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Measurement of interstitial cetirizine concentrations in human skin: correlation of drug levels with inhibition of histamine-induced skin responses

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Key words: cetirizine; human skin; microdialysis; pharmacokinetics.

Background: The purpose of the present study was to measure the concentrations of cetirizine in the extracellular water compartment in intact human skin and assess simultaneously inhibition of histamine-induced wheal and flare reactions.

Methods: Skin cetirizine levels were collected by the microdialysis technique and analyzed by high-pressure liquid chromatography with mass spectrometry detection. Skin levels in 20 subjects were compared to plasma levels for 4 h after a single oral dose of 10 or 20 mg of cetirizine. Skin prick tests were performed with histamine 100 mg/ml.

Results: Plasma cetirizine levels increased within 30 min to reach peak values of 315 ± 10 and 786 ± 45 ng/ml 90–120 min after administration of 10 and 20 mg of cetirizine. This was followed by a slow decline. In the skin, dialysate cetirizine levels (non-protein-bound fraction only) peaked at 1.6 ± 0.1 and 2.4 ± 0.3 ng/ml at 120–180 min. *In vivo* recovery of cetirizine was $14.4 \pm 4.3\%$. It was estimated that the non-protein-bound concentration of cetirizine in the skin was 50–70% of corresponding plasma values. Both 10- and 20-mg doses of cetirizine inhibited wheal and flare reactions over 240 min. The time vs concentration profile of cetirizine in skin dialysate paralleled the inhibition of skin reactions, but no significant correlations were found between individual cetirizine levels in skin or plasma with wheal and flare reactions.

Conclusions: Cetirizine concentrations in the skin could be monitored by the microdialysis technique. The results indicate no simple linear correlation between cetirizine skin levels and inhibition of skin reactions.

Date:

Accepted for publication 12 January 1999

To cite this article:

Petersen L.J., Church M.K., Rihoux J.-P. & Skov P.S.
Measurement of interstitial cetirizine concentrations in human skin: correlation of drug levels with inhibition of histamine-induced skin responses.
Allergy 1999; **54**, 607–611.

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ISSN 0105-4538

Histamine H₁-receptor antagonists (antihistamines) are drugs used for treatment of most cases of urticaria and allergic rhinitis (1, 2). Cetirizine is a second-generation,

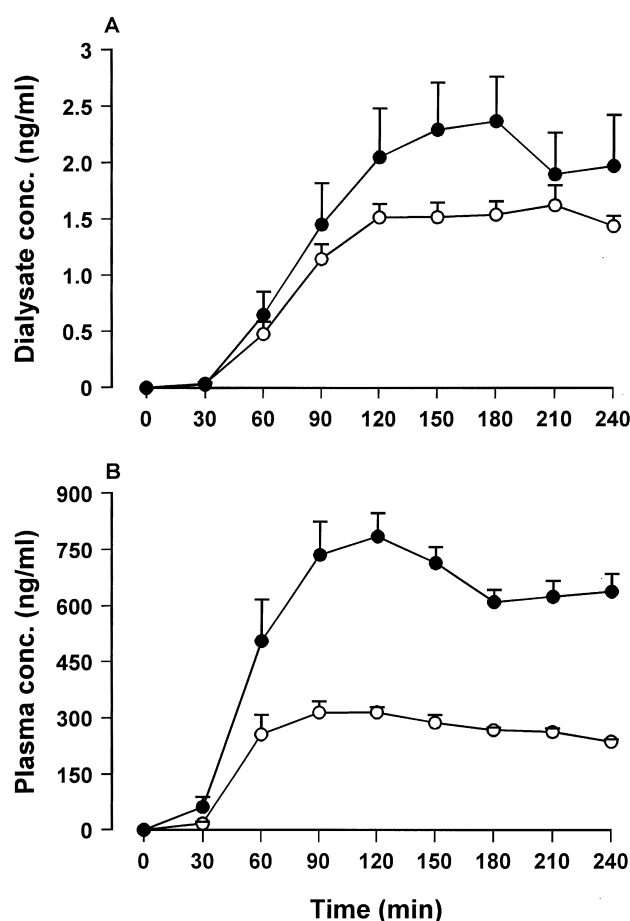


Figure 1. Time-concentration profile of cetirizine in skin dialysate (A) and venous plasma (B) after single oral administration of 10 (○) and 20 mg (●) of cetirizine. Values are mean $\pm$ SEM ($n=10$).

nonsedating antihistamine which is frequently used for such diseases. After oral administration of 10 or 20 mg of cetirizine, peak plasma concentrations are found within 1 h (3, 4). In comparison to its rapid absorption, inhibition of histamine-induced skin reactions reaches its maximum at 4 h (5, 6). Thus, the pharmacokinetic properties of cetirizine in the systemic circulation do not correlate with its pharmacodynamics at skin level.

So far, only very few attempts have been made to measure local drug levels and compare these with pharmacodynamic properties (7). This is mainly due to methodological problems of measuring drug levels chronologically in human skin, especially in intact skin. Microdialysis is a technique that has been used in experimental brain research for decades in studies of neurotransmitter release and pharmacologic experiments (8). Recently, microdialysis techniques have been adapted for used in human skin, and several pharmacokinetic studies have recently been published (9–12). In this study, an intradermal microdialysis technique has been applied to measurement of extracellular

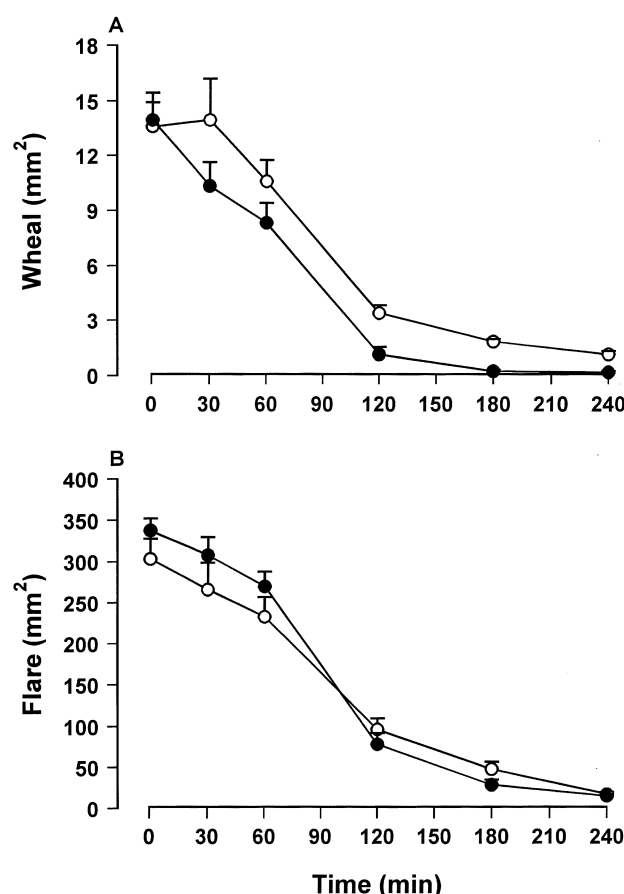


Figure 2. Inhibition of histamine-induced wheal (A) and flare (B) reactions after single oral dose of 10 (○) and 20 mg (●) of cetirizine. Values are mean $\pm$ SEM ($n=10$).

concentrations of cetirizine in the skin of healthy subjects. Levels of cetirizine in the skin were compared to plasma levels, and the drug concentrations were compared to the degree of inhibition of histamine-induced skin reactions.

Material and methods

Subjects

A total of 30 healthy subjects participated in the studies, and 10 of these subjects took part in control experiments described later. The 20 subjects in the trial comprised 16 men and four women aged 29 ± 7 (SD) years. Body weight was 75.8 ± 13.2 kg. Eight subjects were allergic to grass or tree pollens. The study was conducted outside the pollen season, and no patient had taken systemic or topical drugs within the preceding 6 weeks before entering the trial. All subjects gave informed consent, and the study was approved

by the regional ethics committee of Copenhagen and the Danish Board of Health.

Medication

Cetirizine was administered in 10-mg tablets (UCB Pharma SA, Braine l'Alleud, Belgium) in an open trial design. Ten subjects received a single 10-mg tablet, and another 10 subjects received two 10-mg tablets to a total dose of 20 mg of cetirizine.

Microdialysis technique

Microdialysis fibers of 216 μm diameter, 2-kDa molecular mass cutoff, and length of 20 mm available for diffusion, were used, as previously described (13, 14). Six dialysis fibers were inserted intradermally in the right forearm. A recovery phase of 2 h was allowed before sampling of skin dialysate (15). The inlet of each dialysis fiber was connected to individual syringes mounted in microinjection pumps (CMA/100, CMA/Microdialysis, Stockholm, Sweden), each fiber being perfused with Krebs-Ringer bicarbonate (KRB) at a rate of 5 $\mu\text{L}/\text{min}$. Dialysate from six fibers over 30-min periods was pooled into one sample (to a total volume of 900 μL) and rapidly stored at -18°C for further analysis. One sample was collected before administration of the drug, and eight samples were collected for 240 min after administration of the drug.

Blood sampling

A catheter was inserted into the antecubital vein in the left arm for blood samples. After discarding of the first 5 mL of blood, 5 mL of blood was drawn into heparinized vials and centrifuged for 10 min at 800g, and the supernatant was immediately stored at -18°C .

Histamine-induced skin reactions

Skin reactions to histamine prick tests (100 mg/mL) were examined in duplicate before and 0.5, 1, 2, 3, and 4 h after administration of the drug. Skin reactions were evaluated in the left arm. ALK lancets were used. Wheal and flare reactions were transferred onto transparencies 10 min after skin challenge and measured by planimetry.

In vivo recovery of cetirizine

In separate control experiments, *in vivo* relative recovery of cetirizine was determined. None of the subjects in the control experiments had taken any medication, including cetirizine, within the preceding week. Dialysis fibers were inserted intradermally in 10 healthy male volunteers (aged 34 ± 8 years). In these experiments, cetirizine (50 ng/mL) was added to the perfusate. A steady-state perfusion period of 20 min was allowed before sampling of dialysate from three fibers over 40 min, the dialysates being pooled into one sample (total volume 600 μL). Relative recovery in percentage was calculated as the relative loss of cetirizine from the fiber to the skin; i.e., $\text{recovery} = 100 \times (C_i - C_o)/C_i$, where C_i and C_o are the cetirizine concentrations in the inlet (perfusate) and outlet (dialysate), respectively.

Measurement of cetirizine

Cetirizine was assayed by high-pressure liquid chromatography. Chromatographic separation was obtained using a ODS 3 μm , 20 $\times$ 2 mm ID column, a mobile phase of 35% acetonitrile and 65% of 10 mM ammonium acetate, and a flow rate of 0.25 mL/min. The eluent was introduced with a split ratio of 1:15, detection being by mass spectrometry (positive ion, MRM scan mode).

Aliquots of 0.05 mL of plasma were diluted with 0.45 mL of blank human plasma and with 10 mL of 500 ng/mL internal standard cetirizine solution, 1 mL of pH 5 citrate buffer, and 6 mL of chloroform. After vacuum-evaporation, it was reconstituted in 500 μL of water, a sampling volume of 20 μL being analyzed.

Aliquots of 0.5 mL of dialysis fluid were mixed with 10 μL of the internal standard solution and citrate buffer and chloroform. The dry residue was reconstituted in 100 μL of water, and 20 μL was injected onto the HPLC column.

The limit of quantification of cetirizine was 0.1 ng/mL for dialysis fluid and 10 ng/mL for plasma. Within-day reproducibility and accuracy were below 12%.

Statistics

The statistical analyses were performed using the SPSS/PC+ statistical package in an IBM-compatible computer. Values are expressed as geometric means $\pm$ SEM (n =number of observations). Analytic statistics comprised nonparametric Wilcoxon two-sample tests, Friedmann analyses of variance, and Spearman correlation tests.

Results

Time vs concentration profiles of cetirizine in skin dialysate and venous plasma are shown in Fig. 1. Cetirizine concentrations in skin dialysate and plasma samples before administration of the drug were below the analytic sensitivities of 0.1 and 10 ng/ml, respectively. After oral administration of 10 mg of cetirizine, plasma levels increased within 30 min to reach peak values of 315 ± 10 ng/ml at 60–90 min, and it was followed by a slow decline. With a total dose of 20 mg, peak plasma values of 786 ± 45 ng/ml were found at 90–120 min (Fig. 1B). In the skin, dialysate cetirizine levels peaked at 1.6 ± 0.1 and 2.4 ± 0.3 ng/ml at 120–180 min after 10- and 20-mg doses of cetirizine, respectively (Fig. 1A).

Retrodialysis experiments demonstrated *in vivo* recovery of cetirizine to be $14.4 \pm 4.3\%$ ($n=10$).

Fig. 2 shows the time course of inhibition of histamine-induced wheals and flares. Prechallenge wheal and flare reactions were comparable in the two series of experiments. Both 10- and 20-mg doses of cetirizine significantly inhibited wheal and flare reactions over 240 min ($P<0.001$). In comparison to baseline values, significant inhibition of skin reactions were observed at 60 min for both doses of cetirizine. Nearly maximal inhibition of skin reactions was observed with 10- and 20-mg doses at 240 min (wheals, 91–99%; flares, 95–96%).

No significant correlations were found between cetirizine concentrations in skin dialysate or plasma with wheal and flare reactions among individual subjects at any time points with 10 mg of cetirizine. With the 20-mg dose, significant correlations at wheal sizes were demonstrated at 60 and 180 min with cetirizine skin levels and plasma levels of cetirizine at 60 min. No correlations between flare reactions and drug levels were found at any time points. Thus, no consistent statistically significant correlations were found between drug levels and inhibition of skin reactions.

Discussion

In this study, a microdialysis technique was used to measure skin levels of cetirizine after a single oral administration of one or two 10-mg tablets to healthy volunteers. Skin concentrations were compared to plasma concentrations, and drug levels were compared to inhibition of histamine-induced skin reactions.

The concentration of cetirizine in skin dialysate turned out to be very low; skin levels were usually less than 1/100

of plasma concentrations. Therefore, a sensitive high-pressure liquid chromatography method with mass spectrometric detection was adjusted to measure cetirizine levels in the skin. The concentrations of cetirizine in skin dialysate and plasma are not readily comparable, and the significant differences in their concentrations can be explained by two factors. First, the dialysis membrane had a 2-kDa molecular mass cutoff, thus allowing only non-protein-bound cetirizine to diffuse across the membrane. In comparison, plasma concentrations were determined as total plasma cetirizine; i.e., protein-bound and nonbound concentrations. Since cetirizine is a highly protein-bound drug, approximately 95% of the total concentration is bound to plasma proteins (3, 4), and the free unbound peak plasma concentration of this drug was estimated to be 15 and 35 ng/ml at 10- and 20-mg doses. The exact degree of protein-binding of cetirizine was not determined in this study. Second, due to diffusion resistances in the dialysis fiber and the tissue, dialysate concentrations of cetirizine were lower than extracellular unbound skin cetirizine concentrations. The exact *in vivo* recovery of cetirizine was measured in another group of subjects; it was approximately 15%. This indicated peak skin concentrations of cetirizine to be approximately 11 and 17 ng/ml at 10- and 20-mg doses, respectively. Thus, over the 4-h sampling period, it could be estimated that peak unbound cetirizine concentrations in the skin were approximately 50–70% of corresponding values in venous plasma. Previously, it has been shown that total skin cetirizine concentrations were about 20% and 50% of plasma concentrations at 1 and 3 h after a single 10-mg dose (7). For reasons discussed above, direct comparison of drug concentrations in microdialysis experiments and skin biopsies are difficult, but the data suggest that both total and nonbound cetirizine is lower than plasma concentrations within the first 3–4 h after administration. At steady state, identical values have been found in skin and plasma (7).

Plasma peak concentrations were observed approximately 90–120 min after oral administration of the drug, whereas skin peak values were seen at 150–180 min. This difference may reflect the time for diffusion of cetirizine from the systemic circulation into the extracellular water phase. Similar findings were observed by Simons et al. using sequential skin biopsies (7).

Direct comparisons of drug concentrations in the skin and plasma with histamine-induced wheal and flare reactions were performed. No consistent significant correlations were found between cetirizine concentrations in skin or plasma and wheal and flare reactions among individuals. This is in

accordance with previous findings (7). The almost complete inhibition of skin responses made correlation with drug levels in skin and plasma difficult. Probably both the 2.5- and 5-mg doses of cetirizine were needed to make satisfying PK/PD relations. However, Fig. 2 shows that $T_{1/2\max}$ for inhibition of wheal and flare reactions (approximately 80–90 min) was comparable with that of dialysate cetirizine $T_{1/2\max}$ of 80–90 min (Fig. 1A), not with the figure of 45–50 min for plasma cetirizine (Fig. 1B). Thus, the averaged data indicated that the time-concentration profile of cetirizine in the skin seemed to parallel the inhibition of skin reactions better than plasma concentrations.

In conclusion, skin microdialysis was applied to compare simultaneously the pharmacokinetic and pharmacodynamic profiles of cetirizine, a highly protein-bound antihistamine. The data confirmed a temporal discrepancy between plasma cetirizine levels and inhibition of skin responses. In the comparison, a relationship between skin

drug levels and inhibition of skin reactions was suggested. Additional studies are needed to determine more exact PK/PD relations, including measurements at steady-state conditions. In this aspect, the microdialysis method may prove useful; e.g., by local delivery of drugs to the skin via dialysis tubes with subsequent infusion of histamine. Furthermore, assessment of the skin drug concentrations needed to inhibit the release of inflammatory mediators can be evaluated by this approach.

Acknowledgments We thank Ms Inge Aakilde for skillful technical assistance. The cetirizine analyses were performed by the Bioanalysis Laboratory of Bio-Pharma, Wavre, Belgium. Statistical analyses were made by the Data Investigation Company Europe, Brussels, Belgium. The work was supported by UCB Pharma, Braine l'Alleud, Belgium. Dr Petersen was supported by a Weimann Senior Research Fellowship.

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