

Quantitation of H₁-receptor antagonists in skin and serum

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Background: Cetirizine and hydroxyzine produce prompt, long-lasting peripheral H₁-blockade in skin. We hypothesized that after oral administration of these H₁-receptor antagonists, skin concentrations would be higher than serum concentrations and would correlate with peripheral H₁ blockade.

Methods: In a randomized, double-blind, parallel-group study in 13 healthy subjects, skin biopsies, venipunctures, and epicutaneous tests with histamine were performed before ingestion of 10 mg cetirizine or 50 mg hydroxyzine and 1, 3, 6, 9, and 24 hours after administration. Subjects then took 10 mg cetirizine or 50 mg hydroxyzine at 21:00 hours for 6 consecutive days. All tests were repeated at 168 hours (steady state), 12 hours after the last dose.

Results: Skin cetirizine concentrations were lower than serum cetirizine concentrations from 1 to 9 hours but were higher at 24 hours and equivalent at 168 hours (steady state). Skin hydroxyzine concentrations were higher than serum hydroxyzine concentrations at all test times. After hydroxyzine dosing, cetirizine, the active metabolite of hydroxyzine arising *in vivo*, was found in skin and serum. Single doses of cetirizine or hydroxyzine produced highly significant suppression of wheals and flares from 3 to 24 hours inclusive, and this suppression was maintained at steady state.

Conclusions: Cetirizine and hydroxyzine enter the skin readily, and their sustained high concentrations in skin after single or multiple dosing may contribute to their well-known efficacy in symptomatic treatment of urticaria and other skin disorders in which histamine plays a role. (*J ALLERGY CLIN IMMUNOL* 1995;95:759-64.)

Key words: Histamine H₁-receptor antagonists, cetirizine, hydroxyzine, biopsy

H₁-receptor antagonists such as cetirizine and hydroxyzine are widely used for relief of pruritus in allergic skin disorders¹⁻¹⁰ and are particularly helpful in urticaria for reducing itching and the number, size, and duration of wheals.⁴⁻⁸ The pharmacokinetics and pharmacodynamics of these medications have been thoroughly studied. After administration of single oral doses of cetirizine or hydroxyzine, although absorption is rapid and serum concentrations peak within 1 to 2 hours, peripheral H₁-blockade does not usually become

Abbreviation used

E_{max}: Observed maximal effect

maximal until at least 5 hours after administration, when serum concentrations are waning. Peripheral H₁-blockade also may persist for hours after serum H₁-antagonist concentrations have declined to less than the limits of analytic detection.¹¹⁻¹⁵ The concentrations of these H₁-receptor antagonists achieved in human skin after usual doses are unknown, and the relationships of skin concentrations to serum concentrations and to peripheral H₁-blockade have not yet been elucidated.

We hypothesized that cetirizine and hydroxyzine skin concentrations after oral dosing would be higher than serum concentrations and that skin concentrations would correlate better than serum concentrations with the amount of peripheral H₁-

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blockade produced. We tested this hypothesis in a randomized, double-blind, parallel-group study.

METHODS

Subjects

All subjects gave written informed consent before participation. The study protocol was approved by the University of Manitoba Faculty Committee on the Use of Human Subjects in Research.

We recruited healthy men, 18 to 40 years of age, with normal skin. Subjects were excluded if they had a history of any skin disorder, were prone to scar formation, or had very dark skin, precluding accurate visualization and tracing of the flare response. Subjects were also excluded if they were obese or if they could not tolerate drowsiness or sedation during the day; might need to use an H_1 -receptor antagonist other than study medications before or during the study; or had a history of adverse reactions to H_1 -antagonists, or smoking during the previous year.

Study design

The subjects had a preliminary visit to the Health Sciences Clinical Research Centre, during which they were given the opportunity to become familiar with the test procedures. On the first study day they arrived at the Health Sciences Clinical Research Centre at 08:00 hours, after an 8-hour fast. Baseline skin biopsy, venipuncture, and histamine skin tests were performed. The subjects were then randomly assigned to receive either 10 mg cetirizine or 50 mg hydroxyzine, and the biopsies, venipunctures, and skin tests were repeated in the same sequence 1, 3, 6, 9, and 24 hours after administration. The techniques used for these procedures are described later.

The subjects received a standardized breakfast consisting of 250 ml apple juice and a muffin, 2 hours after administration, and a standardized lunch and dinner 4 and 8 hours after administration, respectively.

Beginning 36 hours after receiving their initial dose of cetirizine or hydroxyzine, the subjects took 10 mg cetirizine or 50 mg hydroxyzine (whichever they had received on the first study day) at 21:00 hours for 6 consecutive days. Medications were administered in the evening to minimize daytime somnolence from hydroxyzine and consequent loss of double blinding.¹⁶⁻¹⁸ Skin biopsies, venipunctures, and histamine skin tests were repeated at 168 hours (steady state), exactly 12 hours after the seventh and last H_1 -antagonist dose.

At all test times subjects were questioned about the presence of somnolence or other adverse effects.

Techniques for measuring cetirizine and hydroxyzine in skin

The skin biopsies were performed on the medial surfaces of the thighs, with a 3 mm Accu-Punch device (Acuderm, Ft. Lauderdale, Fla.) (predose baseline bi-

opsy) or a 4 mm Accu-Punch device (subsequent biopsies) 15 minutes after the biopsy site was infiltrated with 1% lidocaine with 0.01 mg/ml epinephrine and 0.1 mEq/ml sodium bicarbonate. A different site was used for each biopsy, and the sequence of sites was the same in each subject. All biopsy samples were blotted free of blood on surgical gauze, transferred to nonabsorbent paper, weighed immediately, and then stored and sealed in a polypropylene tube at -20°C until analyzed for cetirizine or hydroxyzine.

Before analysis skin samples were thawed then digested in 0.25 ml of 2 mol/L sodium hydroxide for 24 hours. Cetirizine and hydroxyzine were extracted from the skin samples by the same procedure as for serum samples, and H_1 -antagonist concentrations were determined in skin by utilizing high-pressure liquid chromatography procedures previously developed in our laboratory.^{11, 14}

Techniques for measuring cetirizine and hydroxyzine in serum

Venipunctures for 7 ml blood were performed 30 minutes after application of EMLA local anesthetic cream (Astra Pharma Inc., Mississauga, Ontario, Canada) to potential sites. Blood samples were centrifuged at 4°C . Serum was transferred into appropriately labeled polypropylene tubes with screw caps, frozen promptly in the upright position, and stored at -20°C until analysis, by utilizing high-pressure liquid chromatography methods previously developed in our laboratory.^{11, 14} In subjects receiving hydroxyzine, in addition to measurement of hydroxyzine in serum, cetirizine arising from hydroxyzine *in vivo* was also measured.

Skin tests

Epicutaneous tests were performed with histamine phosphate, 1 mg/ml (Glaxo Canada Inc., Mississauga, Ontario, Canada) and 10 mg/ml (Sigma Chemical Co., St. Louis, Mo.) in 0.9% NaCl, sterilized by 0.22 μm filtration. A different site on the volar surfaces of the forearm was used for each test, and the sequence of test sites was the same in each subject. The wheal-and-flare circumferences were traced with a pen at 10 minutes and transferred to paper with transparent tape. Wheal-and-flare areas were measured by a computerized digitizing system.^{11, 14, 15}

Data analysis

Mean skin cetirizine or hydroxyzine concentrations were compared with serum cetirizine or hydroxyzine concentrations, respectively, over time with two-way analysis of variance and the Tukey and Bonferroni multiple-range tests.¹⁹ Differences were considered significant at a *p* value less than 0.05.

Mean wheal-and-flare areas at each time were compared with predose values for cetirizine or hydroxyzine by analysis of variance and the Tukey and Bonferroni

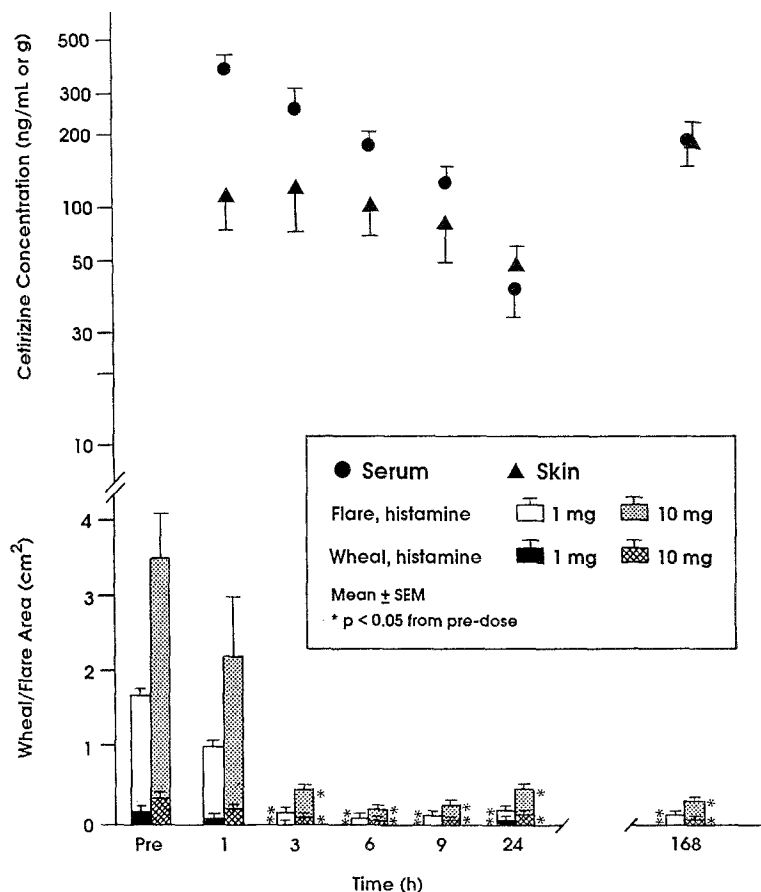


FIG. 1. Skin and serum cetirizine concentration versus time plots, and wheal-and-flare areas after epicutaneous tests with 1 and 10 mg/ml histamine. Tests were performed at baseline and 1, 3, 6, 9, and 24 hours after initial dose of 10 mg cetirizine. Subjects then took 10 mg cetirizine at 21:00 hours for 6 consecutive days, and tests were repeated at 168 hours (steady state), exactly 12 hours after seventh and last dose.

multiple-range tests, with differences being considered significant at a p value less than 0.05. Wheal-and-flare areas after cetirizine administration were compared with those values after hydroxyzine administration by utilizing the same statistical tests and level of significance.

Correlations between skin and serum cetirizine or hydroxyzine concentrations and degree of suppression of histamine-induced wheals and flares after cetirizine or hydroxyzine were evaluated by utilizing linear regression techniques, by which 95% and 99% confidence intervals were calculated.

Plots of absolute wheal-and-flare area versus skin and serum cetirizine and hydroxyzine concentrations were fitted by the observed maximal effect (E_{max}) model of Sheiner et al.^{20, 21}

RESULTS

Thirteen subjects (mean age, 26 ± 6 years; mean weight, 75 ± 11 kg) completed the study; seven

received cetirizine, and six received hydroxyzine. Skin and serum cetirizine concentration versus time curves are shown in Fig. 1. Skin cetirizine concentrations were lower than serum cetirizine concentrations during the first 9 hours after administration but were equivalent at 24 hours and at 168 hours (steady state).

The relationship between skin and serum cetirizine concentrations and the peripheral H_1 -blockade produced in skin by cetirizine is also shown in Fig. 1. After epicutaneous tests with 1 or 10 mg/ml histamine phosphate, cetirizine produced significant suppression of wheals and flares from 3 to 24 hours inclusive, and at steady state, compared with predose values.

Skin and serum hydroxyzine concentration versus time curves are shown in Fig. 2. Skin hydroxyzine concentrations were higher than serum

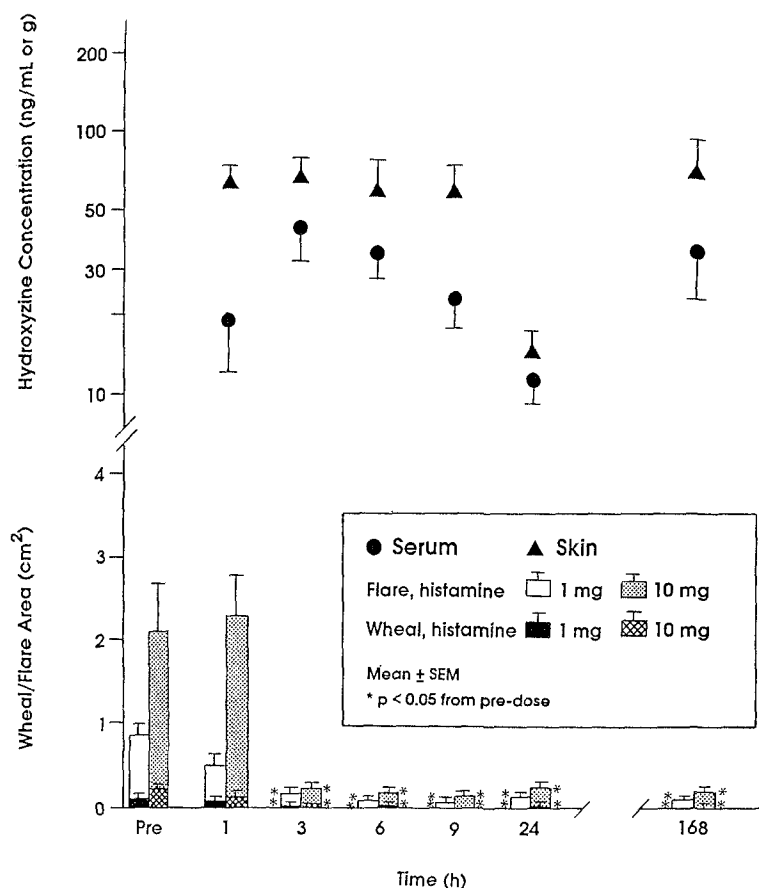


FIG. 2. Skin and serum hydroxyzine concentrations versus time plots, and wheal-and-flare areas after epicutaneous tests with 1 and 10 mg/ml histamine. Tests were performed at baseline and 1, 3, 6, 9, and 24 hours after initial dose of 50 mg hydroxyzine. Subjects then took 50 mg hydroxyzine at 21:00 hours for 6 consecutive days, and tests were repeated at 168 hours (steady state), exactly 12 hours after seventh and last dose.

hydroxyzine concentrations during the 24 hours after the initial dose and at steady state. At steady state mean skin and serum concentrations of cetirizine arising in vivo as the active metabolite of hydroxyzine were 39.7 ± 27.1 and 481.6 ± 85.4 ng/ml, respectively, compared with 181.5 ± 81.0 and 188.4 ± 47.3 ng/ml, respectively, achieved after administration cetirizine itself.

The relationship between skin and serum hydroxyzine concentrations and the peripheral H_1 -blockade produced in skin by hydroxyzine is also shown in Fig. 2. After epicutaneous tests with 1 or 10 mg/ml histamine phosphate, hydroxyzine produced significant suppression of the wheals and flares from 3 to 24 hours inclusive, and at steady state, compared with predose values.

Cetirizine and hydroxyzine did not differ significantly in their ability to suppress the histamine-induced wheals and flares at any test time.

No serious adverse effects from cetirizine or hydroxyzine were reported or noted.

DISCUSSION

The data obtained in this study of human subjects are similar to data obtained previously in rabbits, in which we found that skin concentrations of the H_1 -antagonists cetirizine, hydroxyzine, diphenhydramine, and chlorpheniramine measured after a single intravenous dose were as high as or higher than concomitantly measured serum H_1 -antagonist concentrations throughout a dosing interval.^{22, 23}

The findings in this study help explain the prompt onset of action and prolonged duration of action of cetirizine and hydroxyzine in patients with allergic skin disorders such as urticaria, in which histamine plays a role⁴⁻⁸; these H_1 -receptor antagonists are among the most effective medica-

tions currently available for suppressing wheals and flares.^{11-15, 24}

Cetirizine, the active metabolite of hydroxyzine, is considerably more polar than the parent compound because of oxidation of the terminal alcohol to a carboxylic acid. This may be a factor in limiting the extent of its penetration into the skin, because cetirizine skin concentrations were two to three times lower than concurrent cetirizine serum concentrations except at 24 hours after a single dose, and at steady state during multiple dosing, when they were slightly higher or equivalent to serum concentrations. In contrast, hydroxyzine skin concentrations were two to three times higher than hydroxyzine serum concentrations at all times during the single-dose study and at steady state. The extent of partitioning out of the serum relates to the size of the apparent volumes of distribution, 0.4 L/kg for cetirizine and 16 L/kg for hydroxyzine, as documented in earlier investigations of these H₁-receptor antagonists.¹¹⁻¹⁴

The serum elimination half-life values of cetirizine and hydroxyzine are approximately 9 hours and 15.5 hours, respectively.^{11-13, 25} After seven daily doses no further accumulation would be expected and steady state would be achieved. In this study at steady state, serum cetirizine concentrations were 481.6 ± 85.4 ng/ml after 50 mg hydroxyzine and 188.4 ± 47.3 ng/ml after administration of 10 mg cetirizine. This 2.5-fold difference was not surprising, because approximately 50% of a hydroxyzine dose is converted to the active metabolite cetirizine, *in vivo*²⁵ and subjects receiving 50 mg hydroxyzine may have received the equivalent of 25 mg cetirizine, 2.5 times the amount of cetirizine administered *de novo*. In the subjects receiving hydroxyzine, our study design did not permit investigation of the relative contribution to peripheral blockade of hydroxyzine and of cetirizine arising from hydroxyzine *in vivo*.

When correlations between skin and serum H₁-receptor antagonist concentrations and wheal-and-flare suppression were studied with the E_{max} model,^{19, 20} considerable scatter in the data was found, especially with skin concentrations. Cetirizine and hydroxyzine administration resulted in 100% suppression of the histamine-induced wheal response at peak times, making it almost impossible to calculate reliable values for E_{max} and for the medication concentration producing half-maximal effect. The histamine-induced flares were not 100% suppressed, and some correlation with flare suppression was easier to demonstrate. When the steady-state data at 168 hours were plotted, as

absolute wheal-and-flare area versus serum or skin H₁-receptor antagonist concentrations, no linear correlations were observed.

The design of this study also did not permit us to ascertain whether the H₁-receptor antagonists in the skin were located in the extracellular fluid, were bound to H₁-receptors on cells, or were at both sites. Most H₁-receptor antagonists act by competitive antagonism of H₁-receptors and therefore must be present in the skin in sufficiently large quantities to compete with histamine, to occupy the receptors, and to prevent the histamine released during the allergic reaction from combining with the H₁-receptors and causing symptoms. Although suppression of the wheal-and-flare response to histamine, allergen, or other agents causing histamine release from mast cells has proved to be a reliable objective method for functional assessment of peripheral H₁-blockade in skin, the local concentrations of H₁-receptor antagonists required for peripheral H₁-blockade have not been measured. In this study we have shown that the H₁-receptor antagonists cetirizine and hydroxyzine reach maximum concentrations of 10^{-6} mol/L at steady state in human skin and have thus established that these concentrations are associated with excellent functional antagonism. H₁-receptor antagonist concentrations of 10^{-6} and 10^{-7} mol/L have been well documented to have an antihistaminic effect *in vitro*. In addition, the cetirizine concentrations achieved in this study are within the range of cetirizine concentrations used in some *in vitro* studies of its antiinflammatory effects.^{26, 27}

How these H₁-receptor antagonist concentrations relate to the concentrations of histamine released during an allergic reaction in the skin is unknown, because the latter, although estimated to be as high as 10^{-5} to 10^{-3} mol/L, have not been directly measured.

H₁-receptor antagonists are not equally effective in suppressing the wheal-and-flare response in the skin¹⁵ or in suppressing the itching and other symptoms and signs of allergic skin disorders. This study provides a model whereby H₁-receptor antagonists, including those in early phases of investigation and not yet generally available for use, could be assessed for their ability to enter the skin and be available in sufficiently high local concentrations to have a meaningful effect.

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