The influence of ranitidine, alone and in combination with clemastine, on histamine-mediated cutaneous weal and flare reactions in human skin

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1 Significant, ($P < 0.05$) inhibition of histamine induced cutaneous weal and flare reactions by ranitidine at 10^{-5} M concentration has been demonstrated in a double blind, *in vivo* study; the results support the evidence for cutaneous H_2 -histamine receptors provided by previous studies using cimetidine and metiamide.

2 The degree of inhibition of histamine mediated cutaneous reactions achieved by clemastine was increased by the administration of clemastine and ranitidine together, for ranitidine concentrations 10^{-5} M ($P < 0.001$) and 10^{-6} M ($P < 0.05$).

3 The magnitude of the inhibitory effect of ranitidine, both administered alone and in combination with clemastine, suggests that in the production of histamine induced cutaneous weal and flare reactions, the contribution afforded by stimulation of cutaneous H_2 -histamine receptors is only small.

Keywords ranitidine clemastine histamine human skin

Introduction

Subsequent to the suggestion by Ash & Schild (1966) of at least two classes of histamine receptor, the presence of histamine H_2 -receptors (H_2 -receptors) in human skin has been demonstrated (Marks & Greaves, 1977; Robertson & Greaves, 1978). Pharmacological investigation of the effects of antagonism of these cutaneous H_2 -receptors has been limited to date to studies of the weaker H_2 -receptor antagonists (Marks & Greaves, 1977; Hagermark *et al.*, 1979; Harvey & Schocket, 1980; Carpenter *et al.*, 1983); whilst the results of these studies have not been completely uniform, they have tended to show that cimetidine or metiamide administered alone inhibit histamine induced cutaneous weal and flare reactions, and when administered in combination with an H_1 -receptor antagonist they produce an increase in the inhibition achieved by the H_1 -receptor antagonist alone.

The magnitude of the inhibitory effect of H_2 -receptor antagonists on histamine reactions in

the skin has not been assessed to date, as the previous studies cited examined only a single concentration of H_2 -receptor antagonist (without any assessment of the dose-response characteristics of cutaneous H_2 -receptor antagonism). In the only study to investigate such dose response relationships, no clear dose dependency for the inhibitory effects of cimetidine or metiamide was demonstrated (Hagermark *et al.*, 1979).

In order to explore further the extent to which cutaneous histamine reactions can be inhibited by H_2 -receptor blockade, we have studied the effect on histamine induced weal and flare of ranitidine, an H_2 -receptor antagonist 4–10 times more potent than cimetidine (Bradshaw *et al.*, 1979; Daly *et al.*, 1979; Brittain *et al.*, 1981), over a wide range of concentrations. As far as we are aware, this is the first study to assess quantitatively the contribution afforded by H_2 -receptor stimulation in the production of histamine

mediated weal and flare reactions. It is also the first report to document the effects of cutaneous H_2 -receptor blockade using ranitidine.

Methods

Subjects

After receiving approval from St Bartholomew's Hospital ethics committee, 20 healthy volunteers aged between 19 and 30 years were studied. No subject was receiving any oral, inhaled or topical treatment; no subject was dermographic. All subjects gave their informed consent to participate in the study.

Antagonist drugs and their administration

- Clemastine (H_1 -receptor antagonist) in concentrations tested: 1×10^{-5} M in 10-fold dilutions to 1×10^{-9} M.
- Ranitidine (H_2 -receptor antagonist) in concentrations tested: 1×10^{-5} M in 10-fold dilutions to 10^{-8} M.
- Ranitidine and clemastine together in final concentrations as above in all 20 possible combinations.

All dilutions of ranitidine (from stock solution of 10 mg ml⁻¹, Glaxo Laboratories Ltd) and clemastine (from stock solution of 1 mg ml⁻¹, Sandoz Ltd) were made with sodium chloride 0.9% weight/volume (w/v), which was also used as the control solution.

Antagonist drug administration

All 29 drug solutions, and sodium chloride 0.9% w/v as control were coded and administered double-blind in random order by intradermal injections of 0.05 ml each (using a 1 ml tuberculin syringe with a 25 gauge Sabre needle) in three rows of 10 injections/row, distributed over the volar surface of the right forearm; all the solutions were then administered by the same method to the left forearm, but so as to abolish any influence that differences in forearm site might exert, the order of the rows and the order of the solutions in each row were reversed such that the most distal, medial solution on the right forearm became the most proximal, lateral solution on the left forearm. No inflammation was observed at the site of any of the solutions injected.

Agonist solution and administration

Histamine acid phosphate 16 mg ml⁻¹ was used as agonist. Forty minutes after antagonist drug in-

jection, the histamine acid phosphate was administered over the sites of previous antagonist injection, using the modified prick test technique of Squires (1950). Preliminary experiments had shown that histamine acid phosphate administered by this method evoked a weal area of approximately 20 mm² in most subjects; additional preliminary studies in five subjects had shown that the maximum inhibition of histamine cutaneous reactions achieved by clemastine, ranitidine and the combination of both drugs, was observed when the interval between antagonist injection and subsequent agonist skin prick testing was between 30 and 45 min.

Measurement of skin prick test response

Ten minutes after prick testing with histamine acid phosphate, the perimeter of each weal, and the boundaries of the maximum diameter of erythema for each prick test were outlined in biro and then transferred by adhesive tape to graph paper. Weal areas were then calculated in mm² and erythema diameter in mm.

Analysis of results

On completion of the study, the means of both results for all 30 antagonist solutions were calculated for each subject individually; the code was then broken, and the result for each antagonist solution was calculated for each subject as a percentage of the value obtained for the saline control for that subject. The means of the results for each drug solution for all 20 subjects were then calculated.

Analysis of variance (ANOVAR) adopting a regression approach was used employing the statistical package MINITAB (The Pennsylvania State University), the effects of the three variables—clemastine concentration, ranitidine concentration and inter-subject variability—being included in the analysis.

The effect of clemastine and ranitidine in combination was then compared against the effect of clemastine alone (for each ranitidine concentration) allowing for both the percentage inhibition due to clemastine at different concentrations and for inter-subject variability. Student's *t*-statistics were then used to determine whether the results which were thereby obtained (which represented the percentage inhibition achieved by ranitidine alone at each of the four concentrations) differed statistically significantly from zero (Table 3).

Results

The results are represented graphically in Figures 1, 2 and 3 and are tabulated in Tables 1, 2 and 3.

The mean control (sodium chloride 0.9% w/v) weal area was 19.92 mm² (s.e. mean = $\pm$ 1.52) and the mean control flare diameter was 23.57 mm (s.e. mean = $\pm$ 1.4).

Clemastine alone caused dose related inhibition of both weal and flare reactions over the concentration range 10⁻⁵–10⁻⁷ M (Figures 1 and 2; Tables 1 and 2).

Ranitidine alone caused significant inhibition of weal and flare at 10⁻⁵ M concentration, $P < 0.05$. The slight inhibition of weal and flare at 10⁻⁶ M and 10⁻⁷ M was not statistically significant (Figure 3; Tables 1 and 2).

Clemastine and ranitidine in combination achieved significantly greater inhibition of weal and flare reactions than clemastine achieved alone, over the ranitidine concentration range 10⁻⁵–10⁻⁶ M. The size of increase in inhibition achieved by the combination compared with that achieved by clemastine alone, was concentration related for ranitidine (Figures 1 and 2; Table 3).

There was no evidence of any interaction between clemastine and ranitidine.

Figure 1 Inhibition of histamine induced flare diameter by clemastine, and clemastine in combination with ranitidine, over ranitidine concentrations 10⁻⁵ M–10⁻⁸ M; mean $\pm$ s.e. mean. $\blacktriangle$ — $\blacktriangle$ clemastine alone, $\bullet$ — $\bullet$ clemastine + ranitidine 10⁻⁵ M, $\circ$ — $\circ$ clemastine + ranitidine 10⁻⁶ M, $\square$ — $\square$ clemastine + ranitidine 10⁻⁷ M, $\bullet$ — $\bullet$ clemastine + ranitidine 10⁻⁸ M.

Figure 2 Inhibition of histamine induced weal area by clemastine, and clemastine in combination with ranitidine, over ranitidine concentrations 10⁻⁵ M–10⁻⁸ M; mean $\pm$ s.e. mean. $\blacktriangle$ — $\blacktriangle$ clemastine alone, $\bullet$ — $\bullet$ clemastine + ranitidine 10⁻⁵ M, $\circ$ — $\circ$ clemastine + ranitidine 10⁻⁶ M, $\square$ — $\square$ clemastine + ranitidine 10⁻⁷ M, $\bullet$ — $\bullet$ clemastine + ranitidine 10⁻⁸ M.

Discussion

Several previous studies have investigated the effect of H₂-receptor blockade on histamine mediated cutaneous reactions; although these studies have tended to confirm the existence of cutaneous H₂-receptors, they have not provided uniform results. Marks & Greaves (1977) showed that cimetidine alone significantly inhibited both weal and flare reactions evoked by histamine and that the combination of cimetidine with chlorpheniramine achieved significantly greater inhibition of histamine induced flare (but not weal) than that achieved by chlorpheniramine alone. Carpenter *et al.*, (1983) however, found the combination of cimetidine and chlorpheniramine achieved no greater inhibition of histamine flare than did chlorpheniramine alone. Harvey & Schocket (1980) were unable to show any statistically significant inhibition of histamine weal by cimetidine alone, but the combination of cimetidine with hydroxyzine (a potent H₁-receptor antagonist) produced a significantly greater degree of inhibition of histamine weal than was produced by hydroxyzine alone.

Similarly conflicting results have been obtained from studies of the effect of cimetidine on cut-

Figure 3 Inhibition of histamine induced weal (Δ) area and flare ($\blacktriangle$) diameter by ranitidine; mean $\pm$ s.e. mean.

aneous weal and flare reactions evoked by allergen or compound 48/80 (Marks & Greaves, 1977; Johnson *et al.*, 1984; Smith *et al.*, 1980; Summers *et al.*, 1981).

Variations in the results of these studies may in part reflect the different dosage schedules employed; the influence of such dosage variations cannot be quantified however, as none of the investigations referred to examined dose response relationships for H_2 -receptor antagonism. In the only previous report in which such dose response effects were studied, Hagermark *et al.* (1979), (using both cimetidine and metiamide as H_2 -receptor antagonists) demonstrated a small reduction in histamine flare and itch, but did not find the reduction to be clearly dose related.

Phillips *et al.* (1983) examined the dose response relationships for H_1 -receptor inhibition of histamine (and allergen) induced cutaneous weal reactions by studying different concentrations of the H_1 -receptor antagonists administered intradermally. In order to define more clearly the effect of H_2 -receptor antagonism on cutaneous reactions mediated by histamine, we have employed a slight modification of the same technique to study the effect of ranitidine, an H_2 -receptor antagonist of between 4 and 10 times greater potency than cimetidine (Bradshaw *et al.*, 1979; Daly *et al.*, 1979; Brittain *et al.*, 1981), over a 10^4 fold range of local tissue concentrations. The maximum concentration studied (10^{-5} M) was 35 times greater than the plasma concentration of ranitidine required to achieve 50% inhibition of gastric acid secretion in man (Peden *et al.*, 1979).

We have demonstrated that histamine weal and flare reactions are significantly inhibited by

Table 1 Histamine flare diameter (expressed as percentage of flare diameter in the presence of control solution) in the presence of clemastine, ranitidine and both drugs in combination.

		Clemastine concentration (M)					
		10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁹	Nil
Ranitidine concentration (M)	10 ⁻⁵	25.0	37.7	72.3	85.8	96.5	90.9
	10 ⁻⁶	31.4	48.8	73.7	88.7	95.1	99.5
	10 ⁻⁷	40.1	58.5	87.2	92.9	103.6	97.9
	10 ⁻⁸	40.6	56.3	81.7	94.1	105.1	105.2
	Nil	42.3	54.2	81.3	100.0	100.2	100.0

Table 2 Histamine weal area (expressed as percentage of weal area in the presence of clemastine, ranitidine, and both drugs in combination).

		Clemastine concentration (M)					
		10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁹	Nil
Ranitidine concentration (M)	10 ⁻⁵	4.1	11.0	48.0	72.6	88.3	85.3
	10 ⁻⁶	4.8	15.1	52.6	90.6	91.0	95.7
	10 ⁻⁷	7.0	23.2	71.5	96.0	99.6	96.8
	10 ⁻⁸	11.3	21.2	71.8	96.9	101.8	103.5
	Nil	5.7	24.6	72.0	102.4	105.4	100.0

Table 3 Percentage increase in inhibition of histamine weal and flare achieved by the combination of ranitidine and clemastine compared with that achieved by clemastine alone (for all clemastine concentrations combined).

	Ranitidine concentration (M)			
	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Weal area	15.4%**	8.9%*	0.2%	0%
Flare diameter	15.1%**	10.0%*	1.5%	0%

** = $P < 0.001$, * = $P < 0.05$

ranitidine at 10^{-5} M concentration; the slight, but non-significant, inhibition achieved by ranitidine at 10^{-6} M and 10^{-7} M concentrations, suggests that this effect is concentration related. Furthermore, when ranitidine was administered in combination with the potent H_1 -receptor antagonist clemastine, the inhibition of both weal and flare was significantly increased compared to that achieved by clemastine alone (the size of this increase also being related to ranitidine concentration). However, the maximum percentage increase in inhibition that was achieved by the addition of ranitidine (at the maximum concentration tested) to the H_1 -receptor antagonist was only 15.4%.

In the previous studies using orally administered cimetidine, the percentage increase in inhibition achieved by the combination of cimetidine with H_1 -receptor antagonist over and above that achieved by the H_1 -receptor antagonist alone was only between 5 and 10% (Marks & Greaves, 1977; Harvey & Schocket, 1980; Johnson *et al.*, 1984); where the plasma cimetidine concentrations were measured (Marks & Greaves, 1977; Johnson *et al.*, 1984) the levels were approximately three to four times greater than the IC_{50} of cimetidine for human gastric acid secretion (Brimblecombe & Duncan, 1977). As these studies only examined cimetidine given orally at a single dose, they do not permit any conclusion as to the potential contribution

that H_2 -receptor stimulation might make to the production of histamine mediated cutaneous reactions; they do however suggest that this contribution is likely to be small. By examining the effect of ranitidine over a wide concentration range, our study has demonstrated that the degree of inhibition achieved by H_1 -receptor antagonists can only be increased to a small degree (approximately 15%) by the addition of a potent H_2 -receptor antagonist (even at very high concentration).

There are four possible explanations which would account for the small inhibitory effect exerted by H_2 -receptor antagonism on cutaneous reactions evoked by histamine. First, compared with their effect on gastric H_2 -receptors, ranitidine and cimetidine may be relatively weak antagonists of cutaneous H_2 -receptors; this is, however, unlikely as direct evidence that H_2 -receptors are not a homogeneous population is as yet far from conclusive (Bertaccini & Coruzzi, 1983). Second, H_1 -receptor antagonists may exert some inhibitory effect on cutaneous H_2 -receptors thereby masking some of the influence of the H_2 -receptor inhibitors; there is however no evidence to indicate such receptor cross-reactivity for the H_1 -receptor antagonist studied. Thirdly, ranitidine may be exerting a degree of antagonism of H_1 -receptors at the high concentrations used in this study; however, using concentrations up to 3.2×10^{-4} M, Daly *et al.* (1981) were unable to demonstrate any inhibitory effect on H_1 -receptors, indicating that even at high concentrations, ranitidine is a selective H_2 -receptor antagonist.

We believe that the small effect of cutaneous H_2 -receptor antagonism indicates that H_2 -receptor stimulation plays only a small part in the production of histamine induced weal and flare reactions; this explanation is supported by the observation that H_1 -receptor antagonism alone may achieve 85–90% inhibition of histamine mediated weal and flare reactions (Johnson *et al.*, 1984).

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