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Dose-response Relationship Between Objective Measures of Histamine-induced Weals and Dose of Terfenadine*

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Terfenadine is a safe non-sedative H_1-receptor antagonist. This study aimed to quantify the relative reduction in weal and flare area, thickness and erythema at 4, 8, 12 and 24 h following a single but variable oral dose of terfenadine compared with pre-treatment measurements, in order to compare the dose-effect relationship and time course of the different dosages. In a double-blind randomized cross-over study, 12 healthy volunteers were given 60, 120 or 240 mg of terfenadine or placebo. Twenty micrograms of histamine acid phosphate was then injected intradermally at 4, 8, 12 and 24 h. The weal and flare areas were measured by planimetry, the thickness of the weal by an A-scan pulsed ultrasound device and the redness of both the weal and flare by an erythema-meter. A definite dose-response relationship was demonstrated between the weal and flare areas and the three active treatments. For the weal area, there was a significant difference between 60 mg and 240 mg of terfenadine at 4 h ($p < 0.01$), at 8 and 12 h ($p < 0.05$). For the flare area there was a similar significant difference at 4 h ($p < 0.01$) and at 8 h ($p < 0.05$). A dose-response relationship was demonstrated between weal erythema and 120 mg or 240 mg and 60 mg of terfenadine ($p < 0.05$). There was a correlation between the plasma levels of the major metabolite and the initial dose of terfenadine, demonstrating their expected proportionality. This metabolite was also demonstrated in tissue fluid.

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Terfenadine, a safe non-sedating H_1-receptor antagonist used in the symptomatic treatment of immediate hypersensitivity reactions such as allergic rhinitis and urticaria, is usually prescribed at the recommended dosage of 60 mg twice daily (British National Formulary). This dosage schedule is based on the work of Huther et al. (1). We have demonstrated suppression of the wealing response induced by intradermal histamine following a single oral dose of 120 mg of terfenadine at 12, 18 and 24 h post dosage and suggested that a single daily dose of 120 mg may be effective in clinical practice (2).

The timing of detectable (within 30 min) and peak plasma levels (within 2 h) of terfenadine(3) does not correlate with the observed onset of the clinical action of terfenadine or the time at which the drug exerts its maximum effect. Using the well known model of histamine induced skin weals, inhibition of wealing has an onset at 1 to 2 h after an oral dose of 60 mg and reaches its peak at 3 to 4 h. The reason for this discrepancy is unknown.

The main aim of the study described here was to determine whether a dose-response relationship exists between the dose of terfenadine and objective measures of histamine induced skin weals. A subsidiary aim has been to characterize the tissue and plasma levels of 4-[1-hydroxy-4-(4-hydroxydiphenylmethyl-1-piperidinyl)butyl]-α-α–dimethylbenzene acetic acid (MDL 16455), the major metabolite of terfenadine, in order to understand the difference in timing between peak plasma levels and onset of maximum effect of the drug.

MATERIALS AND METHODS

Twelve healthy, non-atopic volunteers (8 males and 4 females; ages ranging from 20 to 40 years, mean age 26.8) were recruited. None of the subjects was on any regular medication and no alcohol was permitted for the 24 h prior to commencement. The volunteers gave their signed witnessed informed consent for the study which was approved by the Joint Ethics Committee of South glamorgan Area Health Authority and carried out in accordance with the provisions of the Declaration of Helsinki as amended in Venice (1983).

The study was conducted as a double-blind randomized cross-over trial. Each subject received placebo and three different doses of terfenadine, 60, 120 and 240 mg, accord-

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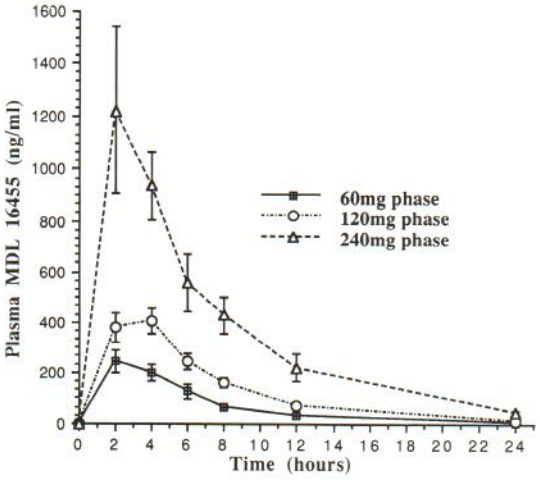

Fig. 1. Mean and standard error plasma concentration profiles of the major terfenadine metabolite, MDL 16455, for the terfenadine 60 mg, 120 mg and 240 mg phases over 24 h.

ing to a predetermined randomization schedule. Each of the four segments was separated by a mean wash out period of 5 days (range 2 to 7 days).

The terfenadine tablets were manufactured to the commercial formulation (Triludan) and differed from the marketed product only in the embossed marking on the tablet surface. The placebo tablets were identical in formulation to the active, with the exception that terfenadine was replaced by lactose.

Each segment of the study began at 8.00 a.m. Blood was taken from all volunteers prior to the ingestion of the tablets and at 2, 4, 6, 8, 12, and 24 h following the treatment. In addition, in 1 subject interstitial tissue fluid from the skin was obtained by raising a suction blister on the flexor surface of this forearm using a vacuum pump. After between 90 and 120 min of exerting a pressure of 150–200 mm of mercury, a subepidermal blister formed (4). The fluid was drained using a fine-gauge needle and syringe. A suction blister was raised before the tablets were taken and at 4, 8, 12, and 24 h following the treatment. Both the plasma and the blister fluid was used to measure the terfenadine metabolite MDL 16455, using the method previously described (2). Briefly, the samples were added to an internal standard which was then extracted along with MDL 16455. The residue was reconstituted in mobile phase for chromatography on an HPLC column.

Before any medication was taken, 20 μg of histamine acid phosphate diluted in 0.1 ml of normal saline was injected intradermally using a 0.5-ml insulin syringe (Monoject) into the flexor aspect of one forearm, 5 cm from the antecubital fossae. The histamine was administered by the same investigator on each occasion. Saline control injections were not performed because the measured criteria; area, thickness and redness were expressed as ratios of 1 dose of terfenadine or placebo against another.

Fifteen minutes after the intradermal histamine injection, the perimeters of the weal and flare were traced onto transparent acetate sheets. At the same time the redness of the weal and flare was measured using an erythema measuring device (erythema-meter, Cutech Ltd). Immediately there after the thickness of each weal was measured using an A-scan pulsed ultrasound device [Cutech Ltd]. The areas were subsequently calculated using a digitizing planimeter linked to a microcomputer. The whole process of intracutaneous injection of histamine and measurement was repeated at 4, 8, 12, and 24 h after the study medications had been taken. The injections were alternated between forearms, an adjacent site being chosen each time.

The erythema measuring device (Cutech erythema-meter) emits light of two colours; green and red. The green is absorbed by haemoglobin and the red is not. The intensity of the reflected light can be measured and the ratio between the intensity of reflection of the two colours is a measure of the redness of the tissue being studied (5). Readings were taken from normal skin adjacent to the flare, from the flare at four adjoining sites which were 1.5 cm distal to the injection point and outside the perimeter of palpable wealing, and from the weal.

The A-scan pulsed ultrasound device [Cutech Ltd], employs a polyvinyledene fluoride transducer of 17 MHz centre frequencies, as previously described (6). In short, the device sends a pulse of ultrasound into the body which is partially reflected at tissue interfaces. The reflected echos are detected by the transducer and displayed on a time base on an oscilloscope, from which the thickness of the skin can be measured. At each of the response times ultrasound measurements were made on the weal at three adjoining sites and on normal skin.

Statistics

Log transformation was used for area and redness measurements, to correct for skewness. These variables were summarized by the geometric mean and an interval fitted to predict where 95% of data values would lie according to the log normal model. Thickness measurements were not transformed and are summarized by mean and standard deviation. The area, thickness and redness measurements at each of the time points 4, 8, 12 and 24 h were analysed by analysis of covariance, using subject, period and dose as factors and time zero response as covariate. Selected dose contrasts were then extracted from the three degress of freedom between-doses comparison. The serum MDL 16455 concentrations were compared using *t*-tests.

RESULTS

Plasma and tissue levels of the principal terfenadine metabolite MDL 16455

MDL 16455 was not detected in 11 of the 12 subjects over the 24 h test period following the placebo tablets. Only the data obtained from these 11 were used in the analysis. In the 1 subject that MDL 16455 was detected, the levels observed in the plasma samples indicated that a dose of terfenadine, probably 60 mg, was taken in error during the placebo phase. Results from the 120 mg and 240 mg phases of this subject

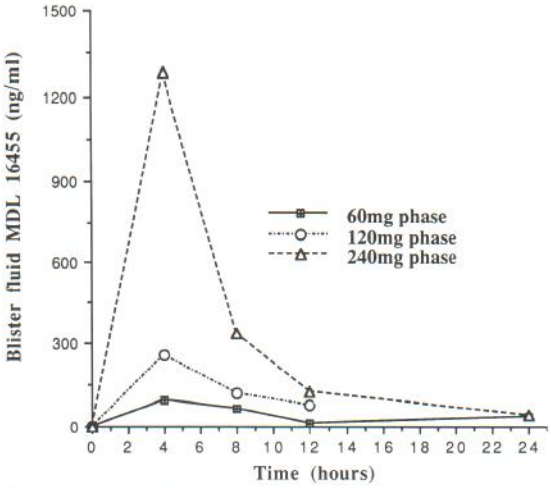

Fig. 2. Comparison of the major terfenadine metabolite, MDL 16455, in interstitial tissue fluid from suction blisters raised on the flexor surface of the forearm of volunteer no. 11 for the terfenadine 60 mg, 120 mg and 240 mg phases over 24 h.

were consistent with those of the other 11 volunteers.

Fig. 1 shows the mean plasma MDL 16455 profiles for the 60, 120 and 240 mg phases. From these the normalized areas under the plasma concentration curves (AUC) and the normalized maximum values (C_{max}) were calculated. A statistically significant result was obtained from the normalized C_{max} data ($p < 0.05$ t-test) demonstrating the expected proportionality between dose of terfenadine and the plasma concentration of MDL 16455. In comparison, the blister fluid of MDL 16455 profiles from the 1 subject in whom suction blisters were raised is shown in Fig. 2. His corresponding plasma levels were similar to the mean values obtained for the whole group.

Area of weal and flare

Descriptive statistics for weal and weal and flare areas, for each treatment, time and dose of the 11 subjects used in the analysis are summarized in Table I. All three doses of terfenadine were significantly more effective in suppressing histamine induced weal and flare reactions than placebo, at times up to 12 h.

To test whether a dose-response relationship existed between the varying doses of terfenadine and weal and flare areas, the differences in weal and

Table I. *Descriptive statistics for weal area, and weal & flare area, measured by computer-aided planimetry, 15 min after a 20 μg intradermal histamine challenge, following a single oral dose of placebo, 60 mg, 120 mg and 240 mg of terfenadine, at 0, 4, 8, 12 and 24 h (n = 11)*

Dose (mg)	Time (hrs)	Weal area (mm^2)		Weal and flare area (mm^2)	
		Geometric mean	Fitted 95% range	Geometric mean	Fitted 95% range
0	0	363	161 to 817	2550	1040 to 6280
	4	250	136 to 458	2320	1110 to 4880
	8	186	68 to 514	1740	740 to 4060
	12	240	101 to 571	2330	1060 to 5130
	24	197	119 to 325	1770	850 to 3710
60	0	319	196 to 518	2350	1250 to 4430
	4	139	90 to 215	1060	370 to 3013
	8	135	81 to 223	950	270 to 3340
	12	195	79 to 484	1290	420 to 3910
	24	210	144 to 304	1310	490 to 3500
120	0	282	133 to 601	2020	880 to 4640
	4	91	29 to 288	880	270 to 2900
	8	107	47 to 246	840	360 to 1970
	12	136	59 to 317	930	300 to 2920
	24	202	115 to 353	1260	560 to 2850
240	0	277	163 to 472	2500	1150 to 5450
	4	85	50 to 145	680	240 to 1910
	8	80	37 to 173	710	330 to 1550
	12	134	83 to 215	920	320 to 2610
	24	173	81 to 368	1130	380 to 3360

Table II. *Dose-response relationship*

Estimated percentage change in weal & flare area and 95% confidence intervals, measured by computer-aided planimetry 15 min after a 20 μg intradermal histamine challenge, following a single oral dose of placebo, 60 mg, 120 mg and 240 mg of terfenadine, at 0, 4, 8, 12 and 24 h by using 240 mg instead of 60 mg terfenadine. NS = not significant ($n = 11$)

	Time (hrs)	Ratio between area using 240 mg and 60 mg (%)	95% confidence interval	*t*-value	
Weal	4	64.6	47.3– 88.3	−2.87	$p < 0.01$
	8	59.8	39.6– 90.4	−2.56	$p < 0.05$
	12	66.1	44.8– 97.7	−2.18	$p < 0.05$
	24	82.1	66.2–101.9	−1.88	ns
Weal & flare	4	62.6	54.4– 86.3	−3.00	$p < 0.01$
	8	71.4	51.3– 99.4	−2.09	$p < 0.05$
	12	69.1	45.5–104.9	−1.82	ns
	24	84.3	59.7–118.9	−1.02	ns

flare areas of the higest and lowest doses were used, at each time point. Table II lists the quantitative assessment of the differences in response to 60 mg and 240 mg. A dose-response relationship is demonstrated for the weal area through to 12 h. For the weal and flare area the dose response relationship is clear at 4 and 8 h but not at 12 h.

The differences in weal and flare areas following 120 mg and 240 mg of terfenadine were used to determine when weal and flare area suppression peaked. These analyses show that the geometric mean area is smaller using the larger dose, however significance is not reached.

Weal thickness

The mean weal thickness for each of the four doses at the specified time points are listed in Table III. For the four-dose comparison there were statistically significant differences in weal thickness at 4 h ($p < 0.001$) and 24 h ($p < 0.05$) demonstrating that terfenadine reduced histamine induced weal thickness. However, these differences did not reach significance at 8 and 12 h. Analysis of covariance to assess the differences in response to 60 and 240 mg of terfenadine revealed no clear evidence of a dose-response relationship as far as weal thickness concerned.

Weal redness

Table IV lists the mean erythema-meter readings at each site. For the four dose comparisons, there were statistically significant differences in weal redness at 4 h ($p < 0.001$) and at 24 h ($p < 0.05$) but not at 8 or 12 h. However, both these significant results were in the opposite, positive direction, indicating that the lowest or least red values were observed in volunteers treated with placebo and the highest values seen in those subjects on 240 mg terfenadine. Comparing the various doses of terfenadine and placebo, using analysis of covariance, weal redness was significantly greater on either 120 or 240 than on 60 mg of terfenadine. There were no consistent differences in redness of the flare.

DISCUSSION

In this study the effects of varying doses of terfenadine on cutaneous weal and flare reactions were evaluated to determine whether a dose response existed between dose of terfenadine and objective measures of histamine induced skin reactions. A

Table III. *Skin thickness of weal*

Mean of three adjoining measurements and standard deviation of weal thickness, using an A-scan pulsed ultrasound device, 15 min after an intradermal histamine challenge of 20 μg, 0, 4, 8, 12 and 24 h following a single oral dose of placebo, 60 mg, 120 mg and 240 mg of terfenadine ($n = 11$)

Time (hrs)	Placebo	Terfenadine 60 mg (mm)	Terfenadine 120 mg (mm)	Terfenadine 240 mg (mm)
0	3.72 (0.62)	3.67 (0.68)	3.58 (0.54)	3.55 (0.46)
4	3.38 (0.54)	2.61 (0.40)	2.50 (0.42)	2.37 (0.37)
8	2.85 (0.55)	2.62 (0.49)	2.51 (0.33)	2.45 (0.44)
12	2.84 (0.50)	2.81 (0.49)	2.57 (0.37)	2.79 (0.43)
24	2.77 (0.37)	3.12 (0.50)	3.14 (0.49)	3.13 (0.63)

Table IV. *Redness of weal & flare*

Descriptive statistics for redness measurements, by erythema-meter, 15 min after a 20 μg intradermal histamine challenge, following a single oral dose of placebo, 60 mg, 120 mg and 240 mg of terfenadine, at 0, 4, 8, 12 and 24 h ($n = 11$)

Dose (mg)	Time (hrs)	Weal redness		Flare redness	
		Geometric mean	Fitted 95% range	Geometric mean	Fitted 95% range
0	0	289	203 to 409	337	257 to 442
	4	293	206 to 416	343	251 to 470
	8	313	220 to 443	346	259 to 461
	12	299	207 to 430	344	240 to 494
	24	293	202 to 427	344	261 to 452
60	0	298	205 to 432	336	237 to 475
	4	313	208 to 469	334	220 to 508
	8	325	235 to 450	344	240 to 492
	12	316	220 to 452	331	232 to 471
	24	300	212 to 426	341	238 to 488
120	0	278	175 to 443	341	245 to 476
	4	333	222 to 500	348	249 to 485
	8	327	228 to 468	335	236 to 475
	12	311	211 to 458	338	248 to 460
	24	323	243 to 430	347	263 to 457
240	0	301	225 to 404	336	251 to 450
	4	340	242 to 478	340	256 to 452
	8	315	221 to 449	337	257 to 441
	12	319	228 to 448	344	267 to 443
	24	315	209 to 474	350	258 to 473

relationship between weal area and the dose of terfenadine was demonstrated for up to 12 h, as well as a relationship between weal redness and dose of terfenadine at 4 h. Terfenadine significantly suppressed weal thickness but no dose relationship was demonstrated.

The presence of a relationship between the dose of terfenadine and the plasma concentrations of MDL 16455 was demonstrated by the normalized C_{max} data but not the normalized AUC data. This discrepancy was due to a greater than normal deviation of the AUC values around the mean, caused primarily by the absence of some samples around the time of maximum concentration, by the use of only a small number of sample time points to approximate the plasma profile, and by performing only a single analysis on each sample. Using a vacuum pump, large enough suction blisters were raised to obtain enough tissue fluid with which to measure MDL 16455 levels. The blister fluid levels of MDL 16455 correlated with the dose of terfenadine ingested. This technique takes approximately 90 min for a blister to develop and only 4 samples could be be obtained for each dose and none earlier than 4 h. From the data we have, MDL 16455 blister fluid levels were maximum at 4 h and we still do not know whether, like plasma levels they peak at 2 h. For this reason we are still unable to explain the time difference between maximum plasma levels of MDL 16455 and maximum clinical effect exerted by terfenadine.

Over the 24 h period some variation in weal and weal & flare areas were observed during the placebo phase. This is consistent with the concept of a circadian rhythm in the skin reaction to intradermal histamine and has previously been described (7). A wide inter-subject variation in the response to intradermal histamine exists as shown by the fitted 95% range but the intra-subject reactions exhibit excellent reproducibility. This inter-subject variation necessitated the statistical analysis chosen as it takes into account this variation.

The ultrasound was useful in demonstrating the difference in weal thickness between terfenadine and placebo at 4 and 24 h, following the single oral dose. This corresponds to previously reported results (6). As a result of the circadian rhythm peak cutaneous reactions to intradermal histamine occur

at about 11 p.m. and trough reactions around 11 a.m. to 3 p.m. This may explain the fact that following a single oral dose of terfenadine the reduction in weal thickness reached significance at 4 and 24 h but not at 8 and 12 h. This explanation is pertinent to the erythema-meter readings from the weal.

The erythema-meter produced what would appear to be paradoxical results. Comparing terfenadine and placebo, the weal was palest on placebo and most red on the highest dose of terfenadine. This may be because histamine constricts endothelial cells of post-capillary venules resulting in the exudation of fluid and weal formation. Pressure from this extracellular oedema restricts blood flow in the post-capillary venules, in the area of the weal, and would be recorded as being pale by the erythema-meter. Presumably terfenadine binds to the H_1-receptors of the post-capillary venules (8) decreasing the amount of oedema produced and causing less restriction of blood flow which would be recorded as redder on the erythema-meter. Interestingly, similar results have been produced in an independent study using the laser doppler flowmeter (Perimed) to measure superficial cutaneous blood flow instead of the erythema-meter (9). No significant change in flare redness was demonstrated.

In conclusion, using objective measures of weal and flare reactions and plasma concentrations of MDL 16455, we have demonstrated that a dose response relationship exists between area of weal and weal & flare, weal redness and plasma levels of MDL 16455 and terfenadine (at doses 60–240 mg) but not between terfenadine and weal thickness or flare erythema.

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