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Pharmacological basis for the induction of gastric carcinoid tumours in the rat by loxtidine, an unsurmountable histamine H_2-receptor blocking drug

R.T. Brittain, D. Jack, J.J. Reeves & R. Stables

Glaxo Group Research Ltd, Ware, Herts, SG12 0DJ

1 The very late occurrence of gastric carcinoids in a life-span carcinogenicity study with loxtidine in the rat might have resulted from continuous achlorhydria induced by this long-acting unsurmountable histamine H_2-antagonist.

2 The nature of the anti-secretory activity of loxtidine was compared with that of ranitidine on histamine-induced acid secretion in the perfused stomach preparation of the rat and in the rat isolated gastric mucosa preparation.

3 Ranitidine and loxtidine had qualitatively different inhibitory effects on acid secretion, ranitidine being a competitive antagonist of histamine even at high concentrations, whereas the effect of loxtidine on both preparations was unsurmountable at relatively low concentrations.

4 These results support the hypothesis that the late formation of gastric carcinoids in rats receiving loxtidine is a consequence of persistent achlorhydria caused by unsurmountable blockade of parietal cell H_2-receptors.

Introduction

Loxtidine, a selectively acting histamine H_2-antagonist which is about 5 times more potent than ranitidine, causes much more prolonged inhibition of gastric acid secretion than that drug in the rat and dog. It also differs from ranitidine in causing unsurmountable rather than competitive blockade of the H_2-receptors in guinea-pig atria *in vitro*. Both the unsurmountable blockade and the long duration of action result from prolonged, but slowly reversible, occupation of the affected receptors (Brittain & Jack, 1983). From the chemical structures shown in Figure 1 it is clear that loxtidine, a phenoxypropylaminotriazole, is substantially different from cimetidine or ranitidine and so these qualitative differences in their pharmacological properties are not too surprising.

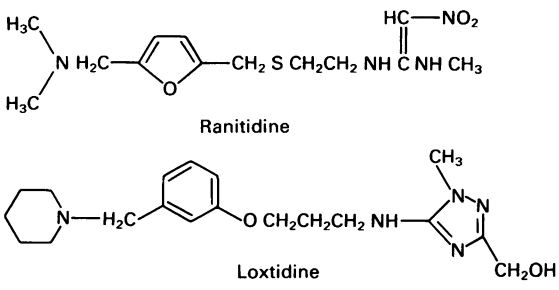

Figure 1 Chemical structures of ranitidine and loxtidine

The possibility of sustained controlled inhibition of acid secretion with a single oral daily dose of loxtidine was considered to be clinically interesting since the end-result would be more akin to that achieved with highly selective vagotomy than that with a competitive H_2-blocker such as ranitidine. However, total suppression of gastric acid secretion was also seen as a possibility with an unsurmountable blocker and this, in turn, brought to mind the possibility of an oncogenic effect in the gastric mucosa after prolonged dosage. Accordingly, it was decided to carry out oncogenicity studies in animals before loxtidine was given to patients.

Loxtidine given orally was well tolerated in chronic toxicity studies of one year's duration in the dog and 18 months in the rat. However, in a life-span carcinogenicity study in the rat, carcinoid tumours (carcinoids) were detected in the fundus of the stomach but only after the drug had been given for 712 days or longer, and the incidence of tumours was not dose-related. Carcinoid tumours are tumours composed of neurosecretory endocrine cells; they are not to be confused with adenocarcinomas whose glandular

origin is often apparent. This is dicussed more fully in a detailed paper on the carcinogenicity study (Poynter *et al.*, 1985). In a similar life-span study carried out earlier using the same strain of rat, ranitidine did not cause gastric tumours in daily doses up to 2000 mg kg^{-1} (Poynter *et al.*, 1982).

Because of the results outlined in the previous paragraph and because loxtidine, its metabolites and its nitroso-product were not mutagenic in micro-organisms commonly used in the Ames test, it seemed likely that the tumours resulted from continuous achlorhydria in the loxtidine-treated animals rather than a direct oncogenic action of the drug. Achlorhydria is, of course, a predictable consequence of unsurmountable H_2-blockade in the gastric parietal cells. To investigate this hypothesis, we have compared the effects of ranitidine and loxtidine on gastric acid secretion in the rat. The experiments were designed to establish firstly, whether there were qualitative differences between the two drugs in their antagonism of histamine-induced gastric acid secretion and secondly, whether, when tested at concentrations equivalent to those achieved in the carcinogenicity studies, loxtidine and not ranitidine would completely suppress histamine-induced acid secretion.

Methods

The effects of ranitidine and loxtidine on gastric acid secretion were studied *in vivo* using an anaesthetized rat perfused stomach preparation (Ghosh & Schild, 1958) and *in vitro* on the rat gastric mucosa preparation (Main & Pearce, 1978).

In vivo *experiments*

Female rats (80 to 120 g) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} intraperitoneally) and a jugular vein cannulated for drug administrations. Two perfusion cannulae were inserted into the stomach to facilitate perfusion of the acid secreting mucosa at 3 ml min^{-1} with 5% dextrose solution at 37°C. The gastric effluent was passed continuously over a microflow pH electrode and the pH recorded via a pH meter on a flat-bed recorder. In each anaesthetized rat one secretory dose-response curve was obtained to histamine by infusing progressively increasing doses for 1 h each. In control experiments saline alone was infused for 1 h and then histamine was tested at 0.01, 0.03, 0.1 and finally 0.3 mg kg^{-1} min^{-1} intravenously. In test experiments an intravenous loading dose of loxtidine or ranitidine was given at time zero followed by intravenous infusion throughout the experiment. One hour later infusion of histamine was started at 0.03 or 0.1 mg kg^{-1} min^{-1} and doses increased hourly up to the maximum tolerated dose of 3 mg kg^{-1} min^{-1}. Gastric acid secretion was measured immediately before starting the histamine infusion and at the peak secretory response to each dose of histamine; results were expressed as change in acid output in nmol H^+ min^{-1}. Mean ± s.e.values were then calculated for each histamine dose level in the control and test groups. Each dose level of ranitidine and loxtidine was tested in at least 6 rats; there were 14 rats in the control group. The intravenous dosing regimes used for ranitidine and loxtidine are given in Table 1 which also relates the plasma levels achieved to those obtained in the rat lifespan carcinogenicity study. To produce a steady state plasma concentration of the H_2-antagonists, it was found necessary to administer a loading bolus dose followed by infusion. The actual plasma levels of ranitidine and loxtidine produced by most of these dosage regimes were determined in parallel experiments using 3 rats per dosage regime. Blood samples (0.05 ml) were taken from anaesthetized rats at hourly intervals during the drug infusions, concentrations of ranitidine and loxtidine were determined by radioimmunossay.

Table 1 Dosage regimes and plasma concentrations of ranitidine and loxtidine in the rat

	Anaesthetized rat perfused stomach experiments		*Rat long-term carcinogenicity study*	
H_2-antagonist	*Intravenous dose bolus (mg kg^{-1}) plus infusion (mg kg^{-1} h^{-1})*	*Plasma conc.* Mean ± s.e. at 1 h (μM)	*Dose group* (mg kg^{-1} day^{-1})	*Basal plasma conc.* (μM)
Ranitidine	9 plus 3	2.99 ± 0.13	H 2000	12.4–28.3
	1 plus 0.3	Not determined	I 450	3.8– 5.1
			L 100	0.3– 0.6
Loxitidine	1.25 plus 0.435	1.44 ± 0.03	H 685	55.6–77.9
	0.42 plus 0.145	0.29 ($n = 2$)	I 185	13.9–16.7
	0.125 plus 0.044	0.064 ± 0.006	L 50	3.1– 3.9

Dose groups: H = high, I = intermediate, L = low

In vitro *experiments*

Female rats (70 to 110 g) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} intraperitoneally), the abdomen opened and the stomach exteriorized. After removing the non-glandular rumen, the fundic mucosa was separated from the underlying muscle layer and tied, mucosal surface inwards, over a small perfusion chamber. The chamber was then placed in a gut bath so that the serosal surface of the tissue was bathed in Krebs solution (serosal solution) at 37°C bubbled with 95% O_2/5% CO_2. The mucosal surface (1.54 cm^2) was then perfused at 0.5 ml min^{-1} with unbuffered Krebs solution (mucosal solution) at 37°C, gassed with 100% O_2, and the pH of the effluent perfusate monitored continuously. The serosal solution contained (mM): NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 1.3 and glucose 11.1. The mucosal solution contained (mM): NaCl 144.7, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.3 and glucose 11.1. Once a steady level of basal secretion was reached (pH 3.5–4.0), 0.05 ml of saline (control experiments) or test drug was added to the serosal solution and 20 min later a cumulative concentration-response curve constructed to histamine (10^{-6} M to 10^{-4} M in control experiments, and up to 10^{-2} M in some test experiments). Ranitidine was tested at 1, 3 and 10 μM and loxtidine at 0.1, 0.3 and 1 μM, each concentration being tested in 6 preparations. The secretory response to each concentration of histamine was expressed as the change in acid output from basal level in nmol H^+ min^{-1}, and the mean ± s.e.values were calculated.

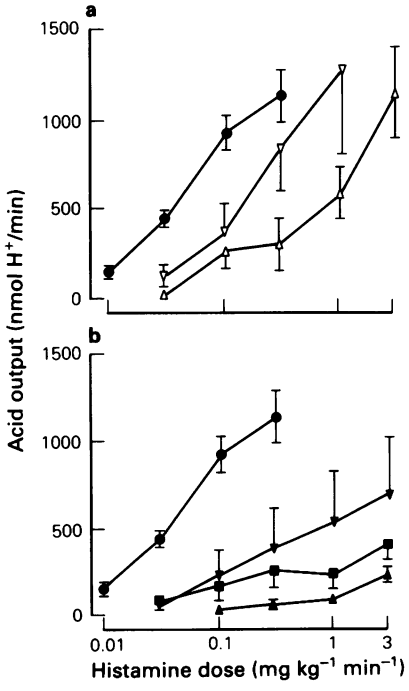

Figure 2 Effects of intravenous ranitidine (a) and loxtidine (b) on histamine-induced acid secretion in the perfused stomach preparation of the anaesthetized rat. Doses (mg kg^{-1} bolus plus mg kg^{-1} h^{-1} infused): ranitidine (▽) 1 plus 0.3, (△) 9 plus 3.0; loxtidine (▼) 0.125 plus 0.044, (■) 0.42 plus 0.145, (▲) 1.25 plus 0.435. Control histamine (●). Values are mean, from 14 rats in the control group and at least 6 rats in each dose schedule of ranitidine and loxtidine; s.e. shown by vertical lines.

Drugs

Ranitidine and loxtidine were synthesized at Glaxo Group Research Ltd.

Results

In vivo *experiments*

The effects of different dosage regimes of ranitidine and loxtidine on histamine-induced acid secretion in the anaesthetized rat are shown in Figure 2. In control animals histamine 0.01–0.30 mg kg^{-1} min^{-1} produced dose-related increases in gastric acid output, the maximum being about 1100 nmol H^+ min^{-1}. Ranitidine, infused at 0.3 and 3.0 mg kg^{-1} h^{-1} after an appropriate loading dose, caused dose-related apparent parallel displacements to the right of the histamine dose-response curves. Thus even during the higher dosage regime which resulted in a plasma ranitidine concentration of 2.99 ± 0.13 μM, histamine was capable of eliciting a full secretory response in the anaesthetized rat. This result contrasts markedly with the effects of loxtidine treatment. All three dosage regimes of loxtidine produced non-parallel displacements of the histamine dose-response curves and the highest loxtidine dose regime which achieved a plasma concentration of 1.44 ± 0.03 μM virtually abolished the gastric secretory response to histamine. Thus loxtidine, behaved as an unsurmountable antagonist of histamine in the rat *in vivo*.

In vitro *experiments*

In control experiments histamine 10^{-6}–10^{-4} M caused concentration-related increases in gastric acid output from rat isolated gastric mucosae, the maximum being about 160 nmol H^+ min^{-1}. As shown in Figure 3 ranitidine, 1, 3, and 10 μM caused concentration-related parallel displacements of the histamine concentration-response curves without depressing the maximum response results which indicated competitive antagonism. The Schild plot slope (Arunlakshana &

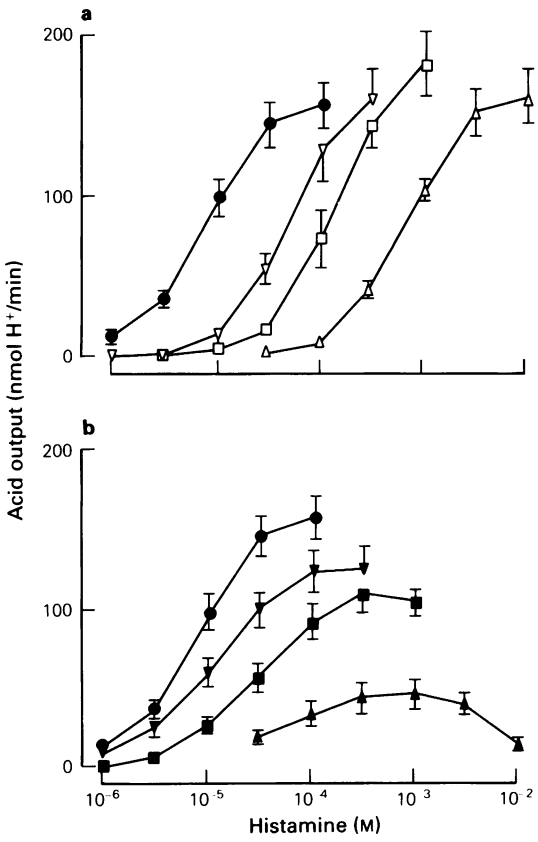

Figure 3 Effects of ranitidine (a) and loxtidine (b) on histamine-induced acid secretion in the rat isolated gastric mucosa preparation. Concentrations (μM); ranitidine (▽) 1, (□) 3, (△) 10; loxtidine (▼) 0.1, (■) 0.3, (▲) 1. Control histamine (●). Values are mean, each response curve being the mean from 6 experiments; s.e. shown by vertical lines.

Schild, 1959) calculated from these results was 1.18 (95% confidence limits 0.87–1.50) and, since this value did not differ significantly from unity, this confirmed that the antagonism observed was competitive. The pA_2 value for ranitidine derived from these data was 6.62 (95% confidence limits 6.36–7.04), which is similar to values previously reported on guinea-pig atrium and rat uterus (Daly *et al.*, 1981b).

The effects of loxtidine, 0.1, 0.3 and 1.0 μM were qualitatively different from those of ranitidine. Loxtidine caused non-parallel displacements of the histamine concentration-response curves and depressed the maximum response (see also Figure 3). At the highest concentration of loxtidine, 1.0 μM, the maximum response obtainable to histamine was only 30% of that obtained in control experiments. Thus loxidine behaves as an unsurmountable H_2-antagonist in this preparation.

Discussion

The results show that ranitidine is qualitatively different from loxtidine as an inhibitor of histamine-induced acid secretion in the rat. In the perfused stomach preparation *in vivo* and on gastric mucosae *in vitro* ranitidine is clearly a competitive H_2-antagonist and this profile of action is consistent with that previously reported for this drug on other preparations containing H_2-receptors such as guinea-pig atrium and rat uterus (Daly *et al.*, 1981b), and for inhibition of histamine-induced acid secretion in the dog (Daly *et al.*, 1981a). It is particularly noteworthy that in the present experiments in the rat, a full secretory response to histamine could still be obtained in the presence of plasma ranitidine concentrations as high as 2.99 μM or 10 μM *in vitro*, provided sufficient histamine was administered. These concentrations are about the same as those in the plasma of rats in the intermediate dose group and approach the levels found in the high dose group in the carcinogenicity study. Accordingly, it is likely that ingestion of food, a strong secretory stimulus, could be capable of eliciting gastric acid secretion even in rats dosed chronically with high doses of ranitidine. Loxtidine, in contrast to ranitidine, is an unsurmountable inhibitor of histamine-induced acid secretion in the rat and this effect was obvious at concentrations less than those achieved in the plasma of rats receiving the lowest dose of loxtidine in the carcinogenicity study. Since loxtidine is also long-acting due to slow disengagement from receptors (Brittain & Jack, 1983) it is highly probable that H_2-receptor-mediated gastric secretory responses in the rats in all the dosage groups in the carcinogenicity study were completely suppressed throughout the test.

These pharmacological findings are consistent with the hypothesis that the late formation of fundic carcinoids seen in some rats given loxtidine for 712 days or longer stemmed from achlorhydria caused by sustained, unsurmountable H_2-blockade in the parietal cells. How drug-induced achlorhydria causes carcinoid tumours in the stomach and why they should occur only after more than 2 years' administration of the drug are, of course, not explained by these experiments. However, if our hypothesis is correct, other drugs which produce achlorhydria by the same or a different mechanism, for example, inhibition of the parietal cell H^+/K^+ ATP-ase should also induce stomach tumours in genuine life-term studies in sensitive rats.

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