
Case report

Cross-reactivity between coconut and hazelnut proteins in a patient with coconut anaphylaxis

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Background: The medical literature reports few cases of severe allergic reactions to coconut. We encountered a patient with anaphylaxis to coconut and oral symptoms to tree nuts.

Objective: To identify cross-reactive antibodies between coconut and other tree nuts.

Methods: We performed commercial radioallergosorbent tests to coconut and various tree nuts using the patient's serum. Skin prick testing was performed to fresh coconut and commercial extracts of coconut, almond, Brazil nut, cashew, pecan, walnut, and hazelnut. Proteins from fresh coconut, commercial coconut extract, and tree nuts were extracted. Immunoblot and inhibition assays were performed to evaluate for cross-reacting IgE antibodies between similar-sized allergens in coconut and hazelnut.

Results: Positive skin test results occurred to the coconut and multiple tree nut extracts. In vitro serum specific IgE was present for coconut, hazelnut, Brazil nut, and cashew. Immunoblots demonstrated IgE binding to 35- and 50-kDa protein bands in the coconut and hazelnut extracts. Inhibition assays using coconut demonstrated complete inhibition of hazelnut specific IgE, but inhibition assays using hazelnut showed only partial inhibition of coconut specific IgE.

Conclusions: Our study demonstrates the presence of cross-reactive allergens between hazelnut (a tree nut) and coconut (a distantly related palm family member). Because there are many potentially cross-reactive allergens among the tree nuts, we recommend patients with coconut hypersensitivity be investigated for further tree nut allergies.

Ann Allergy Asthma Immunol. 2004;92:281–284.

INTRODUCTION

Severe allergic reactions to coconut (*Cocos nucifera*) are rarely reported in the medical literature. To date, only 3 patients with systemic reactions after the consumption of coconut have been described.^{1,2} Two of these patients also had systemic reactions to walnuts and subsequently demonstrated cross-reactivity with walnut allergens.

Serologic studies have shown a high degree of IgE binding to multiple tree nuts.^{3–5} In a study of children with tree nut allergy, Sicherer³ reported that 92% of 111 patients with peanut allergy, tree nut allergy, or both had IgE antibody to more than 1 tree nut. In fact, 37% of 54 patients had experienced convincing reactions and had specific IgE antibody to more than 1 nut.

Coconuts are not tree nuts but are monocotyledonous plants of the palm family Arecaceae. Nut-bearing trees, on the other hand, are dicots and are only distantly related to the palm species. We recently encountered a patient with systemic reactions to coconut who also described oral symptoms

to pecan, almond, and walnut. We sought to identify cross-reactive antibodies between coconut and the commonly consumed tree nuts.

METHODS

Case Report

The patient is a 19-year-old man with a history of mild seasonal allergic rhinitis who presented to the emergency department with anaphylaxis after ingestion of coconut-containing food. He reported similar reactions to coconut since childhood that required endotracheal intubation and mechanical ventilation on 3 previous occasions. His family history is positive for atopy in first-degree relatives. He denied a past medical history of asthma. The patient also noted a penicillin allergy during childhood. In addition, he described oral symptoms to other foods, such as lima beans and various tree nuts (pecan, almond, and walnut). However, he can consume cashews and peanuts without incident. On the most recent episode of coconut ingestion, the patient reported the immediate onset of oral pruritus, generalized hives, and wheezing after the ingestion of a dessert that contained coconut-flavored cream topping.

Radioallergosorbent tests (RASTs) revealed specific IgE levels of 11.60 kU/L to coconut, 1.71 kU/L to hazelnut, 0.55 kU/L to Brazil nut, 0.85 kU/L to cashew, 0.49 kU/L to peanut, and 0.71 kU/L to pea. RASTs demonstrated IgE levels of less than 0.35 kU/L to kiwi, pecan, and walnut. Two

Allergy/Immunology, Wilford Hall Medical Center, San Antonio, Texas. No grant support was obtained for this manuscript, and the authors have no pharmaceutical industry affiliations.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Air Force, the Department of Defense, or the United States Government.

Received for publication March 13, 2003.

Accepted for publication in revised form September 26, 2003.

months after initial presentation, the patient returned to our clinic for skin prick testing. After obtaining written informed consent from the patient, we performed skin testing in our allergy clinic with resuscitative equipment readily available. The results of skin prick tests to fresh coconut, commercial extracts of coconut, canned coconut milk, almond, Brazil nut, cashew, pecan, walnut, and hazelnut were positive, with appropriate responses to positive and negative controls. We used the prick method on the slurry mixture of the flesh of the fresh coconut and the canned coconut milk. Five atopic control subjects tested negative with the similar slurry mixture. We did not perform aeroallergen skin testing.

Extract Preparations

Protein extraction of commercial coconut extract (1:10 wt/vol, Alk Abello, Round Rock, TX) and fresh coconut flesh was performed in 0.125 M NH_4HCO_3 at 4° C overnight. Each extract was centrifuged and the supernatant passed through 0.45- μm pore-sized filters. Each filtrate was dialyzed against distilled water overnight at 4° C in 3,500-Da dialysis tubing. Each resulting extract was lyophilized and stored at 4° C. Extracts of previously mentioned tree nuts were prepared in a similar manner. Extract protein concentrations were determined by a modification of the technique used by Lowry et al.⁶

Sodium Dodecylsulfate–Polyacrylamide Gel Electrophoresis

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of the coconut and hazelnut extracts was accomplished as described previously.⁷ In short, the coconut and hazelnut extracts and cashew, brazil nut, almond, and pecan underwent polyacrylamide gel electrophoresis (Bio-Whittaker, Rockland, ME) on 10% to 20% gradient gels along with low-molecular-weight controls (Pharmacia, Piscataway, NJ), which provided 6 calibration points (phosphorylase B, 94 kDa; albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 20.1 kDa; and α -lactalbumin, 14.4 kDa).

Gels were stained with 0.025% Coomassie blue R-250. The molecular weight of each stained protein was calculated by comparison of the protein's gel position to a standard curve constructed from the molecular weight standards.

Human IgE Immunoblots

Immunoblots were accomplished as previously described.⁷ In short, coconut and hazelnut proteins from the extracts were transferred from unstained SDS-PAGE gels to 0.45- μm nitrocellulose membranes. After transfer, the molecular weight standards were cut from the nitrocellulose membrane and stained with Coomassie blue.

The nitrocellulose membranes were washed, blocked, and incubated overnight at room temperature with either 1:10 dilution of the patient's serum in 10% fetal bovine serum (FBS) or 1:10 pooled cord serum used as a negative control. The membranes were washed and subsequently incubated for 6 hours at room temperature in mouse anti-human IgE monoclonal antibody (Sigma Chemical Company, St Louis, MO)

diluted 1:5,000 in 10% FBS. Finally, the membranes were incubated overnight in alkaline phosphatase–conjugated goat anti-mouse IgG (Chemicon International, Temecula, CA) diluted 1:2,000 in 10% FBS. Membranes were developed, and the molecular weight of each allergen was determined from the molecular weight standards.

Inhibition IgE Immunoblots

Inhibition immunoblots were accomplished as previously described.⁷ The procedure duplicated that of the immunoblots as described herein, with preincubation of 10 mL of 1:10 serum with 10 mg of coconut protein and 10 mg of hazelnut protein separately before the immunoblot procedure.

RESULTS

Protein Lowry

Fresh coconut, commercial coconut extract, and hazelnut contained 232 μg , 817 μg , and 606 μg of protein per milligram of extract, respectively.

Sodium Dodecylsulfate–Polyacrylamide Gel Electrophoresis

The fresh coconut and coconut commercial extracts contained protein bands in the 15-, 23-, 35-, 50-, and 68-kDa ranges, and similar-sized proteins were shared by the fresh hazelnut (Figure 1).

Human IgE Immunoblots

IgE binding to the hazelnut and coconut extracts was demonstrated at the 35- and 50-kDa proteins as demonstrated in the uninhibited lane in Figure 2 and Figure 3. The cord blood showed no IgE binding.

Inhibition IgE Immunoblots

The inhibition blots performed with fresh coconut completely inhibited IgE to itself and the commercial coconut extract and

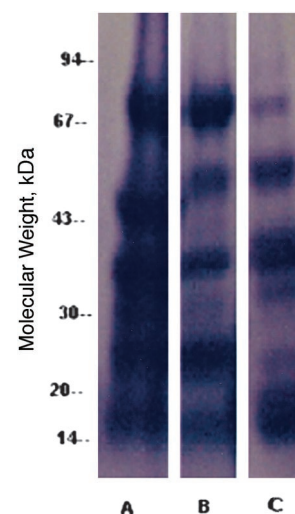


Figure 1. Sodium dodecylsulfate–polyacrylamide gel electrophoresis for commercial coconut extract (A), fresh coconut (B), and hazelnut (C).

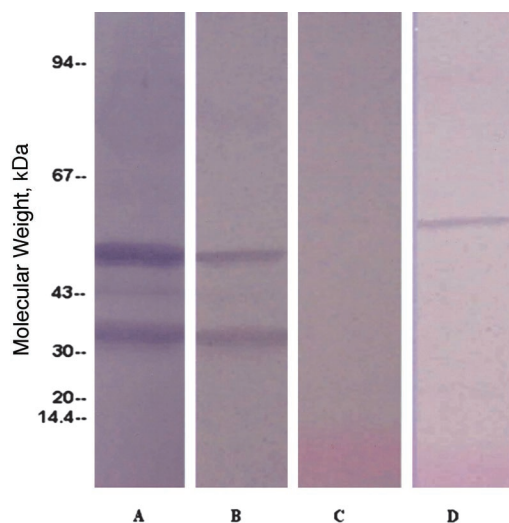


Figure 2. Fresh coconut IgE immunoblots. A, Uninhibited; B, preincubated with fresh hazelnut (demonstrates partial inhibition at 50- and 35-kDa bands); C, preincubated with fresh coconut (demonstrates complete inhibition at 50- and 35-kDa bands); and D, cord blood negative control.

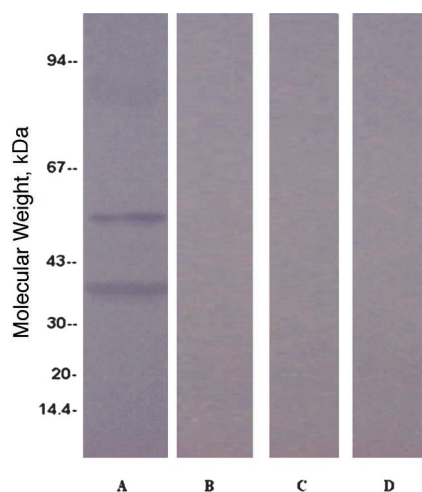


Figure 3. Hazelnut IgE immunoblots. A, Uninhibited; B, preincubated with hazelnut (demonstrates complete inhibition at 50- and 35-kDa bands); C, preincubated with fresh coconut (demonstrates complete inhibition at 50- and 35-kDa bands); and D, cord blood negative control.

hazelnut. On the other hand, hazelnut inhibited itself but only partially inhibited the 2 coconut extracts.

DISCUSSION

Teuber and Peterson¹ described 2 patients with walnut allergy who later developed systemic reactions after the consumption of coconut. They showed that serum IgE from both patients recognized coconut allergens with sizes of 35 and 36.5 kDa. Preincubation of allergic serum with tree nut extracts (walnut, almond) inhibited IgE binding to coconut proteins. Preincubation of serum with coconut inhibited binding to walnut

allergens of 35 and 36 kDa. The 35-kDa protein has been shown to be a subunit of the coconut 11S globulin, which is similar to other described 11S globulins (legumin storage protein).⁸ This large globulin in coconut was originally described in 1930 and named *cocosin*.⁹

Carr et al⁸ have shown in their studies that cocosin is a hexamer with a molecular weight of approximately 300 kDa, with each 54-kDa subunit comprising an acidic and basic chain linked by a disulfide bond. On electrophoresis, these bands migrate to approximately 35 and 32 kDa (acidic chains) and a complex of 22 kDa (basic chains). This 22-kDa basic chain region may explain the common band identified on SDS-PAGE as the 23-kDa protein in hazelnut.

An 18-kDa protein isolated in hazelnuts similar to the hazel pollen allergen Cor a 1 has been linked with oral allergy syndrome in patients with allergy to the major birch pollen allergen Bet v 1.¹⁰ This protein is homologous to pathogenesis-related 10 type proteins found in celery, apple, apricot, pear, parsley, and potato.¹¹ Our patient had oral symptoms with pecan, walnut, and almond. However, he only had mild seasonal rhinitis, which did not warrant skin testing for aeroallergen allergy at the time of the original evaluation. He is no longer in the area and not available for further testing.

In our SDS-PAGE (Figure 1) and our protein Lowry result, the protein content in commercial coconut appears to be more than 3 times higher than fresh coconut. We speculate that the fresh coconut may have retained more water and not completely lyophilized during the protein extraction stage.

We have demonstrated that coconut and hazelnut proteins contain cross-reactive allergens in the 35 and 50-kDa molecular weight range. This result is similar to the data shown by Teuber and Peterson in their study of cross-reactivity between coconut and walnut. In our inhibition immunoblot assay, coconut protein completely inhibited hazelnut specific IgE, but hazelnut protein only partially inhibited coconut specific IgE. However, this partial inhibition does not necessarily mean that these proteins are different allergens. Proteins from trees in general are poor immunoabsorbents, even with their own antiserum.¹² Similar findings were reported by More et al.¹³ Complete inhibition may have been achieved if more hazelnut protein had been used. We were unable to perform further investigations of cross-reactivity to other legumes and tree nuts given a limited amount of the patient's serum.

Our study demonstrates the presence of cross-reactive allergens between hazelnut (a tree nut) and coconut (a distantly related palm family member). Because there are many potentially cross-reactive allergens among the tree nuts, we recommend patients with coconut hypersensitivity be investigated for further tree nut allergies.

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