Actions of terfenadine and cimetidine on histamine wheal formation

D. W. VERE

Department of Clinical Pharmacology and Therapeutics, The London Hospital Medical College, Turner Street, London E1 2AD

- 1 A Latin square design was used to compare the effects of four masked oral drugs, placebo, cimetidine, terfenadine and cimetidine plus terfenadine, at single standard doses against histamine injected at concentrations of 0.3 mM and 0.074 mM subepidermally in eight healthy volunteers studied at weekly intervals.
- 2 Wheal growth occurred in three phases, an initial delay, a phase of rapid growth where drugs were effective, and a phase of slow growth where there were no significant drug effects.
- 3 Terfenadine was an effective antihistamine.
- 4 The data were consistent with a terfenadine effect continued into the next treatment block.
- 5 Under these conditions cimetidine apparently reduced the effects of terfenadine during the rapid wheal growth phase both simultaneously and after a week's interval from terfenadine dosing.

Keywords histamine skin terfenadine cimetidine

Introduction

Former work [1] had suggested that histamine wheal formation in human skin takes place by stepwise accretion of small territories to a central area about the injection site. Also, some work in animals and in man [2, 3] had pointed to histamine H₂-receptor involvement in the vascular response to histamine in skin. It was, therefore, decided to use the former methods [1] to study histamine wheal growth in those who had taken either an H₁- or an H₂-receptor antagonist or both, by exploring potential interaction between terfenadine and cimetidine as antagonists of injected histamine in skin.

Methods

Since repeated histamine injections into the same area of skin might alter histamine responses locally, but varying the injection site is also likely to do the same, it was decided to use the same sites in each subject but to inject at not less than weekly intervals.

The experiments were designed for maximum sensitivity to crossover effects, whether from the histamine injections themselves or from the antagonist drugs.

Eight healthy adults, aged from 23 to 53 years, and taking no medication volunteered for the experiments, which were accepted by Tower Hamlets District Ethics Committee. There were three females and five males of

varied Indo-European stock. Each subject came to the laboratory on four occasions. Histamine tests were made between 11.00 h and 12.00 h in an air conditioned laboratory at 21°C ± 1.5°C. The methods used have already been described in detail [1, 4]; a solution of histamine acid phosphate in isotonic saline 1 mg ml⁻¹, was diluted to 1 in 4 and 1 in 16 to yield solutions equivalent to histamine free base, 0.3 mM and 0.074 mM. These were injected subepidermally, with a saline control in volumes of 0.1 ml of each, by tuberculin syringes with 25 gauge needles, into the palmar surface of the left forearm. Wheal areas were recorded at minute intervals for up to 9 min by drawing round them on cellulose acetate sheet, which was then photocopied against graph paper. Areas were measured as the sum of complete graph squares plus half the sum of incomplete squares within each ring. Reasons for measuring area, not volume, of the wheals have been discussed before [4].

Volunteers took placebo or terfenadine 120 mg or cimetidine 800 mg, or both terfenadine 120 mg and cimetidine 800 mg in single oral doses exactly 90 min before each histamine test. These drugs were marketed formulations, masked by packing the tablets in size 00 (8 × 22 mm) opaque white gelatin capsules; the tablets were surrounded by coloured sugar granules so that all the doses resembled one another exactly within the capsules.

The antihistamine drugs were allocated using the Williams' version of a Latin square design [5, 6]. This balances and minimizes the sequential effects of cross-

Table 1 Latin squares for treatment allocations. One is a Williams square (left) and the other a reversed square used as one block in these experiments (right). P, placebo, T, terfenadine, C, cimetidine, T/C, terfenadine plus cimetidine. In the reversed square P was always preceded by T/C and C by T except for one row

Subjects	Weeks				Subjects	Weeks			
	1	2	3	4		1	2	3	4
1	P	T	T/C	C	1	C	T/C	P	T
2	T	C	P	T/C	2	T	C	T/C	P
3	C	T/C	T	P	3	P	T	C	T/C
4	T/C	P	C	T	4	T/C	P	T	C

Table 2 Analysis of variance with repeated measures. P, placebo; B, both terfenadine and cimetidine; C, cimetidine; T, terfenadine

Mean	Effect	n	Effect mean	s.e. mean	Mean square	F ratio	df	Probability () = values for data with initial values subtracted
All data untransformed	Between Latin square	288	first square: 118.32	3.392				
		288	second square 91.88	2.772	25135.48	10.82	1,6	0.017 (0.29) (n = 256)
	Between drugs	P144	122.21	4.86				
		B144	94.49	3.67				
		C144	106.65	5.30				
		T144	97.08	3.67	5664.33	2.68	3,18	0.078 (0.015) (n = 128)
	Drug × time interaction				512.64	2.58	24,144	0.014 (0.011)
	(T + B) vs (C + P)				12516	5.78	1,6	0.053 (0.012)
	(T + B) vs (C + P) contrast × Latin Square interaction				1684.4	0.78	1,6	0.4 (0.74)
Differenced data, 2 to 4 min	Between Latin Squares				852.28	3.20	1,6	0.12
	Between drugs	P48	27.46	3.80				
		B48	19.54	2.36				
		C48	27.67	4.54				
		T48	11.96	2.26	673.24	3.20	3,18	0.048
	Drugs × Latin Square interaction				130.64	0.62	3,18	0.61
	(T + B) vs (C + P) contrast				1674.44	9.16	1,6	0.023
	(T + B) vs (C + P) contrast × Latin Square interaction				128.38	0.70	1,6	0.434
	T vs B contrast				345.04	5.24	1,6	0.062

overs; each crossover occurs only once; all possible treatment sequences are given an equal chance of appearing in the square; each treatment sequence occurs in only one row of the square. But in these experiments two squares were used with different sets of four subjects in each; one block was allocated by a true Williams square as just described; the other block was allocated using a reversed Williams square, with the same four treatments as in the other block (Table 1). Though the treatments are still in a balanced Latin square array when a Williams square is reversed, the crossovers are not; their effects are unbalanced and are maximized for the sequences selected, whereas other possible sequences are ignored. Terfenadine seemed to be the drug most likely to cause crossover effects, so the sequences used in the reversed square design were those which might be expected to maximise crossover effects from terfenadine (Table 1). When these two Latin squares had been completed, they were contrasted as a grouping variable in analysis of variance (ANOVA), so as to maximise the sensitivity of detection of crossover effects from terfenadine.

The remaining variables (drug, histamine strength, time from injection) were analysed in the same ANOVA as within subject variables. ANOVA was done using the BMDP4 programme with repeated measures [7]. Selected contrasts amongst significant results were tested either by the contrast step in P4V, or by paired difference *t*-tests [8].

Regression analysis was done by BMDP 6D. Because time trends were not of prime interest in these experiments, having been analysed previously [1], the rates of growth or reduction in wheal size were measured by differencing the data, subtracting each from its predecessor, thus producing increments between the minute intervals of measurement and so removing trend [9]. Another reason for the differencing procedure is that wheal growth is a self-correlated process in time, each successive wheal area representing small accretions and removals of fluid from the preceding area.

Differencing removes this serial auto-correlation, permitting the analysis of a truly random, stochastic variation (i.e. the small increments or decrements of wheal area) with their random error variation.

Results

The median saline control wheal area on placebo days was 32 mm², the range being from 4 to 108 mm². The saline wheals are not considered further. Figure 1 shows the mean wheal growth patterns for both histamine dilutions and various drug treatments in both Latin squares, one the first block or reversed Williams square, the other the second, or true Williams square. After a delay of 1 to 2 min there was rapid growth; this occurred from the maximum area covered by a hemispherical injection volume of 0.1 ml, i.e. 41.3 mm². Thereafter, growth rate differed, depending upon the concentration of injected histamine, but at a slower rate than in the first 2–4 min.

Figure 1 Composite graphs showing the means of untransformed wheal areas ($n=4$). a) Placebo, b) terfenadine with cimetidine, c) cimetidine and d) terfenadine. Abscissae are minutes from injection; ordinates are wheal area in mm². The horizontal lines represent the area which corresponds to the injection volume, 41.3 mm², and 200 mm². Open symbols, first Latin square; closed symbols second Latin square. Triangles, 0.074 mM histamine; circles, 0.3 mM histamine.

The means of the differenced data for each of the treatments are shown in Figure 2. The rates of wheal growth are similar at the first difference point, which is 2 min from the time of injection. There were apparent differences from the second to the fourth difference points (minutes 3–5), but after this growth rates fell sharply, even becoming negative in some cases. After 5 min, growth rates were small and seemed indistinguish-

Figure 2 Means of differenced data ($n=4$). □ placebo, ● terfenadine, ■ cimetidine, and ○ terfenadine with cimetidine.

able between treatments, but rose again in most cases towards the end of the observations. These findings were true for both histamine dilutions used. Two ANOVA tests were made, first with all the data to assess the difference between the two Latin squares as a grouping variable, and a second to study only minutes 2–4 for the whole block of data to examine the effects of drug, histamine strength and time, as within-subject variables. Table 2 presents these analyses. For the total, untransformed data ($n=576$) there was a strongly significant difference between the first and second Latin squares ($P=0.017$). The difference between all drug means did not quite reach significance ($0.1 > P > 0.05$), but the contrast between the two terfenadine treatments and the two without terfenadine was close to significance ($P=0.053$); this difference was not attributable purely to that between the Latin squares, since the interaction between terfenadine *vs* non-terfenadine contrast and the Latin square difference was not significant ($P=0.4$). The power of this ANOVA against a false null hypothesis was small ($1-B=0.43$). The analysis was then repeated with the data transformed by subtracting the initial value from each individual's results. This analysis is also shown in Tables 2 and 3. The effects of terfenadine alone, or in combination with cimetidine are now very significant, as is an apparent effect of cimetidine on the terfenadine response both in the same week, and after a week from terfenadine dosing. The difference between the two Latin squares disappears when initial values are subtracted, showing that it was largely related to the general level of drug response. However, the contrast between the squares for the two treatments where terfenadine preceded the present week, i.e. the placebo and cimetidine responses, is reinforced by the subtraction of initial values.

The ratios between these various treatment blocks is given in Table 3, which summarizes the evidence for a washover effect from terfenadine and its apparent

weakening by cimetidine. The analysis of the differenced data between minutes 2 and 4 (Table 2), showed that whereas the difference between Latin squares was no longer significant in this part of the data ($P=0.124$), the difference between the four drug treatments was significant ($P=0.048$); again this was not attributable purely to any interaction with the Latin square difference ($P=0.61$). The difference between the two terfenadine treatments and those without terfenadine was strongly significant ($P=0.023$), and once more this could not be attributed solely to interaction with the Latin square difference ($P=0.43$).

The apparent difference (Figure 2) in the differenced data between terfenadine alone and terfenadine plus cimetidine did not reach significance in the ANOVA ($P=0.062$), but a *t*-test for the difference of means at the peak (3 min) using a pooled standard error estimate was significant ($t_{31}=3.514$, $P<0.01$).

A last question of interest was the apparent lack of drug effects during the slow, last 5 min period of wheal growth. This was tested by regression analysis, BMDP 6D. The results are given in Table 4. Whereas several means differed significantly, there were no significant differences between slopes, some of which included zero within their 95% confidence intervals.

Discussion

In earlier work it was shown the histamine wheal growth begins after a delay of 1–2 min, and proceeds for up to 9–10 min from injection, growth occurring by piecemeal accretion of small territories, though at a general rate which accords with assisted diffusion in the skin from an initial area corresponding to that of the subepidermal injection bleb, i.e. about 41 mm² [1, 4]. In those analyses, data were fitted best by linear

Table 3 Treatment block means for the two Latin Squares; reversed square (total data), A, with initial values subtracted, C; Williams square (total data), B, with initial values subtracted, D

Treatment		A	C	B	D	A - B	A/B	C - D	95% CI for C - D means
		(n = 36)	(n = 32)	(n = 36)	(n = 32)				
Histamine 0.3 mM	Placebo	34.39	25.19	32.5	28.13	1.89	1.06	-2.94	±2.26
	Cimetidine	35.76	30.38	29.88	24.05	6.89	1.18	6.33	±4.00
	Terfenadine	29.19	17.09	22.49	13.20	6.71	1.30	3.89	±7.21
	Cimetidine + terfenadine	29.61	19.53	22.72	16.0	5.38	1.30	3.53	±2.08
Histamine 0.074 mM	Placebo	30.97	21.34	24.35	20.08	6.63	1.27	1.27	±1.49
	Cimetidine	23.61	17.0	17.92	11.16	5.69	1.32	5.84	±2.53
	Terfenadine	28.00	14.25	17.40	8.75	10.60	1.61	5.50	±4.99
	Cimetidine + terfenadine	26.39	13.94	16.54	10.03	9.85	1.59	3.91	±2.72

Table 4 Regression analysis; last 5 min of wheal growth, untransformed data

Drug	Histamine concentration (mM)	Slope	95% CI for slope	Mean	95% of CI for mean
Placebo	0.3	7.224	±8.267	177.1	±12.906
	0.074	7.752	±5.713	141.7	±8.919
Cimetidine	0.3	4.628	±12.097	171.5	±18.884
	0.074	4.524	±8.238	105.8	±12.86
Terfenadine	0.3	7.424	±7.114	126.8	±11.106
	0.074	6.176	±9.720	106.9	±15.174
Cimetidine + terfenadine	0.3	7.924	±7.803	254.7	±12.181
	0.074	2.852	±6.370	105.2	±9.943

regression slopes. That approach now seems to have been too simple; though the onset delay and growth times are confirmed by the present work, drug antagonism of the histamine response occurred chiefly during the early rapid phase of wheal growth (2–4 min); wheal growth was slower during the third phase (5–9 min) and drug effects were then too small to measure.

A sensitive way to test for crossover effects confirmed that these occurred with terfenadine even though the least interval between tests was 7 days.

The mechanism of neither effect is known. Terfenadine would be expected to be well absorbed, to be effective in a single dose of 120 mg, to have no significant affinity for histamine H₂, α or β adrenergic receptors, and to have its maximal effect against histamine whealing after 4 h, retaining activity for at least 24 h [10]. It is oxidized rapidly to at least one active metabolite which is moderately protein bound (70%). These findings are little affected by food or age. The dose-response relationships of terfenadine for histamine wheals were described by Shall *et al.* [11].

There have been studies of combinations of H₂-receptor antagonists combined with terfenadine in chronic urticaria [10] and in histamine release by anaesthetic agents [12] which showed reductions in whealing which varied from small to nil. The most directly relevant work is that of Sharpe & Shuster [13] who studied dermatographic urticaria, showing that the addition of an H₂-receptor antagonist to terfenadine increased wheal threshold and produced a small signifi-

cant reduction in whealing response, without symptomatic benefit. They concluded that involvement of the H₂-receptor in this form of urticaria is minimal. Honig *et al.* [14] found no evidence that co-administration of cimetidine altered the pharmacokinetics of either terfenadine or its active metabolite. There has been no evidence hitherto that cimetidine may, under certain conditions, inhibit some of the effects of terfenadine on wheal formation.

The mechanism of the later wheal growth which seems unaffected by drugs is also unclear. Bull *et al.* [15] concluded that prostaglandin E₂ release is facilitated by histamine H₁-receptors; they found that this release was inhibited only slightly by cimetidine. The release of prostaglandin E₂ was potentiated by interleukin 1, but not by substance P. Prostaglandin E₂ would seem to be a potential candidate for the substance which drives the slow late phase of wheal growth; the present work presents no evidence on this, but it must be remembered that in the latter phases, wheal growth is very clearly the resultant of extension and resorption; the present results show this clearly since wheal growth checks and may even reverse temporarily around the 5–7 min times from injection (Figure 2).

The mechanism of the crossover effect of terfenadine after 1 week is also unclear; it is possible that the drug still has some effect even at that interval from a single dose. But it could be that terfenadine influences the restoration of tissue stores discharged by histamine. Sciberras *et al.* [16] showed that two consecutive daily

injections of compound 48/80 depleted skin sites of histamine such that the response to PAF, which discharged histamine from the dermis, was restored only very slowly being complete after a 2 week interval. It could be that histamine injection also discharges tissue histamine, but that the restoration of tissue mechanisms is impeded by terfenadine. The present work throws no light on the question; further work is needed to explore the mechanism of this effect.

The author gratefully acknowledges substantial contributions to the planning and execution of the first phase of this work by Drs D. W. Littlejohns and J. A. Lopez-Gil, and to the tabulation and manuscript preparation by Miss H. Titre.

References

- 1 Carey C, Clark M, Lopez-Gil JA, Vere DW. The basis of the histamine assay in human skin: II wheal formation. *Br J Clin Pharmacol* 1993; **36**: 39–43.
- 2 Altura BM. Reticuloendothelial system function and histamine release in shock and trauma: relationship to microcirculation. *Klin Wochenschr* 1982; **60**: 882–890.
- 3 Garrison JC. Histamine, bradykinin, 5-hydroxytryptamine and their antagonists. In *The Pharmacological Basis of Therapeutics*, Eighth Edition, eds Gilman AG, Rall TW, Nies AS, Taylor P, New York; Pergamon Press 1990; p 580.
- 4 Byrom H, Clark M, White V, Vere DW. The basis of the histamine assay in skin. *Br J Clin Pharmacol* 1991; **32**: 367–370.
- 5 Cox DR. *Planning of Experiments*. Wiley, London, 1958; pp 272–274.
- 6 Williams EJ. Experimental designs balanced for the estimation of residual effects of treatments. *Aust J Sci Res* 1949; A; **2**: 149–154.
- 7 *BMDP Statistical Software*. Ed Dixon WJ. University of California Press, Los Angeles and Oxford 1990.
- 8 Dixon WJ, Massey FJ Jr. *Introduction to statistical analysis*. Fourth Edition, pp 189–195.
- 9 Anderson OD. *Time Series Analysis and Forecasting*. Butterworth, London, 1976.
- 10 McTavish D, Goa KL, Ferrill M. Terfenadine. An up-dated review of its pharmacological properties and therapeutic efficacy. *Drugs* 1990; **39**: 552–574.
- 11 Shall L, Newcombe RG, Marks R. Assessment of the duration of action of terfenadine on histamine induced wheals. *Br J Dermatol* 1989; **119**: 525–531.
- 12 Treuren BC, Galletly DC, Robinson BJ, Short TG, Ure GW. The influence of the H₁ and H₂ receptor antagonists terfenadine and ranitidine on the hypotensive and gastric pH effects of the histamine releasing drugs morphine and tubocurarine. *Anaesthesia* 1993; **48**: 758–762.
- 13 Sharpe GR, Shuster S. In dermographic urticaria H₂ receptor antagonists have a small but therapeutically irrelevant additional effect compared with H₁ antagonists alone. *Br J Dermatol* 1993; **129**: 575–579.
- 14 Honig PK, Wortham DC, Zemani K, Conner DP, Mullin JC, Cantilena LR. Effect of concomitant administration of cimetidine and ranitidine on the pharmacokinetics and electrocardiographic effects of terfenadine. *Eur J Clin Pharmacol* 1993; **45**: 41–46.
- 15 Bull HA, Cohen J, Dowd PM. Responses of human dermal microvascular endothelial cells to histamine and their modulation by interleukin 1 and substance P. *J Invest Dermatol* 1991; **97**: 787–792.
- 16 Sciberras DG, Jordan S, Gill D, Baber NS, James I. The role of histamine in the acute inflammatory responses to intradermal platelet activating factor. *Br J Clin Pharmacol* 1991; **32**: 85–90.

(Received 12 July 1994,
accepted 10 August 1995)