

Comparison of On-Site and Photographic Evaluations of the Suppressive Effects of Cetirizine, Loratadine, and Fexofenadine on Skin Response to Histamine Iontophoresis: A Double-Blind, Crossover Study in Healthy Volunteers

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ABSTRACT

Background: The standard method used to determine the potency of antihistamines is to assess the degree of suppression of skin response to histamine challenge.

Objectives: The aims of this study were to compare the efficacy of 3 antihistamines using a histamine challenge test and the usefulness of on-site evaluation with that of photographic evaluation of skin-test reactions.

Methods: In this prospective, double-blind, crossover study, healthy volunteers were given cetirizine 5 mg (CTZ-5) and 10 mg (CTZ-10), loratadine 10 mg (LOR), fexofenadine 60 mg BID (FEX), and placebo (PLC), in a randomly assigned order, with an interval of at least 1 week between treatments. Before and 0.5 to 24 hours after administration, the areas of flare and wheal induced by histamine iontophoresis were measured directly (on site) by 1 evaluator and by another evaluator using photographic images on a computer monitor.

Results: Ten healthy volunteers (6 men, 4 women; mean age, 28.2 years [range, 20–39 years]; mean weight, 60.7 kg [range, 41–81 kg]) were enrolled. The data from 9 subjects were analyzed; the data from 1 subject were omitted because the subject used an over-the-counter cold medication containing diphenhydramine several times during the study. By both methods, all antihistamines were shown to suppress flare significantly from 4 to 24 hours after administration. CTZ was most potent in suppressing both flare and wheal. For flare, the areas as measured using on-site evaluation were larger overall than those measured using photographic evaluation, but the shapes of the time-course graphs were similar for both. Overall, the flare area measurements started to decrease significantly from baseline values 4 hours after drug administration, reached a nadir at 10.5 hours, and remained significantly lower com-

pared with baseline values at 24 hours. Comparisons between antihistamines showed significant differences in mean flare areas between the 2 doses of CTZ and LOR from 8 to 12 hours after administration in both evaluation methods. The wheal areas were significantly reduced from baseline values by most of the antihistamines 4 to 12 hours after drug administration, reached their lowest values at 10.5 hours, and returned to near-baseline values at 24 hours. Comparisons with PLC values at each time point, however, showed significant differences only for CTZ-5 and CTZ-10 from 4 to 12 hours after administration. Comparison between antihistamines showed significant differences in mean flare areas between the 2 doses of CTZ and LOR from 8 to 12 hours after administration in both evaluation methods. Although the flare areas measured by both methods correlated linearly ($r = 0.90$; $P < 0.001$), the correlation for wheal areas was weaker ($r = 0.76$; $P < 0.001$).

Conclusions: In this study in healthy volunteers, single doses of CTZ 5 mg and CTZ 10 mg were more potent compared with single-dose LOR 10 mg and FEX 60 mg BID in suppressing skin response. Although linear correlations were found between skin-response areas, as measured by on-site and photographic evaluation, it was difficult to differentiate between wheal and flare by photographic evaluation, especially when a typical wheal was suppressed to slightly edematous erythema by antihistamines. (*Curr Ther Res Clin Exp.* 2005;66:307–322) Copyright © 2005 Excerpta Medica, Inc.

Key words: antihistamine, histamine iontophoresis, histamine challenge test, ImageJ, fexofenadine, loratadine, cetirizine, wheal, flare.

INTRODUCTION

Antihistamines are routinely used for the treatment of skin disorders accompanied by pruritus, most commonly urticaria. Most second-generation (non-sedating) antihistamines are associated with less sedation and greater efficacy compared with first-generation antihistamines (eg, hydroxyzine, diphenhydramine).^{1–3} However, the latter might be more potent and faster acting for dermographism compared with non-sedating antihistamines.⁴

The effect of antihistamines is typically determined by assessing the degree of suppression of skin reaction induced by histamine challenge tests (eg, prick test, intracutaneous injection test).^{2,3,5–8} In many studies of antihistamines, the degree of skin reaction suppression has been assessed using direct measurements of the area of wheal and flare (on-site evaluation) over time after single-dose study drug administration.^{2,3,5–8}

The primary aim of the present study was to compare the suppressive effects of 3 widely used second-generation antihistamines—cetirizine, loratadine, and fexofenadine—at standard daily dosages (including number of doses per day and timing of administration [postprandial]). We also compared the usefulness of on-site evaluation with that of photographic evaluation of skin-test reactions.

SUBJECTS AND METHODS

This prospective, double-blind, crossover study was conducted at the Department of Dermatology, School of Medicine, University of Tokushima, Tokushima, Japan. Healthy volunteers of both sexes and aged ≥ 18 years were eligible for the study. Patients who had any acute or chronic diseases; were receiving any medications; or who had a history of urticaria, including dermographism, were ineligible. Pregnant or breast-feeding patients also were excluded. Institutional review board approval was waived because, in the informal collective opinion of the university board members, the study was not thought to be dangerous, and all individuals were required to provide written informed consent to participate. All subjects were compensated for their involvement in the study.

Study Drug Administration

During the 3-month study period, subjects received, in a randomly assigned order, cetirizine hydrochloride 5 mg (CTZ-5), cetirizine hydrochloride 10 mg (CTZ-10), loratadine 10 mg (LOR), fexofenadine hydrochloride 120 mg (FEX), and pantetheine 200 mg (placebo [PLC]). Randomization of the order of administration was performed using a computer-generated list of random numbers. Cetirizine and loratadine were given as a single dose in the morning (9:30 AM), with a matching placebo (pantetheine 100 mg) given in the evening (7:30 PM). Fexofenadine was divided into 2 doses of 60 mg, given once in the morning and once in the evening. Pantetheine was divided into 2 doses of 100 mg, given once in the morning and once in the evening. All study drugs were given by mouth with an unspecified amount of water 30 minutes after meals. An interval of at least 1 week separated the administration of each study drug. To ensure blinding, all study drugs were given an identical appearance by a third party, who wrapped each dose in a thin, soluble wafer.

Two doses of CTZ were studied to compare dose responses. Pantetheine was used as the placebo because it was of similar size compared with the 3 active antihistamines and, according to the manufacturer (personal communication, Daiichi Pharmaceutical Company, Ltd., Tokyo, Japan), has not been associated with histamine-induced skin reactions.

Exercise and alcohol consumption were prohibited during the tests. The subjects were instructed not to use any medications, including over-the-counter drugs containing antihistamine or anti-inflammatory agent or any systemic or topical steroid, for at least 1 week before each test.

Introduction of Histamine

Before and 0.5, 1, 2, 4, 8, 10.5, 12, and 24 hours after the administration of the morning dose of each drug, histamine was applied to 1 of 9 sites (chosen by lottery) on the skin of the flexor forearm. Following the method described by Furue et al,⁷ histamine 10 mg/mL, dissolved in distilled water, was dripped onto cotton packed into the applicator of an iontophoreser (UI-2060, BS Medical,

Tokyo, Japan) with a round skin-contact surface area of 19.6 mm², and a 0.1-mA electric current was applied for 1 minute.

On-Site and Photographic Evaluations

One dermatologist (H. Tsuda) (investigator A), blinded to the study drugs, was responsible for the on-site evaluations before (0 hour) and 0.5, 1, 2, 4, 8, 10.5, 12, and 24 hours after dosing. For each on-site evaluation, the degree of the skin reaction was determined 15 minutes after the completion of iontophoresis, using direct measurement of flare and wheal, in which outlines of the central wheal and surrounding flare were traced with a fine-point marker onto a transparency sheet. The digital image of this transparent sheet and the 10-cm scale was then obtained using an image scanner (CanoScan D2400U, Canon Inc., Tokyo, Japan).

For each photographic evaluation, a color transparency of the entire area of the skin reaction, together with a scale, was obtained from a constant distance of 35 cm using a single-lens reflex camera equipped with a ring-shaped flash bulb (OM-2 and T10 Rich Flash 1, Olympus Corporation, Tokyo, Japan). These slides were digitized in high resolution using a slide scanner (DS Elite II, Konica Minolta Holdings, Inc., Tokyo, Japan) and saved, in random order, to a computer. Another blinded dermatologist (H. Takiwaki) (investigator B) was responsible for the quantification of the outlined area on the transparent sheet and the photographic evaluations after all clinical trials were completed. The areas of flare (everything within the outer edge of the erythema) and wheal were measured using ImageJ⁹ software for image analysis (National Institutes of Health, Washington, DC) by tracing the images with a computer mouse on a 17-inch monitor with 1280 × 1024-pixel resolution (FMV Deskpower C5/80LR, Fujitsu Ltd., Tokyo, Japan).

Tolerability

Adverse effects (AEs) were monitored using subject interview and physical examination by investigator A.

Statistical Analysis

The results were analyzed using SAS version 8.2 (SAS Institute Inc., Cary, North Carolina). The data were analyzed using analysis of variance. The Dunnett test was used to compare the mean area of skin reaction of each drug with baseline and placebo values, and the Tukey test was used to compare the between-group differences in the efficacy of the drugs. A *P* value <0.05 was considered statistically significant. The Pearson correlation coefficient was used to determine correlations between the data as measured by the 2 evaluators.

The power analyses using the Dunnett test indicated that 10 subjects would be necessary to provide 80% power if the mean (SD) area of skin response was assumed to be 100 (20) mm² for the 4 treatment groups and 150 (20) mm² for the PLC group and the baseline values. Using the Tukey test, 8 subjects would be needed for 80% power if the mean (SD) area of skin response was assumed to be 100 (15) mm² for the 4 treatment groups and 150 (15) mm² for the PLC group.

RESULTS

Study Population

Ten healthy volunteers (6 men, 4 women; mean age, 28.2 years [range, 20–39 years]; mean weight, 60.7 kg [range, 41–81 kg]) participated in the study. The data from 9 subjects were analyzed; the data from 1 subject were omitted because the subject used an over-the-counter cold medication containing diphenhydramine several times during the study.

Suppression of Skin Reaction

Representative skin reactions from 1 subject are shown in **Figure 1**. The changes in mean values of both flare and wheal areas from baseline to 24 hours after administration of each of the drugs are shown in the **table** and plotted in **Figures 2** (flare) and **3** (wheal).

Flare

For flare, the areas as measured using on-site evaluation were larger overall than those measured using photographic evaluation, but the shapes of the time-course graphs were similar for both. Overall, the flare area measurements started to decrease significantly from baseline values 4 hours after drug administration, reached a nadir at 10.5 hours, and remained significantly lower compared with baseline values at 24 hours.

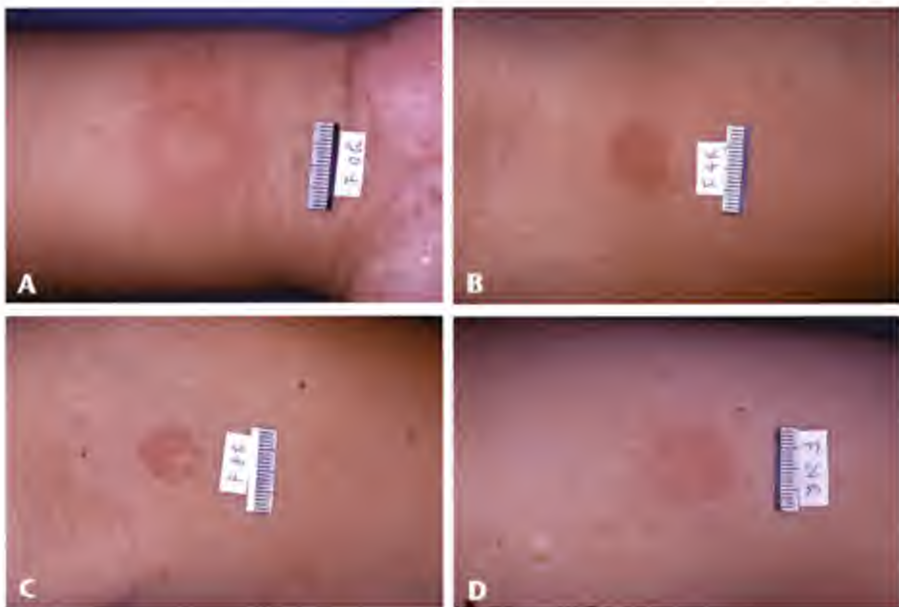


Figure 1. Representative skin reactions from 1 subject (A) before and (B) 4 hours, (C) 8 hours, and (D) 24 hours after the administration of cetirizine 5 mg.

Table. Areas of histamine-induced flare and wheal (mm²) as measured using on-site and photographic evaluation, before (baseline) and after treatment with an antihistamine or placebo (n = 9 in each group). Values are presented as mean (SD).

Lesion Type/ Evaluation Type/ Treatment	Baseline 0 H	0.5 H	1 H	2 H	4 H	8 H	10.5 H	12 H	24 H
Flare									
On-site									
CTZ-5	1272 (719)	1170 (962)	1522 (815)	1251 (863)	512 (484)*†	236 (92)†‡§	239 (95)†§	315 (229)†§	721 (553)¶
CTZ-10	1384 (668)	1098 (635)	1209 (857)	1111 (692)	472 (489)†‡	247 (165)†§	174 (59)†¶	243 (187)†¶	665 (398)*†
LOR	1365 (665)	1236 (650)	1339 (775)	1570 (656)	1041 (764)	759 (505)†#	798 (537)†#	977 (608)¶**	1046 (604)
FEX	1377 (753)	1316 (760)	1168 (547)	1104 (783)	655 (487)†‡	678 (495)†#	433 (338)†‡	775 (441)*†	895 (573)¶**
PLC	1045 (254)	1437 (497)	1301 (399)	1392 (605)	1373 (434)	1091 (355)	1142 (464)	1534 (444)	1430 (439)
Photographic									
CTZ-5	703 (387)	646 (437)	835 (486)	648 (395)	256 (222)†‡	144 (54)†§	182 (78)†§	242 (196)†§	454 (252)‡
CTZ-10	759 (321)	567 (360)	682 (359)	600 (296)	260 (221)†‡	162 (60)†§	117 (31)†¶	141 (56)†§	377 (221)†***
LOR	670 (193)	596 (263)	658 (324)	728 (266)	563 (405)	422 (303)	465 (283)	538 (323)‡	602 (236)
FEX	771 (379)	635 (362)	683 (309)	677 (440)	376 (191)†#	376 (232)†	289 (151)†‡	411 (251)*,***	457 (283)‡#
PLC	660 (247)	699 (290)	738 (294)	682 (387)	696 (291)	582 (221)	618 (266)	781 (299)	691 (284)

(continued)

Table. (Continued)

Lesion Type/ Evaluation Type/ Baseline Treatment	0 H	0.5 H	1 H	2 H	4 H	8 H	10.5 H	12 H	24 H
Wheal									
On-site									
CTZ-5	178 (60)	165 (62)	169 (43)	150 (27)	93 (73) [#]	40 (61) ^{††}	32 (56) ^{††§††}	36 (71) ^{††¶††}	124 (22)
CTZ-10	174 (56)	142 (67)	130 (40)	149 (30)	65 (55) ^{‡‡*}	37 (76) ^{††}	28 (58) ^{††§††}	34 (68) ^{††¶††}	116 (70)
LOR	201 (75)	171 (37)	167 (33)	166 (39)	114 (56) [*]	130 (79)	116 (60) [*]	140 (54)	153 (30)
FEX	167 (46)	152 (45)	154 (27)	151 (56)	115 (49)	127 (61)	107 (50)	113 (51)	146 (51)
PLC	170 (40)	169 (45)	180 (44)	160 (25)	150 (42)	176 (69)	147 (44)	162 (27)	163 (38)
Photographic									
CTZ-5	169 (40)	155 (67)	162 (37)	134 (44)	65 (72) [#]	22 (45) ^{††##}	20 (41) ^{††§##}	62 (77) ^{††§††}	161 (44)
CTZ-10	185 (67)	134 (60)	130 (45)	138 (61)	44 (44) ^{‡‡*}	15 (29) ^{††§##}	13 (38) ^{††§##}	23 (41) ^{††§§}	165 (62)
LOR	179 (60)	147 (31)	142 (39)	143 (43)	102 (49) [*]	93 (74) [*]	86 (70) [†]	124 (38)	155 (30)
FEX	166 (34)	143 (41)	143 (30)	146 (54)	98 (47) [*]	127 (51)	109 (58)	123 (23)	156 (39)
PLC	150 (45)	156 (48)	144 (53)	136 (44)	140 (52)	136 (66)	137 (56)	151 (23)	160 (45)

CTZ-5 = cetirizine 5 mg; CTZ-10 = cetirizine 10 mg; LOR = loratadine; FEX = fexofenadine; PLC = placebo.
^{*}*p* < 0.01 versus baseline; [†]*p* < 0.001 versus PLC; [‡]*p* < 0.001 versus baseline; [§]*p* < 0.05 versus LOR; ^{||}*p* < 0.01 versus LOR;
[#]*p* < 0.05 versus PLC; ^{**}*p* < 0.01 versus PLC; ^{††}*p* < 0.05 versus FEX; ^{†††}*p* < 0.01 versus FEX; ^{‡‡}*p* < 0.01 versus FEX; ^{§§}*p* < 0.001 versus LOR; ^{|||}*p* < 0.001 versus FEX.

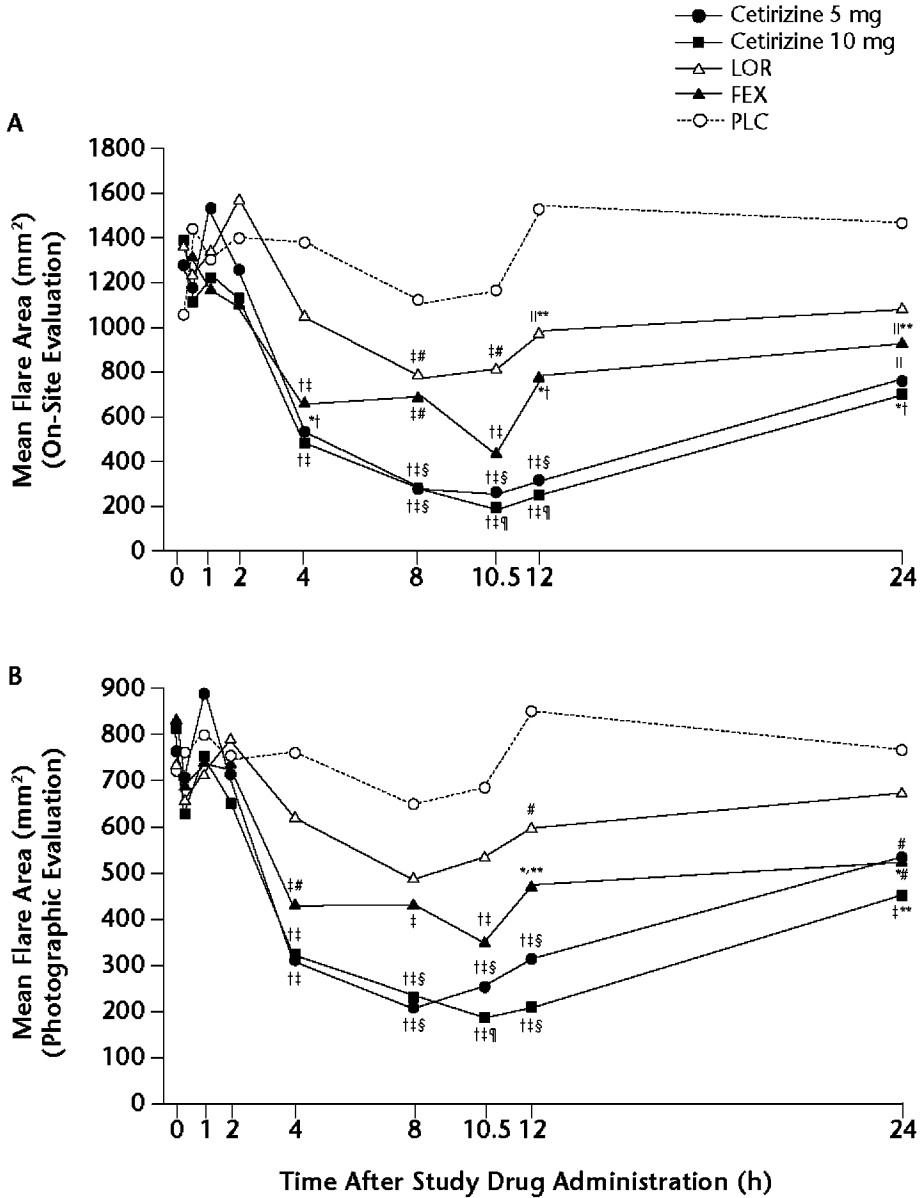


Figure 2. Areas of histamine-induced flare before (time 0) and after the administration of an antihistamine or placebo (PLC) as measured using (A) on-site and (B) photographic evaluations (n = 9 in each treatment group). FEX = fexofenadine. **P* < 0.01 versus baseline; †*P* < 0.001 versus PLC; ‡*P* < 0.001 versus baseline; §*P* < 0.05 versus loratadine (LOR); ||*P* < 0.05 versus baseline; ¶*P* < 0.01 versus LOR; #*P* < 0.05 versus PLC; ***P* < 0.01 versus PLC.

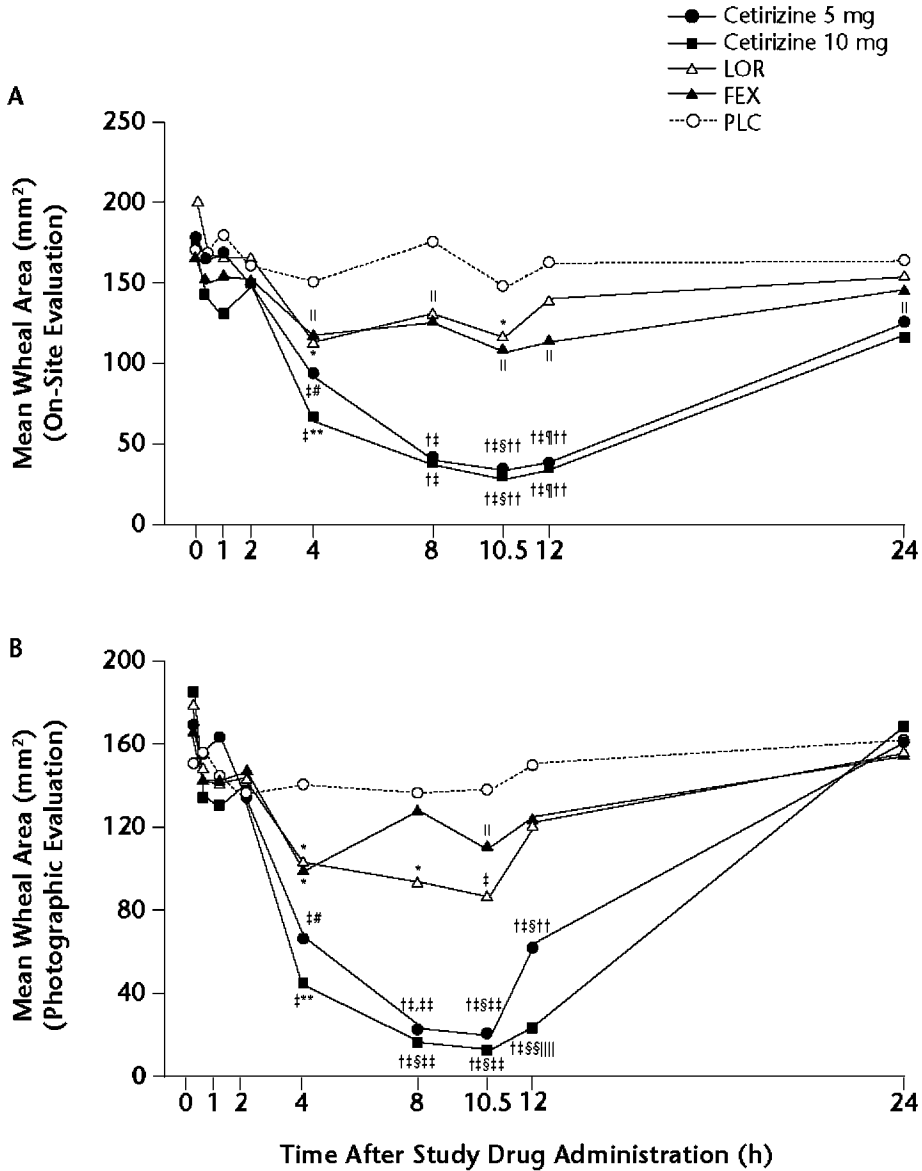


Figure 3. Areas of histamine-induced wheal before (time 0) and after the administration of an antihistamine or placebo (PLC) as measured using (A) on-site and (B) photographic evaluations (n = 9 in each treatment group). **P* < 0.01 versus baseline; †*P* < 0.001 versus PLC; ‡*P* < 0.001 versus baseline; §*P* < 0.05 versus loratadine (LOR); ¶*P* < 0.05 versus baseline; ¶¶*P* < 0.01 versus LOR; #*P* < 0.05 versus PLC; ***P* < 0.01 versus PLC; ††*P* < 0.05 versus fexofenadine (FEX); †††*P* < 0.01 versus FEX; §§*P* < 0.001 versus LOR; ††††*P* < 0.001 versus FEX.

For CTZ-5, mean flare areas as measured using on-site evaluation were significantly less compared with baseline at 4, 8, 10.5, 12, and 24 hours after administration ($P < 0.01$, <0.001 , <0.001 , <0.001 , and <0.05 , respectively) and compared with PLC at the same time points (all, $P < 0.001$). These values as measured using photographic evaluation were significantly less compared with baseline at 4, 8, 10.5, and 12 hours after administration (all, $P < 0.001$), and compared with PLC at 4, 8, 10.5, 12, and 24 hours ($P < 0.001$, <0.001 , <0.001 , <0.001 , and <0.05 , respectively).

For CTZ-10, mean flare areas as measured using on-site evaluation were significantly less compared with baseline at 4, 8, 10.5, 12, and 24 hours after administration ($P < 0.001$, <0.001 , <0.001 , <0.001 , and <0.01 , respectively), and compared with PLC at the same time points (all, $P < 0.001$). These values as measured using photographic evaluation were significantly less compared with baseline at 4, 8, 10.5, 12, and 24 hours after administration (all, $P < 0.001$), and compared with PLC at the same time points ($P < 0.001$, <0.001 , <0.001 , <0.001 , and <0.01 , respectively).

For LOR, mean flare areas as measured using on-site evaluation were significantly less compared with baseline at 8, 10.5, and 12 hours after administration ($P < 0.001$, <0.001 , and <0.05 , respectively), and compared with PLC at the same time points ($P < 0.05$, <0.05 , and <0.01 , respectively). These values as measured using photographic evaluation were statistically similar compared with baseline at all time points, and were significantly less compared with PLC at 12 hours after administration ($P < 0.05$).

For FEX, mean flare areas as measured using on-site evaluation were significantly less compared with baseline at 4, 8, 10.5, 12, and 24 hours after administration ($P < 0.001$, <0.001 , <0.001 , <0.01 , and <0.05 , respectively), and compared with PLC at the same time points ($P < 0.001$, <0.05 , <0.001 , <0.001 , and <0.01 , respectively). These values as measured using photographic evaluation were significantly less compared with baseline at 4, 8, 10.5, 12, and 24 hours after administration ($P < 0.001$, <0.001 , <0.001 , <0.01 , and <0.01 , respectively), and compared with PLC at 4, 10.5, 12, and 24 hours ($P < 0.05$, <0.001 , <0.01 , and <0.05 , respectively).

For PLC, no significant differences versus baseline were found.

Comparisons between antihistamines showed significant differences in mean flare areas between the 2 doses of CTZ and LOR from 8 to 12 hours after administration in both evaluation methods (all, $P \leq 0.05$).

When the AUC_{0-24} was regarded as being negatively correlated with the potency of overall suppression of the flare, the order of potency was CTZ-10 > CTZ-5 > FEX > LOR > PLC.

Wheal

The wheal areas were significantly reduced from baseline values by most of the antihistamines 4 to 12 hours after drug administration, reached their lowest values at 10.5 hours, and returned to near-baseline values at 24 hours. Comparisons with PLC values at each time point, however, showed significant differences only for CTZ-5 and CTZ-10 from 4 to 12 hours after administration.

For CTZ-5, mean wheal areas as measured using on-site evaluation were significantly less compared with baseline at 4, 8, 10.5, 12, and 24 hours after administration ($P < 0.001$, <0.001 , <0.001 , <0.001 , and <0.05 , respectively), and compared with PLC at 4, 8, 10.5, and 12 hours ($P < 0.05$, <0.001 , <0.001 , and <0.001 , respectively). These values as measured using photographic evaluation were significantly less compared with baseline at 4, 8, 10.5, and 12 hours after administration (all, $P < 0.001$), and compared with PLC at the same time points ($P < 0.05$, <0.001 , <0.001 , and <0.001 , respectively).

For CTZ-10, mean wheal areas as measured using on-site evaluation were significantly less compared with baseline at 4, 8, 10.5, and 12 hours after administration (all, $P < 0.001$), and compared with PLC at the same time points ($P < 0.01$, <0.001 , <0.001 , and <0.001 , respectively). These values as measured using photographic evaluation were significantly less compared with baseline at 4, 8, 10.5, and 12 hours after administration (all, $P < 0.001$), and compared with PLC at the same time points ($P < 0.01$, <0.001 , <0.001 , and <0.001 , respectively).

For LOR, mean wheal areas as measured using on-site evaluation were significantly less compared with baseline at 4, 8, and 10.5 hours after administration ($P < 0.01$, <0.05 , and <0.01 , respectively). These values as measured using photographic evaluation were significantly less compared with baseline at 4, 8, and 10.5 hours after administration ($P < 0.01$, <0.01 , and <0.001 , respectively). No statistically significant differences between LOR and PLC were found.

For FEX, mean wheal areas as measured using on-site evaluation were significantly less compared with baseline at 4, 10.5, and 12 hours after administration (all, $P < 0.05$). These values as measured using photographic evaluation were significantly less compared with baseline at 4 and 10.5 hours after administration ($P < 0.01$ and <0.05 , respectively). No statistically significant differences between FEX and PLC were found.

For PLC, no significant differences versus baseline were found.

Comparisons of the antihistamines showed significant differences between the 2 doses of CTZ and LOR and FEX at 10.5 and 12 hours after administration in both methods of evaluation (all, $P \leq 0.05$).

Using AUC_{0-24} , the strength of wheal suppression was CTZ-10 > CTZ-5 > FEX > LOR > PLC on on-site evaluation, but the order of LOR and FEX was reversed on photographic evaluation.

Comparison of Results Obtained Using On-Site or Photographic Evaluation

Figure 4A is a comparison of the area of flare as measured using on-site and photographic evaluation. Despite excellent linear correlation ($r = 0.90$; $P < 0.001$), the slope of the regression line was 0.45, indicating that the flare area as measured using photographic evaluation was approximately half that measured using on-site evaluation. There was also good linear correlation in the case of the area of wheal (Figure 4B). The slope of the regression line was 0.75, indicating that the absolute measurements obtained using the 2 methods were closer

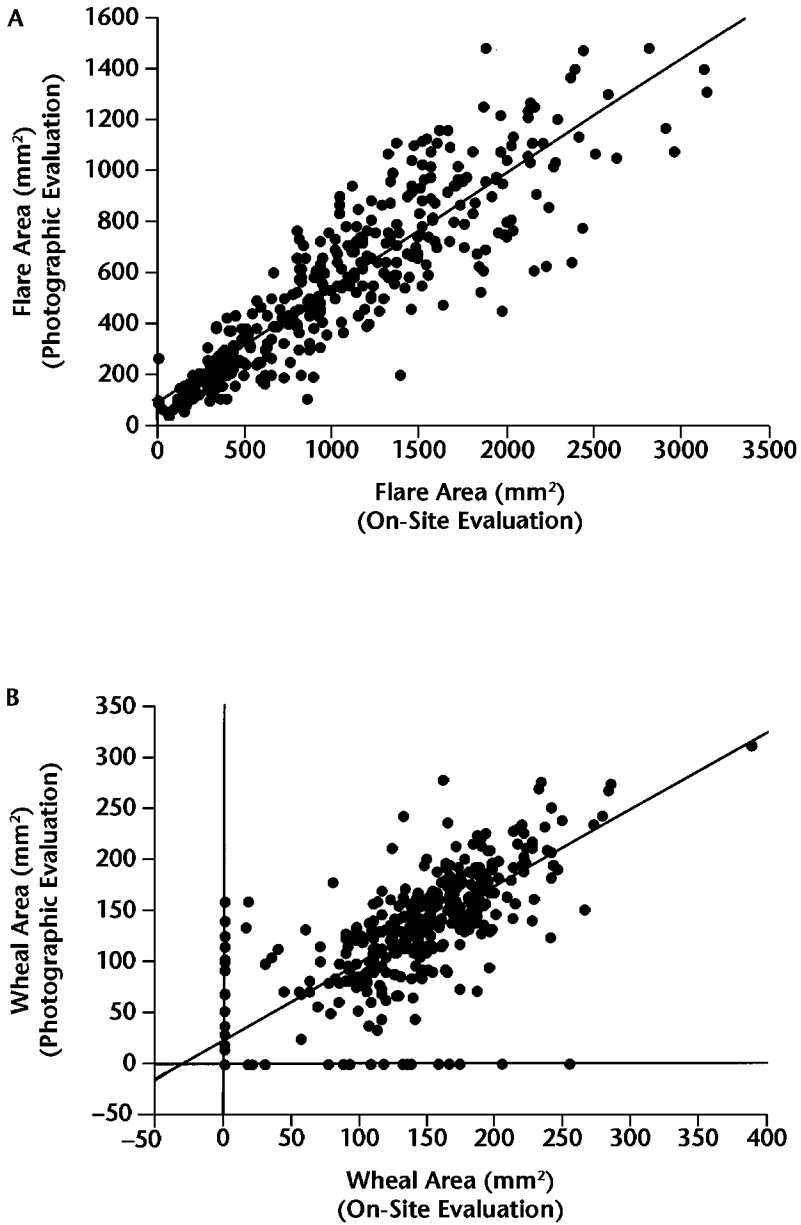


Figure 4. Correlation between area measurements obtained using on-site and photographic evaluations of (A) flare and (B) wheal from the same skin-reaction area. The diagonal lines represent regression. A, $r = 0.90$, $P < 0.001$; B, $r = 0.76$, $P < 0.001$.

for wheal than for flare. However, because many isolated points were plotted only on the *x*-axis or only on the *y*-axis, the correlation coefficient for the wheal was 0.76 ($P < 0.001$). These points represent reactions that were regarded as wheals by 1 evaluator but only as flares by the other.

Tolerability

No AEs were experienced by the subjects during the study.

DISCUSSION

Many studies have compared the suppressive effect of second-generation antihistamines on histamine skin-test reactions.¹⁻⁸ The results of the present study agreed, for the most part, with those of earlier studies. We were especially interested in the suppressive effect of CTZ-5 because 5 mg is half of the standard dose prescribed in Japan. Although the duration of the suppressive effect of CTZ-5 was somewhat shorter than that of CTZ-10, the speed of its manifestation and its maximal strength were approximately the same as those of CTZ-10. Therefore, although the standard dose of CTZ in Japan is 10 mg QD, the option of 5 mg BID should be considered in future studies.

However, the results of the present study were obtained with only 1 or 2 administrations on a single day. The results of one study³ showed that even if the antihistamine effects of 2 drugs were different in the single-dose trial, the efficacy became similar when they were administered for 5 consecutive days. Because the results of the present study appear to be related to differences in the effect on the first day of treatment, it might not be reasonable to assume that the same findings can be used to predict the clinical effects in disorders that require continued drug use, such as chronic urticaria.

Manifestation of the suppressive effects of the drugs examined in the present study was slower than in studies reported in the literature to date (MEDLINE search; key words: *cetirizine* and *histamine-induced wheal*; years: 2000–2005), most of which showed that suppression of the skin reactions was observed 1 to 2 hours after antihistamine administration.^{7,8,10-16} Although the reason for these differences is unclear, they may have resulted from differences in the absorption rates of the drugs. In previous studies, medications were administered with a large amount of water under fasting conditions,^{3,5,6,8} whereas the subjects in the present study received the medication after meals with an unspecified amount of water. The method by which histamine was introduced, and its concentration, might also explain why the suppressive effect of antihistamines in other studies occurred sooner compared with that in the present study.^{7,17} The concentration of histamine introduced in the skin might have been unexpectedly high for unknown reasons.

Pantetheine was chosen as the placebo partly because the manufacturer indicated that the drug had no effect on skin-test reactions. However, this drug might have a weak suppressive effect on urticaria and therefore might not have been an ideal placebo for use in this study.¹⁸

We examined the differences between the results of on-site and photographic assessments of wheals and flares. Because the area of skin reaction changes each minute, and to avoid interinvestigator differences in area evaluations, it is typical in this type of clinical study that only 1 investigator measures the area of wheal and flare.² If photographic assessment was shown to be reliable, evaluations could be performed by several examiners, which might increase objectivity and save time in this kind of clinical study. However, it is necessary to confirm that the areas of skin reaction measured on-site and on photographic images show good linear correlation. Although our study showed good linear correlation between the corresponding flare areas, the absolute measurements obtained from the images were approximately half of those obtained with on-site assessment. In addition to the fact that defining the border for tracing depends on the subjective judgment of the evaluator, underestimation of the area might be inevitable with the photographic measurements because the resolution of photographs is inferior to on-site inspection and because the curved, 3-dimensional surface of the forearm is projected onto the flat, 2-dimensional surface of the computer monitor. Another problem may be discrimination between wheal and flare. When histamine is introduced into the skin, a wheal with a clearly defined border develops that would be difficult to confuse with the surrounding erythema. However, when an antihistamine suppresses the reaction, erythema accompanied by slight edema develops instead of the typical wheal. Because a choice must be made between wheal and flare (ie, not wheal) in a clinical trial, assessments by the evaluators might differ, especially in the case of low-resolution photographic evaluation. This difference might then influence the assessment of the efficacy of drugs with similar potency. In our study, this was thought to be one reason for the reverse order of the suppressive effects of LOR and FEX on wheals as assessed using the 2 methods. Further studies of the accuracy of photographic evaluation are needed to establish this method as an appropriate alternative to on-site measurements.

CONCLUSIONS

In this study in healthy volunteers, single doses of CTZ 5 and 10 mg were more effective in suppressing histamine-induced skin response, as measured using on-site and photographic evaluation, compared with LOR and FEX. Although linear correlations were found between skin-test areas using the 2 methods of evaluation, it was difficult to differentiate between wheal and flare on photographic evaluation, especially if a typical wheal was suppressed to slightly edematous erythema by antihistamines.

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REFERENCES

1. Juhlin L. A comparison of the pharmacodynamics of H1-receptor antagonists as assessed by the induced wheal-and-flare model. *Allergy*. 1995;50(Suppl 24):24–30.
2. Simons FE, McMillan JL, Simons KJ. A double-blind, single-dose, crossover comparison of cetirizine, terfenadine, loratadine, astemizole, and chlorpheniramine versus placebo: Suppressive effects on histamine-induced wheals and flares during 24 hours in normal subjects. *J Allergy Clin Immunol*. 1990;86:540–547.
3. Simons FE, Silver NA, Gu X, Simons KJ. Clinical pharmacology of H1-antihistamines in the skin. *J Allergy Clin Immunol*. 2002;110:777–783.
4. Kaplan AP. Chronic urticaria: Pathogenesis and treatment. *J Allergy Clin Immunol*. 2004;114:465–474.
5. Rihoux JP, Dupont P. Comparative study of the peripheral and central effects of terfenadine and cetirizine 2 HCl. *Ann Allergy*. 1987;59:235–238.
6. Grant JA, Danielson L, Rihoux JP, DeVos C. A double-blind, single-dose, crossover comparison of cetirizine, ebastine, epinastine, fexofenadine, terfenadine, and loratadine versus placebo: Suppression of histamine-induced wheal and flare response for 24 h in healthy male subjects. *Allergy*. 1999;54:700–707.
7. Furue M, Terao H, Koga T. Effects of cetirizine and epinastine on the skin response to histamine iontophoresis. *J Dermatol Sci*. 2001;25:59–63.
8. Grant JA, Riethuisen JM, Moolaert B, DeVos C. A double-blind, randomized, single-dose, crossover comparison of levocetirizine with ebastine, fexofenadine, loratadine, mizolastine, and placebo: Suppression of histamine-induced wheal-and-flare response during 24 hours in healthy male subjects. *Ann Allergy Asthma Immunol*. 2002;88:190–197.
9. ImageJ—Image Processing and Analysis in Java. Available at: <http://rsb.info.nih.gov/ij/>. Accessed August 1, 2005.
10. Devalia JL, De Vos C, Hanotte F, Baltés E. A randomized, double-blind, crossover comparison among cetirizine, levocetirizine, and ucb 28557 on histamine-induced cutaneous responses in healthy adult volunteers. *Allergy*. 2001;56:50–57.
11. Purohit A, Duvernelle C, Melac M, et al. Twenty-four hours of activity of cetirizine and fexofenadine in the skin. *Ann Allergy Asthma Immunol*. 2001;86:387–392.
12. Roongapinun S, Wajajamreon S, Fooanant S. Comparative efficacy of wheal-and-flare suppression among various non-sedating antihistamines and the pharmacologic insights to their efficacy. *J Med Assoc Thai*. 2004;87:551–556.
13. Purohit A, Melac M, Pauli G, Frossard N. Comparative activity of cetirizine and desloratadine on histamine-induced wheal-and-flare responses during 24 hours. *Ann Allergy Asthma Immunol*. 2004;92:635–640.
14. Takahashi H, Ishida-Yamamoto A, Iizuka H. Effects of bepotastine, cetirizine, fexofenadine, and olopatadine on histamine-induced wheal-and-flare response, sedation, and psychomotor performance. *Clin Exp Dermatol*. 2004;29:526–532.
15. Purohit A, N'Gom AS, Deslandes B, et al. Similar rapid onset of action and magnitude of effect of fexofenadine and cetirizine as assessed by inhibition of histamine-induced wheal-and-flare reaction. *Ann Allergy Asthma Immunol*. 2004;93:562–567.

16. Morita E, Matsuo H, Zhang Y. Double-blind, crossover comparison of olopatadine and cetirizine versus placebo: Suppressive effects on skin response to histamine iontophoresis. *J Dermatol.* 2005;32:58–61.
17. Leroy T, Tasset C, Valentin B, Van Neste D. Comparison of the effects of cetirizine and ebastine on the skin response to histamine iontophoresis monitored with laser Doppler flowmetry. *Dermatology.* 1998;197:146–151.
18. Kobayashi S. Investigation of the roles of the substances in serum lipids and their constitutive fatty acids in chronic urticaria. *J Dermatol.* 1989;16:196–206.

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