Clinical and immunologic effects of H1 antihistamine preventive medication during honeybee venom immunotherapy

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Background: H1 antihistamines increase safety during allergen-specific immunotherapy and might influence the outcome because of immunoregulatory effects.
Objective: We sought to analyze the influence of 5 mg of levocetirizine (LC) on the safety, efficacy, and immunologic effects of ultrarush honeybee venom immunotherapy (BVIT).
Method: In a double-blind, placebo-controlled study 54 patients with honeybee venom allergy received LC or placebo from 2 days before BVIT to day 21. Side effects during dose increase and systemic allergic reactions (SARs) to a sting challenge after 120 days were analyzed. Allergen-specific immune response was investigated in skin, serum, and allergen-stimulated T-cell cultures.
Results: Side effects were significantly more frequent in patients receiving placebo. Four patients receiving placebo dropped out because of side effects. SARs to the sting challenge occurred in 8 patients (6 in the LC group and 2 in the placebo group). Seven SARs were only cutaneous, and 1 in the placebo group was also respiratory. Difference of SARs caused by the sting challenge was insignificant. Specific IgG levels increased significantly in both groups. Major allergen phospholipase A_2-stimulated T cells from both groups showed a slightly decreased proliferation. The decrease in IFN-γ and IL-13 levels with placebo was not prominent with LC, whereas IL-10 levels showed a significant increase in the LC group only. Decreased histamine receptor (HR)1/HR2 ratio in allergen-specific T cells on day 21 in the placebo group was prevented by LC.
Conclusions: LC reduces side effects during dose increase without influencing the efficacy of BVIT. LC modulates the natural course of allergen-specific immune response and affects the expression of HRs and cytokine production by allergen-specific T cells. (J Allergy Clin Immunol 2008;122:1001-7.)

Key words: *Venom immunotherapy, antihistamine preventive medication, T cells, cytokines, histamine receptors*

Hymenoptera venom allergy is a major cause for severe and potentially fatal anaphylaxis.[1] Immunotherapy with hymenoptera venoms was shown to be highly effective.[2] However, in patients with honeybee venom (BV) allergy, it might cause systemic allergic side effects in up to 20% to 40%, mainly during the dose-increase phase. For this reason, preventive medication with antihistamines is often used during the initial phase of honeybee venom immunotherapy (BVIT) and was shown to significantly reduce large local and generalized cutaneous reactions in several double-blind, placebo-controlled trials.[3-5] Preventive medication with antihistamines was also effective in reducing side effects from immunotherapy with tree and grass pollen.[6,7] The antihistamines used were terfenadine, loratadine, cetirizine, and fexofenadine. Thus reduction of side effects seems to be a histamine receptor (HR) 1–mediated class effect of antihistamines.

The mechanism by which immunotherapy induces protection is associated with changes in the fine balance between allergen-specific regulatory T cells and T_H2 cells, T_H1 cells, or both.[8] Histamine, originally considered a mediator of acute inflammatory and immediate hypersensitivity responses, has also been demonstrated to regulate antigen-specific T_H1, T_H2, and regulatory T cells, as well as related antibody isotype responses. Histamine enhances T_H1-type responses by triggering HR1, whereas both T_H1- and T_H2-type responses are negatively regulated by HR2.

There is some evidence that the expression of HRs is altered during immunotherapy.[9-11] The question of whether preventive medication with H1 antihistamines during allergen immunotherapy could influence the immune response to this treatment for better or worse arose. Previously, the long-term efficacy of BVIT, as indicated by the reaction to a field sting or a sting challenge, has only been analyzed retrospectively in the 52 patients of the first double-blind, placebo-controlled trial on H1 antihistamine preventive medication during allergen immunotherapy.[12] The results of this retrospective study suggested an increased efficacy in patients with antihistamine preventive medication

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Abbreviations used
BV: Honeybee venom
BVIT: Honeybee venom immunotherapy
HR: Histamine receptor
LC: Levocetirizine
PLA: Phospholipase A_2, a major allergen of honeybee venom
SAR: Systemic allergic reaction
SE: Side effect
STEPC: Skin test end point concentration
TT: Tetanus toxoid

during early BVIT compared with efficacy in those without. We therefore performed a prospective, double-blind, placebo-controlled trial, which is presented here. This study includes a sting challenge after 4 months of BVIT, as well as extensive investigation of allergen-specific skin tests and antibody and T-cell response, including proliferation, cytokine secretion, and HR expression.

METHODS

Study protocol

Fifty-four adult patients aged 18 to 65 years with a history of moderate-to-severe systemic allergic reactions (SARs) to honeybee stings grade II to IV,[13,14] positive intracutaneous skin tests to BV of less than or equal to 10^{-4} g/L, and BV-specific serum IgE (sIgE) levels of 0.7 kU/L or greater in the Immuno-CAP FEIA were included in the study. Exclusion criteria were pregnancy, breast-feeding, severe systemic and psychiatric disease, intake of β-blockers, angiotensin-converting enzyme inhibitors, and treatment with antihistamines within a week and systemic corticosteroids within a month before the start of the study.

Twenty-seven patients each were randomly assigned to preventive medication with 1 tablet daily of either 5 mg of levocetirizine (LC) or placebo from day −2 to day 21 of an ultrarush BVIT protocol.[15,16] During the ultrarush protocol on day 0, patients received 6 injections of BV starting at 0.1 μg, with a top dose of 50 μg and a cumulative dose of 111.1 μg. During the dose-increase (ultrarush) phase of BVIT, patients were monitored for blood pressure, pulse, electrocardiography, and peak flow in the intensive care unit, and a venous access with infusion of sodium chloride 0.9% was established before the first injection. On day 7, they received 2 injections of 50 μg, and on day 21, they received 1 injection of 100 μg. After the dose-increase phase, further injections of the maintenance dose of 100 μg of BV were administered on days 50, 80, and 110. Skin tests with BV, BV sIgE, and BV sIgG were repeated on day 110, and whole blood for PBMC cultures was taken on days −3 to −7, 21, and 110.

Primary end points were as follows: (1) occurrence of SARs and need for rescue medication after BVIT injections during the preventive treatment phase on days 0 to 21 and (2) occurrence of SARs and need for rescue medication after the sting challenge on day 120.

Secondary end points were as follows: (1) intracutaneous skin tests, (2) BV sIgE and sIgG serum antibodies, (3) phospholipase A_2 (PLA)–specific T-cell proliferation, (4) cytokine secretion in PLA-specific T-cell cultures, and (5) HR1 and HR2 receptor expression in PLA-specific T cells from before to day 110 of BVIT.

Written informed consent was obtained from each patient. The study protocol was approved by the Ethical Committee of the Canton of Bern, Switzerland.

Assessment of allergic reactions

SARs after BVIT injections or after the challenge were classified as purely subjective, such as itch, heat sensation, headache, and dizziness (grade 1); cutaneous, such as flush, urticaria, erythema, and angioedema (grade 2); gastrointestinal, such as abdominal cramps, vomiting, and diarrhea (grade 3); respiratory, such as dyspnea, wheezing, and decrease in peak flow of greater than 10% (grade 4); and cardiovascular, such as tachycardia, arrhythmia, decrease in blood pressure of greater than 20 mm Hg, collapse, and unconsciousness (grade 5).

Rescue medications were 0.3 mg of epinephrine administered subcutaneously for grade 2 and grade 3 reactions and 0.3 mg of epinephrine administered subcutaneously or intramuscularly, 2 mg of clemastine, and 125 mg of methylprednisolone administered intravenously for grade 4 reactions. Additional volume substitution and epinephrine administered by means of infusion were recommended for grade 5 reactions.

Sting challenge

The sting challenge with a live honeybee was performed on day 120 in the intensive care unit, with constant monitoring of pulse, blood pressure, electrocardiography, and repeated peak flow measurements before and after the challenge.[17] Intravenous access with an infusion of sodium chloride 0.9% was established before the challenge and remained for 2 hours. The sting was applied on the volar side of the forearm, and the stinger was left in the skin for 1 minute. Honeybees were kindly provided by the Swiss Institute for Agricultural Research in Bern.

Skin tests, BV sIgE, and BV sIgG serum antibodies

Lyophilized BV (Pharmalgen) for skin tests and venom immunotherapy was obtained from ALK-Abelló (Hørsholm, Denmark). Skin test end point concentration (STEPC) was determined by means of intracutaneous injection of 0.02 mL of serial dilutions of BV at 10^{-8}, 10^{-6}, and 10^{-4} g/L, as described earlier.[17] The lowest concentration resulting in a wheal reaction of 5 mm or greater in diameter with erythema is defined as STEPC. BV sIgE, sIgG, and tryptase levels were determined by means of Immuno CAP FEIA (Phadia, Uppsala Sweden).

Immunologic analyses

Material. Recombinant PLA (Api m 1) of BV *(Apis mellifera)* was used. Purified protein derivative of *Mycobacterium bovis* and tetanus toxoid (TT) were used as control antigens. None of the allergens contained detectable amounts of LPS, and all were more than 99% pure.

T-cell proliferation and cytokine detection. Allergen-specific T-cell proliferative response was determined by means of stimulation of 2×10^5 PBMCs for 5 days with 0.3 μmol/L PLA, 1 μg/mL TT, and purified protein derivative of *Mycobacterium bovis* in 200 μL of medium in 96-well flat-bottom tissue-culture plates in triplicate in RPMI 1640 medium supplemented as previously described.[18] Antigen-specific responding T cells were expanded until day 12 and restimulated with anti-CD2/CD3/CD28 mAbs for RNA expression. Solid-phase sandwich ELISAs for IFN-γ, IL-10, and IL-13 were performed in supernatants obtained after 5 days.[19]

Quantitative real-time PCR. T cells were lysed with RNeasy lysis buffer, and the RNA was isolated with the RNeasy mini kit (Qiagen, Hamburg, Germany) and eluted in 30 μL of double-distilled H_2O. Reverse transcription was performed with TaqMan reverse transcription reagents with random hexamers (Applied Biosystems, Rotkreuz, Switzerland). The PCR primers and probes were designed based on sequences reported in GenBank. Primers were as follows: EF-1α forward primer 5′CTG AAC CAT CCA GGC CAA AT 3′, EF-1α reverse primer 5′GCC GTG TGG CAA TCC AAT 3′, HR1 forward primer 5′-TCT CGA ACG GAC TCA GAT ACC A-3′, HR1 reverse primer 5′-CCT GTG TTA GAC CCA CTC CTC AA-3′, HR1 probe FAM-ACA GAG ACA GCA CCA GGC AAA GGC AA-TAMRA; HR2 forward primer 5′-GCT GGG CTA TGC CAA CTC A-3′, HR2 reverse primer 5′-GGT GCG GAA GTC TCT GTT CAG-3′, and HR2 probe FAM-CCC TGA ACC CCA TCC TGT ATG CTG C-TAMRA (all were from Microsynth AG, Balgach, Switzerland). cDNAs were amplified with SYBR-PCR Mastermix (Applied Biosystems) according to the recommendations of the manufacturer in a total volume of 25 μL in an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Relative quantification was performed as previously described.[20] All amplifications were carried out in duplicates.