

At age 38 years, a trial of aspirin and prednisone decreased the severity of the anaphylactic episodes. However, the development of an aspirin allergy associated with anaphylaxis as well as the typical adverse effects associated with chronic use of corticosteroids required discontinuation of that regimen. From age 39 to 41 years, he reported episodes of anaphylaxis at approximately 90-day intervals, although not all required epinephrine therapy. At age 48 years, he had another severe episode of anaphylaxis requiring an intensive care unit admission. Since age 48 years, he has had 15 to 16 episodes of anaphylaxis per year and during these episodes has associated wheezing, vomiting, and hives, but no angioedema. For each episode he self-administered epinephrine and activated the emergency medical system.

He has a history of mild intermittent asthma, allergic rhinitis with seasonal symptoms diagnosed by CAP-RAST to aeroallergens, penicillin allergy implicated by history, and aspirin sensitivity with associated symptoms of anaphylaxis.

His medications at the time of initiation of omalizumab therapy included ranitidine, fluticasone-nasal, cromolyn sodium, montelukast, diphenhydramine, albuterol metered dose inhalant, hydroxyzine, esomeprazole, and bupropion. Before omalizumab therapy, his serum IgE level was 104 IU; his serum tryptase was 54.9 ng/mL.

Omalizumab therapy was initiated at 300 mg every 4 weeks. The patient has not experienced any episodes of anaphylaxis requiring epinephrine in 5 months since starting therapy (Fig 1). The patient has had 1 episode of diarrhea lasting 2 hours that did not necessitate a visit to the emergency department or activation of the emergency medical system. The drug has been well tolerated with the exception of local swelling and erythema at the injection site. He has discontinued cromolyn sodium and montelukast since starting omalizumab.

This is the first observation of the potential efficacy of omalizumab in the treatment of patients with mastocytosis and unprovoked anaphylaxis. These episodes of anaphylaxis were not associated with food, drugs, exercise, insect stings, or any other identifiable allergen. Both patients had symptoms consistent with anaphylaxis in the emergency department manifested by hypotension, flushing, and diarrhea that required epinephrine, hemodynamic support, occasional hospitalization, and documented elevated serum tryptase levels above baseline with values of >200 ng/mL and 186 ng/mL in patients 1 and 2, respectively. Angioedema was not observed in either patient.

The ability of omalizumab to decrease the frequency of episodes of hypotension in these 2 patients within a short time did not appear to rely on the ability of omalizumab to decrease mast cell numbers, because the serum tryptase levels in these patients did not change during the period of response. Further, because mast cells live for months within tissues, an effect of omalizumab on the turnover rate of mast cells is unlikely. However, serum free IgE as a result of omalizumab therapy has been shown to significantly decrease after 1 dose,² and downregulation of FcεRI on the surface of basophils has been documented

at day 7.⁴ Moreover, in the latter study, there was no change in mast cell numbers from skin biopsy specimens with a documented decrease in FcεRI on the surface of mast cells from the same biopsy specimen between day 7 and day 70. We thus hypothesize, but have not proven, that this downregulation of receptors is accompanied by an increase in the threshold above which degranulation is triggered. The mechanism by which omalizumab decreases episodes of mast cell–dependent hypotension in these patients with mastocytosis thus remains unknown but may relate more to a decrease in the activation state of mast cells concurrent with a decrease in surface IgE and associated decreases in receptor (FcεRI) number.

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Editor's note: Readers interested in this topic may also be interested in a forthcoming Letter to the Editor in which Siebenhaar and colleagues present a case report of "Successful treatment of cutaneous mastocytosis with anti-IgE." Look for this article in the July 2007 issue of the JACI, or ahead of print publication in the Articles in Press section of the JACI Web site, www.jacionline.org.

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Relevance of H₁-receptor occupancy to H₁-antihistamine dosing in children

To the Editor:

The H₁-histamine receptor is, like all known types of histamine receptors, a heptahelical transmembrane molecule that transduces extracellular signals to intracellular second messenger systems by way of G proteins. Like

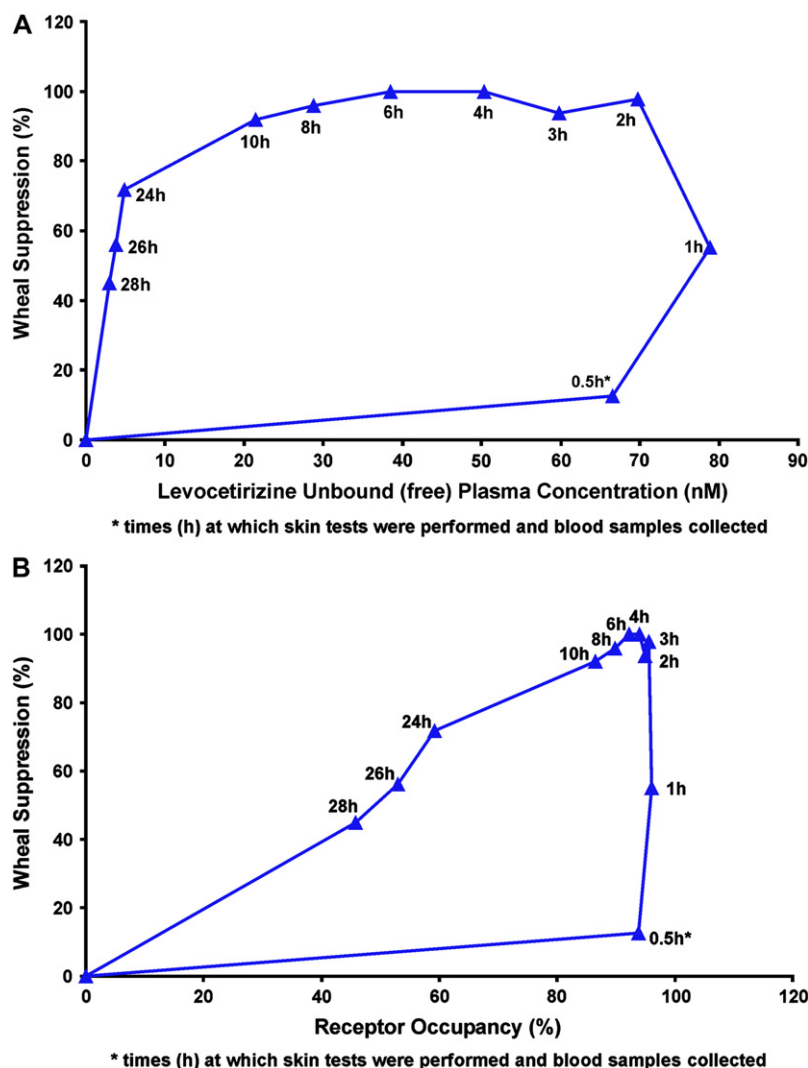


FIG 1. A, Suppression of histamine-induced wheal versus levocetirizine unbound (free) plasma concentrations. B, Suppression of histamine-induced wheal versus levocetirizine H₁-receptor occupancy.

other histamine receptors, it has constitutive activity, which is defined as the ability to trigger downstream events in acute and chronic allergic inflammation, even in the absence of ligand binding. The inactive and active states of the H₁-receptors exist in equilibrium. H₁-antihistamines act as inverse agonists that combine with the H₁-receptor, stabilizing it in the inactive form and shifting the equilibrium toward the inactive state.^{1,2}

For most of the second-generation, relatively non-sedating H₁-antihistamines, despite faster clearance rates and shorter terminal elimination half-life values reported in the pediatric population, the dose interval recommended for children is identical to the dose interval recommended for adults.³⁻⁵ These recommendations are based on traditional pharmacodynamic studies involving measurement of H₁-antihistamine-induced suppression of the histamine-induced wheal-and-flare response over time. A novel approach is to calculate H₁-antihistamine occupancies at H₁-receptor sites by using receptor binding

constants and unbound (free) H₁-antihistamine plasma concentrations (ie, the fraction of medication not bound to plasma proteins) and to correlate these values with the pharmacodynamic response.⁶⁻⁸

Using this approach, we studied levocetirizine, the active enantiomer of the racemic piperazine H₁-antihistamine cetirizine, which is widely used in Europe in children and adults with allergic rhinitis or urticaria and is currently an investigational drug in North America. We hypothesized that levocetirizine H₁-receptor occupancy in children 6 to 11 years of age after a 5-mg oral dose would be similar to levocetirizine H₁-receptor occupancy in adults after this dose. Our objective was to calculate H₁-receptor occupancy by levocetirizine and use these data to predict the onset and duration of suppression of the histamine-induced wheal-and-flare response after the administration of a 5-mg levocetirizine dose in children.

The pharmacokinetics of levocetirizine in children 6 to 11 years of age have been previously described after

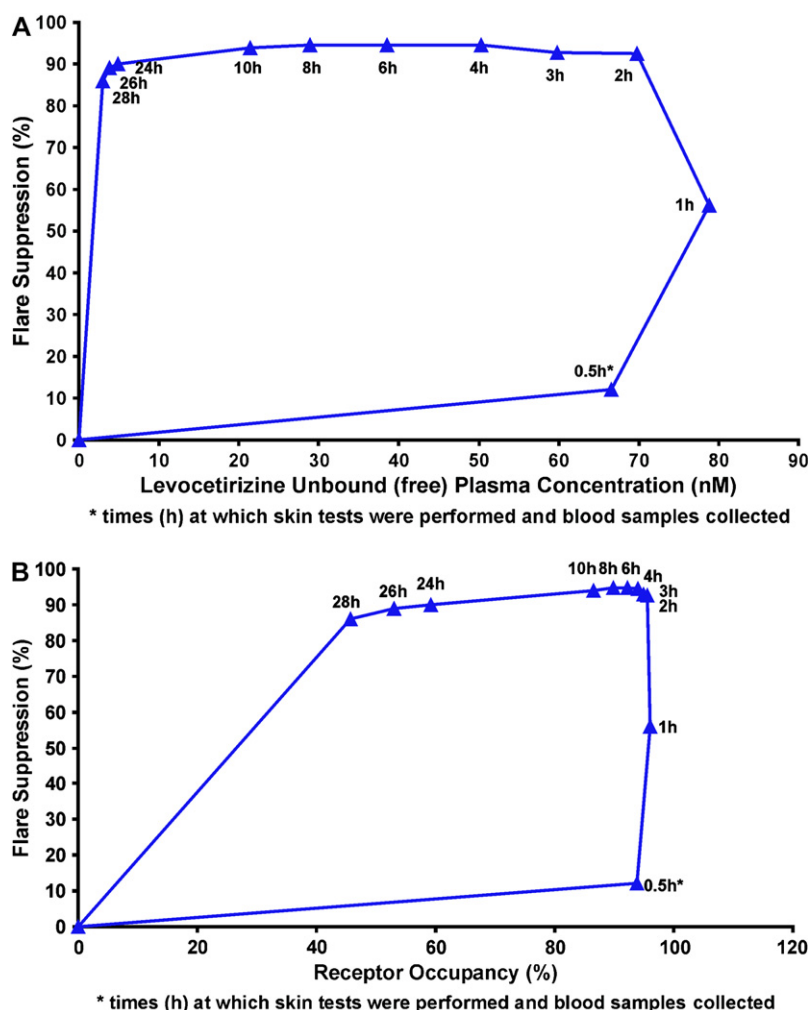


FIG 2. A, Suppression of histamine-induced flare versus levocetirizine unbound (free) plasma concentrations. B, Suppression of histamine-induced flare versus levocetirizine H₁-receptor occupancy.

administration of a single 5-mg dose.⁴ Peak plasma concentrations of 450 ± 37 ng/mL ($\pm$ SEM) occur at 1.2 ± 0.2 hours. The oral clearance is 0.82 ± 0.05 mL $\cdot$ kg $\cdot$ min⁻¹, and the apparent volume of distribution, 0.4 ± 0.02 L/kg, is small. The terminal elimination half-life is 5.7 ± 0.2 hours, and the mean residence time is 6.8 ± 0.3 hours. Based on these pharmacokinetic parameters, and considering that in adults oral levocetirizine clearance is slower (0.62 ± 0.002 mL $\cdot$ kg $\cdot$ min⁻¹ [$\pm$ SEM]) and the terminal elimination half-life is longer (7.8 ± 0.3 hours),⁵ twice-daily dosing might appear to be optimal for children in this age range.

It has been found in a pediatric pharmacodynamic study, however, that after a 5-mg dose of levocetirizine, histamine-induced wheal-and-flare responses were suppressed ($P < .05$) from 1 to 28 hours, with maximal suppression of wheals ($97\% \pm 1\%$) from 2 to 10 hours and maximal suppression of flares ($93\% \pm 1\%$) from 2 to 24 hours.⁴

Estimating the *in vivo* receptor occupancy might provide additional data to confirm the observed pharmacodynamic response and to establish this response as a better

predictor of optimal H₁-antihistamine pediatric dose regimens than are clearance rates and terminal elimination half-life values. Therefore by using the unbound (free) levocetirizine plasma concentrations and *in vitro* affinity data, medication concentrations at receptor sites were calculated, and the kinetics of wheal-and-flare suppression were compared with the kinetics of receptor occupancy.⁷

The percentage of the H₁-histamine receptors occupied by levocetirizine was calculated at each time after dosing by using the following equation^{6,7}:

$$RO (\%) = 100\% \times ([L]/([L] + K_i)) \times (1 - e^{-(k_{on} \times [L] + k_{off}) \times t}),$$

where RO is defined as receptor occupancy, [L] is defined as unbound (free) concentration of levocetirizine, K_i is defined as affinity of levocetirizine for the H₁-histamine receptor, and k_{on} and k_{off} are the association and dissociation constants for levocetirizine. The values used in calculations were as follows: levocetirizine unbound (free)

fraction = 0.0879; kinetic $k_{on} = 2.3 \text{ min}^{-1} \times 1 \times 10^{-6} \text{ mol}^{-1}$; $k_{off} = 0.005 \text{ min}^{-1}$; and $k_{off}/k_{on} = K_i = 3 \text{ nmol/L}$ (equilibrium $K_d = 3 \text{ nmol/L}$).

After a 5-mg dose, unbound (free) levocetirizine concentrations were $50 \pm 4 \text{ nmol/L}$ at 4 hours and $5 \pm 1 \text{ nmol/L}$ at 24 hours compared with 28 nmol/L at 4 hours and 4 nmol/L at 24 hours in adults. Levocetirizine H_1 -receptor occupancies at 4 and 24 hours were $94\% \pm 0.4\%$ and $60\% \pm 3\%$ compared with values of 90% and 57%, respectively, in adults.

Percentage of wheal suppression versus levocetirizine unbound (free) plasma concentrations compared with percentage of wheal suppression versus levocetirizine H_1 -receptor occupancy is shown in Fig 1. Percentage of flare suppression versus levocetirizine unbound (free) plasma concentrations compared with percentage of flare suppression versus levocetirizine H_1 -receptor occupancy is shown in Fig 2. When the percentage of suppression of wheal-and-flare responses is plotted versus the percentage of H_1 -receptor occupancy by levocetirizine, the counterclockwise loop of hysteresis is reduced, corroborating the hypothesis that the kinetics of receptor occupancy are a better representative of the time course of pharmacodynamics than are the plasma pharmacokinetics.

The H_1 -receptor occupancy data therefore provide additional pharmacodynamic support for the appropriateness of once-daily levocetirizine dosing in children age 6 to 11 years,⁴ as is recommended for adults,⁸ despite faster clearance and shorter terminal elimination half-life values in the pediatric age group.

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Identification of 2 new sesame seed allergens: Ses i 6 and Ses i 7

To the Editor:

Sesame seed allergy is a common cause of food allergy in many countries, including Israel, Japan, the United States, and various European countries.¹ Seed storage proteins have been identified as common allergens in peanut, tree nut, and seed allergy.² Recently, several seed storage and oil body proteins in sesame seed have been identified as allergens by us and others, namely two 2S albumins (Ses i 1 and Ses i 2),^{3,4} a 7S globulin (Ses i 3),³ and 2 oleosins (Ses i 4 and Ses i 5).⁵ The purpose of this study was to determine whether 11S globulins in sesame seed, a third seed storage protein family, also have allergenic potential.

A cDNA library was constructed from green, unripe sesame seeds (*Sesamum indicum*). Total RNA was extracted and mRNA was purified on Oligotex mRNA Spin-column from Qiagen (Valencia, Calif). Synthesis and cloning of cDNA was performed by using a cDNA synthesis kit from Stratagene (La Jolla, Calif). The double-stranded cDNA was ligated into the lambda vector Uni-ZAP, packaged *in vitro* (Gigapak Gold; Stratagene), and then transfected into *Escherichia coli* XL1-Blue cells.

By using known sequence data from two 11S globulins (accession nos. AF091842 and AF240004), oligonucleotide primers were synthesized and used to screen the library. Full-length cDNA clones were isolated and sequenced at the Mount Sinai Core Facility. The sequenced clones were 100% identical to the two 11S globulins described above. These 11S globulins have only 36% identity (Fig 1).

The protein-coding regions of the 11S globulins were amplified by means of PCR with the specific primers, which allowed the introduction of unique sites suitable for ligation into the expression pET24b(+) (Novagen, Madison, Wis) vector. The ligated insert-vector constructs were introduced into the *E coli* XL1-Blue strain by means of chemical transformation, according to a protocol provided by the cell manufacturer (Stratagene). The plasmids were purified, and the inserts' orientation and nucleotide sequences were confirmed. Subsequently, each individual expression plasmid was introduced into the BL21 expression *E coli* strain. The recombinant protein was expressed on induction of the bacterial culture at 0.4 to 1.0 OD at 600 nm with 1 mM of isopropyl β -D-1-thiogalactopyranoside for 16 hours at 30°C. The recombinant protein expression