–0.82)	0.79 (0.72–0.86)	0.82 (0.74–0.91)	0.89 (0.80–0.99)
JB-9322	1.96 (1.12–3.69)	1.54 (1.29–1.88)	1.66 (1.40–2.00)	1.86 (1.52–2.32)	2.30 (1.80–3.04)

Each value represents ID₅₀ with the 95% confidence limits in parentheses.

Table 3 ID₅₀ of JB-9322 and ranitidine for inhibition of carbachol-stimulated gastric acid secretion in lumen-perfused rats

Treatment (i.v.)	ID ₅₀ (mg kg ⁻¹)				
	Time after drug administration (min)				
	90–150	90–210	90–240	90–270	90–300
Ranitidine	2.36 (0.98–5.73)	0.88 (0.87–0.90)	1.00 (1.00–1.15)	1.16 (0.85–1.59)	1.36 (0.85–2.21)
JB-9322	2.78 (2.43–3.25)	2.30 (2.08–2.54)	2.83 (2.40–3.37)	4.19 (2.62–6.78)	4.27 (2.95–6.68)

Each value represents ID₅₀ with the 95% confidence limits in parentheses.

Experimentally induced gastric lesion studies

Aspirin and indomethacin-induced gastric lesions The selection of the ulcerogenic drugs was determined from preliminary studies. Administration of indomethacin at doses between 10 and 60 mg kg⁻¹ orally or subcutaneously caused slight gastric ulceration. No gastric lesions were observed in animals treated with aspirin orally at a dose of 250 mg kg⁻¹. In our experience, the most suitable conditions for evaluation of JB-9322 and ranitidine were the combined effect of administration of indomethacin (40 mg kg⁻¹, s.c.) plus aspirin (200 mg kg⁻¹, oral).

No gastric lesions were observed in animals treated with aspirin vehicle (1% carboxymethylcellulose). Administration of aspirin plus indomethacin resulted in the production of gastric lesions in the glandular segment of the stomach, primarily the corpus, but occasionally the antrum also, in 100% of the control animals. Pretreatment with JB-9322 and ranitidine 30 min before the NSAIDs gavage produced a significant and dose-dependent reduction in the severity and incidence of these lesions (Figure 7). Some of the animals that received the highest dose of JB-9322 (20 mg kg⁻¹) were completely protected from ulcer formation. The estimated oral ID₅₀ values and 95% confidence limits were 2.63 mg kg⁻¹ (1.82–3.74) and 4.93 mg kg⁻¹ (4.13–5.76) for JB-9322 and ranitidine, respectively.

Therefore, in this model the inhibitory activity of JB-9322 was higher than that of ranitidine.

Ethanol-induced gastric lesions Oral administration of absolute ethanol produced severe gastric haemorrhagic lesions visible from the outside of the stomach as thick black or red lines. After opening the stomach, lesions were found in the

Figure 4 Effect of 5 mg kg⁻¹ h⁻¹ of JB-9322 (■) and ranitidine (▽) on basal acid secretion in lumen-perfused rats. Control (●). Data were expressed as the difference between the [H⁺] at each 10-min interval during drug infusion and the [H⁺] just before the initiation of infusion. Each point indicates the mean ± s.e.mean obtained from 5 to 7 rats.

glandular mucosa and consisted of elongated bands, 2–12 mm long by 2–4 mm wide, usually parallel to the long axis of the stomach. They were located mostly in the corpus, the antrum was less affected. The control rats with vehicle had gastric lesions of 159.2 ± 29.8 mm². When rats were orally pretreated with JB-9322, 30 min before the ethanol treatment, the formation of these haemorrhagic lesions was inhibited in a dose-dependent manner and the ID₅₀ and 95% confidence limits were 1.33 mg kg⁻¹ (1.06–1.68). In the group

Figure 5 Effect of saline (solid columns), JB-9322 (open columns) and ranitidine (hatched columns) on basal gastric secretion in pylorus-ligated rats. (a) Gastric acid secretion; (b) gastric juice volume; (c) pepsin secretion. Each drug was given intraperitoneally immediately after surgery. Animals were killed 4 h after pylorus ligation. Each column represents the mean $\pm$ s.e. mean of 8 animals. Significantly different from control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

receiving JB-9322 (50 mg kg^{-1}), the pinpoint round and linear lesions were only partly found in the glandular portion.

In similar experiments, no reduction in ethanol-induced haemorrhagic lesions was seen following the oral administration of ranitidine (50 mg kg^{-1}) (Figure 8).

In order to determine whether JB-9322 acted locally or systemically, its cytoprotective activity against ethanol-induced gastric lesions was tested by i.p. administration. In comparison to the control value of $18.6 \pm 2.6\%$, ethanol lesions were reduced to $16.4 \pm 3.5\%$ (not significant) and $1.6 \pm 0.5\%$ ($P < 0.001$) of lesioned corpus in the groups ($n = 8$ each) pretreated with JB-9322, 3 and 10 mg kg^{-1} i.p., respectively.

Effect of indomethacin on the protective activity of JB-9322

The role of endogenous prostaglandins on the protective effect of JB-9322 was examined in rats that were subcutaneously pretreated with 10 mg kg^{-1} of the cyclo-oxygenase inhibitor, indomethacin. Indomethacin itself significantly increased the amount of lesioning approximately 2 times. The protective effect of JB-9322, however, was not diminished when prostaglandin biosynthesis had been inhibited before-

Figure 6 Duration of antisecretory effect of JB-9322 (open columns) and ranitidine (hatched columns) in pylorus-ligated rats. Rats were intraperitoneally dosed with saline (solid columns) or drugs 7 h before the pylorus ligation. Four hours after ligation the rats were killed and gastric acid output (a) and gastric juice volume (b) determined as described. Each column represents the means $\pm$ s.e. mean of 6 animals. * $P < 0.05$ compared to control.

Figure 7 Inhibition by JB-9322 and ranitidine of aspirin and indomethacin-induced gastric lesions. Rats were orally dosed with saline (solid columns), JB-9322 (open columns) or ranitidine (hatched columns) 30 min before each rat received 200 mg kg^{-1} of aspirin orally plus 40 mg kg^{-1} of indomethacin subcutaneously. Each column represents the mean $\pm$ s.e. mean of at least 6 animals. Significantly different from control * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

hand by indomethacin. In the placebo-pretreated groups, JB-9322 (10 mg kg^{-1}) reduced ethanol-induced lesions from 14.7 ± 1.3 to $5.5 \pm 1.9\%$ of lesioned corpus. In the indomethacin-pretreated groups, the gastric lesions due to ethanol were 30.9 ± 2.3 and $4.8 \pm 1.5\%$ of lesioned corpus for vehicle control and JB-9322-treated groups, respectively. The inhibition of mucosal lesion formation by JB-9322 compared to the respective control group, was highly significant in each case ($P < 0.001$).

Figure 8 Effect of JB-9322 and ranitidine on ethanol-induced gastric haemorrhagic lesions in the rats. Rats were orally given saline (solid column); JB-9322 (open columns) or ranitidine (hatched column) 30 min before receiving an oral gavage of 1 ml of absolute ethanol. Each column represents the mean $\pm$ s.e.mean of at least 7 animals. Significantly different from control: * $P < 0.05$; ** $P < 0.01$.

Discussion

JB-9322 is a histamine H_2 -receptor antagonist possessing mucosal protective properties as well as gastric antisecretory activity.

In guinea-pig atrium assay, ranitidine produced parallel shifts and surmountable antagonism. The pA_2 value determined by us for ranitidine agrees with values reported by previous authors (Daly *et al.*, 1981; Lumma *et al.*, 1982; Katz *et al.*, 1986a). JB-9322, like ranitidine, shifted the histamine concentration-response curve in a parallel, surmountable manner after either a 5 or 30 min period of incubation. Our results are not consistent with the findings of other investigators that have observed noncompetitive kinetics in the guinea-pig isolated atrial assay with certain H_2 -receptor antagonists having, as a part of their substructure, a [(piperidinylmethyl)phenoxy] propylamine moiety (Brittain & Jack, 1983; Torchiana *et al.*, 1983; Katz *et al.*, 1986b; Santilli *et al.*, 1988) as does JB-9322. In those papers, the initial concentration-response curve shifts elicited at low concentrations of antagonists were rightward and parallel, but as the antagonist concentration was increased, the concentration-response curve shifts became increasingly nonparallel, accompanied by diminished maxima, that were decreased in a time-dependent manner. A recent paper (Sekiguchi *et al.*, 1993a), as in our study, has demonstrated that using a similar phenoxy derivative H_2 -blocker, the positive chronotropic response of guinea-pig right atrium to histamine was shifted to the higher concentrations with the increase of H_2 -antagonist concentration without changing the maximum response as shown in Figure 2 and showing the same antagonism with 5- and 30-min incubations. These differences might be due to the effects of other structural changes in each molecule.

Selectivity of JB-9322 for the H_2 -receptor was demonstrated by a lack of significant inhibition against histamine in guinea-pig isolated ileum and acetylcholine in rat isolated duodenum.

The antagonism of the histamine H_2 -receptor by JB-9322 is reflected *in vivo* by gastric antisecretory activity. In submaximally histamine-stimulated rats, the maximum intravenous antisecretory activity of JB-9322 and ranitidine was reached 30 min after the administration but as shown in Figure 3, the recovery from the inhibitory effect was faster with JB-9322 than with ranitidine. On the pentagastrin- and carbachol-stimulated acid secretion, the potency of the inhibitory effect of ranitidine was slightly greater than that of JB-9322 and the duration of the antisecretory action of JB-9322 was also shorter than that of ranitidine. These results agree well with

the higher gastric acid antisecretory activity and longer duration of action of ranitidine in pylorus-ligated rats, as evidenced by the suppression of gastric acid output for 7 h after i.p. administration of ranitidine, compared with the lack of antisecretory action by JB-9322 in this paradigm.

Despite JB-9322 having an affinity for the histamine H_2 -receptor which was approximately 2 times greater than that of ranitidine, its gastric acid antisecretory effect was weaker in lumen-perfused and pylorus-ligated rats. This means that JB-9322 might be rapidly metabolized *in vivo* and lose its activity due to its pharmacokinetic characteristics.

The inhibitory effect of histamine H_2 -receptor antagonists on experimental lesion formation has been reported (Okabe *et al.*, 1977; Takeda *et al.*, 1982; Isobe *et al.*, 1990; Shibata *et al.*, 1990; Hakkinen *et al.*, 1991; Sekiguchi *et al.*, 1993b), showing that these drugs can be very useful for peptic ulcer patients. Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) are associated with gastrointestinal damage in man and animals (Rainsford, 1975; Kuwayama *et al.*, 1990). Measures such as vagotomy and pretreatment with anticholinergics, histamine H_2 -receptor antagonists and H^+/K^+ -ATPase inhibitors that are known to decrease gastric acidity, all cause a marked amelioration in gastric damage by NSAIDs (Kasuya *et al.*, 1979; Long *et al.*, 1983; Shibata *et al.*, 1990; Sekiguchi *et al.*, 1993b). Therefore, our findings that both JB-9322 and ranitidine could prevent the development of aspirin plus indomethacin-induced gastric lesions were expected from their antisecretory action, which might improve gastric tolerance to nonsteroidal anti-inflammatory drugs as indicated by Grassi *et al.* (1991). Furthermore, some work shows that the presence of HCl throughout the experiment removes the inhibitory effect of histamine H_2 -receptor antagonists on the NSAIDs-induced erosions that was demonstrated when HCl was not present (Carmichael *et al.*, 1978; Katsura *et al.*, 1994). That the prevention of this ulcer formation by ranitidine is ascribed to the suppression of acid secretion is also based on the results that its effective doses in the inhibition of these ulcers are sufficient to decrease the acid secretion in pylorus-ligated rats. Our results clearly demonstrate that JB-9322 has a higher potency than ranitidine in this model, as in the cold stress plus indomethacin-induced lesion model (Palacios *et al.*, 1993).

Cytoprotection was first demonstrated in rats by Robert *et al.* (1979), who showed that many prostaglandins protected the mucosa of the stomach against the haemorrhagic and erosive effects of intragastric administration of absolute ethanol and other necrotizing agents. This pharmacological property is independent of inhibition of gastric secretion. Possible mechanisms include stimulation of mucus and bicarbonate secretion, stimulation of the sodium pump, strengthening of the gastric mucosal barrier, etc. (Guth, 1982). In the present study, we showed that JB-9322 prevented the development of gastric lesions provoked by intragastric ethanol in a dose-related manner, indicating presence of cytoprotective activity. The antisecretory and cytoprotective activities are exhibited in the same dose-range. Ranitidine at 50 mg kg^{-1} orally was inactive, in agreement with the prevailing view that ranitidine is not cytoprotective (Del Soldato *et al.*, 1985; Hakkinen *et al.*, 1991).

Two lines of evidence indicate that the mucosal protective effect of JB-9322 is a direct action of the compound and not the result of adaptive cytoprotection, which occurs following the application of mild irritants to the gastric mucosa and which may be mediated by increased levels of tissue prostaglandins (Robert *et al.*, 1983). First, the protective effect of JB-9322 is not only present when administered orally but also intraperitoneally, thus the protective effect is not dependent on contact of the drug with the gastric mucosa. Second, in a separate series of experiments, rats were pretreated subcutaneously with indomethacin prior to drug treatment and the ethanol challenge, which eliminates the production of prostaglandins in the gastric mucosa and inhibits the protective effect of mild irritants (Robert *et al.*, 1983). Treatment

1983). Treatment with indomethacin alone significantly increased the injury after the ethanol gavage, probably due to the removal of the protective endogenous prostaglandins. Despite the indomethacin pretreatment, the full protective effect of JB-9322 on the gastric mucosa remained, indicating that the presence of endogenous prostaglandins is not essential to the expression of mucosal protective activity of JB-9322.

Ranitidine, having a more potent antisecretory effect than JB-9322, revealed weaker activity than JB-9322 on NSAIDs and cold stress plus indomethacin-induced gastric lesion models. This might indicate that the gastroprotective effect of JB-9322 against these induced injuries is not completely dependent on the ability of gastric antisecretion, but also it depends on its potent cytoprotective effect, since JB-9322

prevented the ulcer formation at doses less than the ID₅₀ for reduction in the acid output in pylorus-ligated rats.

In summary, JB-9322 is a specific, competitive histamine H₂-receptor antagonist with strong antiulcer and gastro-protective properties. Advantages of JB-9322 over ranitidine include mucosal protective activity and this activity is at doses similar to its antisecretory ID₅₀ value. Thus, JB-9322 may prove to be a useful agent in the treatment of peptic ulcer disease.

The authors would like to thank C. Caballero, M. Grande and J.J. Navarro for the synthesis of JB-9322 and J.C. Villoria for technical assistance. Our thanks to the Junta de Castilla y León for a fellowship to B.P.

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(Received May 23, 1994

Revised January 3, 1995

Accepted January 23, 1995)