Sublingual immunotherapy for hazelnut food allergy: A randomized, double-blind, placebo-controlled study with a standardized hazelnut extract

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Background: Food allergy may be life-threatening, and patients affected need to receive accurate diagnoses and treatment. Hazelnut has often been implicated as responsible for allergic reactions, and trace quantities can induce systemic reactions.
Objective: The aim of this study was to evaluate the efficacy and tolerance of sublingual immunotherapy with a standardized hazelnut extract in patients allergic to hazelnut.
Methods: This was a randomized, double-blind, placebo-controlled study. Inclusion criteria were a history of hazelnut allergy and positive skin prick test and double-blind placebo-controlled food challenge results. Patients were then randomly assigned into 2 treatment groups (hazelnut immunotherapy or placebo). Efficacy was assessed by double-blind, placebo-controlled food challenge after 8 to 12 weeks of treatment. Blood samples were drawn for measurement of specific IgE, IgG_4, and serum cytokines before and after treatment.
Results: Twenty-three patients were enrolled and divided into 2 treatment groups. Twenty-two patients reached the planned maximum dose at 4 days. Systemic reactions were observed in only 0.2% of the total doses administered. Mean hazelnut quantity provoking objective symptoms increased from 2.29 g to 11.56 g ($P = .02$; active group) versus 3.49 g to 4.14 g (placebo; NS). Moreover, almost 50% of patients who underwent active treatment reached the highest dose (20 g), but only 9% in the placebo. Laboratory data showed an increase in IgG_4 and IL-10 levels after immunotherapy in only the active group.
Conclusion: Our data confirm significant increases in tolerance to hazelnut after sublingual immunotherapy as assessed by double-blind, placebo-controlled food challenge, and good tolerance to this treatment. (J Allergy Clin Immunol 2005;116:1073-9.)

Key words: *Sublingual immunotherapy, food immunotherapy, hazelnut allergy, biological standardization in mass units, double-blind, placebo-controlled food challenge, Cor a 1, Cor a 8*

Food allergy, like other atopic disorders, appears to be on the increase. Moreover, food allergy remains a leading cause of anaphylaxis treated in emergency departments in several countries, and the general public has become increasingly aware of the problem.[1]

Peanut and tree nuts are some of the most allergenic foods worldwide.[2] In the United States, peanut and tree nut allergy affects approximately 1.1% of the population.[3] In the tree nut group, hazelnut (*Corylus avellana*) is frequently implicated in allergic reactions.[4] In a recent report, Pastorello et al[5] analyzed the allergen profile of patients with hazelnut allergic reactions and positive double-blind, placebo-controlled food challenge (DBPCFC). All sera from patients recognized an 18-kd allergen; other major allergens recognized were at molecular weights of 47, 32, and 35 kd. The 18-kd allergen, Cor a 1, is a protein homologous to Bet v 1 allergen. Patients with severe allergic reactions to hazelnut showed IgE reactivity to a 9-kd allergen.[5] This allergen was shown to be a hazelnut lipid transfer protein, registered as Cor a 8, and it is a major allergen for Spanish patients with allergy to hazelnut without birch pollen allergy.[6]

In Europe, it has been estimated that hazelnut allergy affects between 0.1% and 0.5% of the population.[7,8] Hazelnut is largely used in prepackaged foods, particularly in the production of pastry and ice creams. The wide use of hazelnut in the food industry represents a considerable risk for subjects with hazelnut allergy, because trace quantities can induce systemic allergic reactions in highly sensitized individuals. Moreover, some food labels may not list a small quantity of a foodstuff, or this may be included as a contaminant, with accidental ingestion

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Abbreviations used
AU: Allergen unit
DBPCFC: Double-blind, placebo-controlled food challenge
n: Native
r: Recombinant
SLIT: Sublingual immunotherapy
SPT: Skin prick test

possible despite efforts at avoidance in unexpected food products.[9]

The management of food allergies continues to consist of educating patients on how to avoid relevant allergens, recognize early symptoms of an allergic reaction in cases of accidental ingestion, and initiate the appropriate emergency therapy. Nevertheless, because of the high risk of life-threatening allergic reactions and the difficulty in avoidance of the culprit food, allergen-specific immunotherapy has been studied as a treatment option. However, subcutaneous immunotherapy was accompanied by a high rate of systemic reactions.[10,11]

The aim of this study was to evaluate the response to sublingual immunotherapy (SLIT) with hazelnut extract standardized in unit masses of major allergens, Cor a 1 and Cor a 8, in a large group of patients allergic to hazelnut. Efficacy was assessed by double-blind, placebo-controlled food challenges with hazelnut before SLIT and after 2 months of immunotherapy maintenance.

METHODS

Patients and study design

This was a randomized, double-blind, placebo-controlled, multicenter study. Patients provided written informed consent. The study was approved by the ethics committees of the participating hospitals. Potential subjects were preselected on the basis of a clear history of hazelnut food allergy and positive skin prick test to hazelnut. Definitive inclusion criteria then also included a positive DBPCFC with hazelnuts. Eligible patients could not be pregnant, and any asthma had to be under control. Systemic corticosteroids and β-blockers were prohibited before screening and throughout the study, and antihistamines and antidepressants were prohibited for 1 week and 2 weeks, respectively, before skin testing and oral food challenge. Patients with systemic diseases and/or oral infection or inflammation not compatible with the correct, easy, and safe administration of the treatment were excluded from the study.

Before the onset of treatment, a titrated skin prick test (SPT) with hazelnut extract was performed, and blood samples were drawn for *in vitro* standardization of a hazelnut extract in mass units of the hazelnut major allergens Cor a 1 and Cor a 8.[5,6]

Patients were later randomly assigned to 2 treatment groups (hazelnut extract or placebo). They then underwent a final DBPCFC 8 to 12 weeks later. At that time, a titrated SPT with hazelnut extract was repeated, and blood samples were again drawn for *in vitro* studies.

SPT

All patients underwent SPT performed according to standard procedure with timothy, parietaria, mugwort, olive, plane tree, birch, and hazel extracts (Diater Laboratorios, Madrid, Spain). A wheal size equal to or larger than the wheal obtained with histamine 10 mg/mL 15 minutes after testing was judged as positive.

Each patient was also skin prick tested in duplicate on the volar surface of the forearm with four 10-fold serial dilutions (10, 1, 0.1, and 0.01 mg/mL) of a raw hazelnut extract (Diater Laboratorios). Wheal areas were marked with a fine-tipped ball-point pen and transferred by transparent adhesive tape onto paper for subsequent planimetric evaluation and statistical analysis. Skin wheal areas were determined by computer scanning (AutoCAD; Autodesk, Inc, San Rafael, Calif) in all patients.

DBPCFC

Double-blind, placebo-controlled food challenges to hazelnut were performed in all patients. A single common protocol, based on a previous European multicenter study, was adhered to in the 3 centers.[4] Patients who had systemic reactions or anaphylaxis were also challenged, but they were started with a modified schedule proposed by Alonso et al,[12] after which they continued with the normal protocol, always within the double-blind, placebo-controlled schedule.

Allergen sources and extracts

Raw hazelnuts were used for oral challenges and SPT and as source material for production of allergen extracts. The extract was prepared as follows: nuts were defatted with diethyl ether in Soxhlet system and proteins extracted with PBS with 4% Tween 20 at a 50% (wt/vol) ratio by stirring for 1 hour at 4°C. The soluble fraction was separated by centrifugation at 22,000*g* for 20 minutes at 4°C. The hazelnut extract was then dialyzed against distilled water, filtered, and lyophilized.

The hazelnut extract was used for SPT, IgE, and IgG_4 measurements. Recombinant hazelnut allergens recombinant (r) Cor a 1 (Biomay, Vienna) and rCor a 8 (Paul Ehrlich Institut, Langen, Germany) were used to obtain rabbit polyclonal antibodies α-rCor a 1 and α-rCor a 8, and biotinylated antibodies against native (n) Cor a 1 and nCor a 8 by affinity purification.[13,14] Protein concentration was determined by the Bradford[15] method.

Specific IgE

Specific IgE against hazelnut was measured by an enzyme allergosorbent test (Hycor Biomedical Inc, Garden Grove, Calif) according to the manufacturer's instructions. Levels higher than 0.35 IU/mL were considered positive (Hytec-specific IgE enzyme immunoassay, Hycor Biomedical Inc).

ELISA for specific IgE

nCor a 1 and nCor a 8–specific IgE antibodies were measured by an ELISA experiment similar to that described previously[16,17] with some modification. In brief, plates were coated with 0.02 mg/mL of nCor a 1 and nCor a 8. A 50-μL serum sample from each patient (before and after immunotherapy) was incubated for 2 hours at room temperature. The bound IgE were detected by biotinylated mouse monoclonal antihuman IgE antibody (1:1000, 50 μL/well; Operon, Cuarte de Huerva, Zaragoza, Spain) followed by streptavidin–horseradish peroxidase—labeled antimouse IgG antibody (1:5000, 50 mL/well; Sigma-Aldrich, St Louis, Mo); IgE binding was detected by using a solution of 3,3′,5′-tetramethyl-benzidine (50 μL/well; Sigma), and optical densities were read at 450 nm.

Human serum cytokines

Human serum IL-4, IL-5, IL-10, TGF-β, and IFN-γ concentrations were measured by ELISA using reagent kits of BLK (Biolink 2000, Barcelona, Spain), according to the manufacturer's instructions.