CASE REPORT

Allergy to Eggplant (*Solanum melongena*) Caused by a Putative Secondary Metabolite

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Abstract

We describe a case of allergy caused by ingestion of eggplant in an atopic subject. Symptoms included urticaria, itching of the throat, and hoarseness. Skin prick test (SPT) was positive with 4 varieties of eggplant; however, allergen-specific immunoglobulin E was not detected. SPT with fractions of green long eggplant extract obtained by dialysis and ultrafiltration suggested the allergen to be less than 10 kd. SPT following acetone precipitation of eggplant extract revealed that the allergen was present in the supernatant portion. Further analysis by size-exclusion chromatography of the 10 kd filtrate of eggplant extract on Sephadex G-25 followed by SPT of fractions revealed that the causative allergen was a low molecular weight nonprotein secondary metabolite of less than 1 kd. To our knowledge, this is the first report of allergy to the ingestion of eggplant in which a nonprotein secondary metabolite has been detected as an allergen.

Key words: Allergens. Aubergine. Eggplant. Food allergy. Secondary metabolite. *Solanaceae . Solanum melongena.*

Resumen

Describimos un caso de alergia causada por la ingestión de berenjena, en un sujeto atópico. Los síntomas fueron urticaria, picor de garganta y ronquera. La prueba cutánea resultó positiva para 4 variedades de berenjena, no obstante, no se detectó inmunoglobulina E específica al alérgeno. Se realizó la prueba cutánea con fracciones de extracto de berenjena obtenida por diálisis y ultrafiltración que indicó que el alérgeno era de menos de 10 kd. La prueba cutánea después de la precipitación de acetona de un extracto de berenjena reveló que el alérgeno se encontraba en el sobrenadante. Un análisis adicional mediante cromatografía por exclusión de tamaño del filtrado de 10kd del extracto de berenjena en una *Sephadex G-25,* seguida por una prueba cutánea de fracciones reveló que el alérgeno implicado era un metabolito secundario no proteico de un peso molecular inferior a 1 kd. Hasta donde alcanza nuestro conocimiento, este es el primer informe de alergia a berenjena en el que se ha detectado un metabolito secundario no protéico como alérgeno.

Palabras clave: Alérgenos. Berenjena. Alergia alimentaria. Metabolito secundario. *Solanaceae. Solanum melongena.*

Introduction

Vegetables are a minor class of allergenic foods that can cause allergic reactions in sensitized individuals. Among the members of the nightshade (*Solanaceae*) family, tomato, potato, bell pepper, and eggplant (aubergine) [1] are commonly consumed throughout the world. Allergic reactions to tomato, potato, and bell pepper have been widely reported in the medical literature, and several allergens have been identified in these vegetables [2,3]. In contrast to these vegetables, allergic reactions to eggplant are rare and have only appeared in recent years. Three proteins in the molecular weight range of 60-71 kd have been described in 3 cases from India [4], and some proteins in the range of 22-50 kd have been described in a case of anaphylaxis to eggplant from Korea with crossreactivity to latex [5]. In a case from Spain, a latex-allergic subject also had banana and eggplant allergy based on case history and skin prick test (SPT) [6].

Eggplant (*Solanum melongena*), commonly known as brinjal in the Indian subcontinent [1], is consumed

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Table. Results of Skin Prick Test With Eggplant Extract and its Fractionated Components.

Sample	Wheal/Flare Diameter, mm[a]
Negative control[b]	0-1/0
Positive control[c]	5-6/30
Eggplant extract (50% w/v)	
Green long	5/30
Green round	4/20
Purple long	4/25
Purple round	4/20
Undialyzed (green long)	5/20
Dialyzed (green long)	-
Acetone precipitation	
Precipitate	-
Supernatant	4/20
Ultrafiltration	
10 kd retained fraction	-
10 kd filtrate	4/15

[a]The result of skin prick test was considered positive if the wheal diameter was 3 mm or more. Value for various eggplant extracts: 1-2/0 (normal subjects); values for other eggplant-allergic subjects (n = 3): 4.5-5/20-30.
[b]50% glycerinated phosphate-buffered saline.
[c]Histamine dihydrochloride, 1.66 mg/mL (equivalent to 1 mg/mL histamine base).

widely in India. An allergic reaction to eggplant appears to be commonly experienced by a considerable number of individuals in the Indian population [7]. During the course of the identification and characterization of the 60-71 kd eggplant allergens in our laboratory, we came across an interesting case of allergy caused by ingestion of eggplant wherein the subject experienced severe allergic reactions not caused by the already-described protein allergens. Here, we describe this unusual case using in vivo and in vitro methods for diagnosis of food allergy.

Case Description

A 31-year-old man with no family history of food allergy reported allergy to eggplant that first occurred at the age of 10 years, although it was first noticed by his mother at the age of 6. He reported experiencing itching or discomfort while eating curry or other foods containing eggplant, followed by immediate development of skin rashes. He stated that within 1 to 2 hours of consuming foods containing eggplant, skin rashes appeared with itching all over the body, along with itching of the throat and hoarseness. He had moderate dust allergy (positive SPT to house dust mite: wheal/flare diameter, 4/12 mm). He had not experienced any allergic reactions to other foods, pollens, or latex. Complete blood count and liver and renal profiles were all normal. Serum concentrations of total immunoglobulin (Ig) E were slightly above normal limits (210 IU/mL). At present, he avoids eating any food containing eggplant.

After approval by the Institutional Ethics Committee and provision of informed consent by all study subjects, attempts were made to identify the allergen responsible for the reaction. Although 4 varieties of eggplant are commonly available in India [1], the green long (Mysore) and the purple round varieties are popular in the southern region. Detailed analyses were only performed with the green long variety in this study.

SPT [8] was performed with fresh 50% (weight by volume [w/v]) eggplant extracts (protein content: 0.5 mg/mL based on the Bradford assay [9]) prepared as described previously [4]. The SPT results were found to be positive (Table). SPT using eggplant extracts was negative in 5 healthy control subjects.

The eggplant extract was concentrated by ultrafiltration using a DIAFLO YM10 membrane (molecular weight cutoff, 10 kd; Millipore, Bedford, MA, USA); the retained fraction and the filtrate were tested by SPT. The retained fraction gave a negative SPT whereas the filtrate was positive (Table). Acetone precipitation of 50% (w/v) eggplant extract was performed as described previously [4]. SPT was performed using both redissolved acetone precipitate (containing mostly proteins) and reconstituted supernatant (containing nonprecipitable nonprotein compounds). The allergic subject showed positive reactions to the supernatant and negative reactions to the precipitate in SPT. Based on the results of SPT with materials obtained from acetone precipitation and ultrafiltration, the suspected allergen in this study appeared to be an eggplant component of less than 10 kd.

Allergen-specific IgE was analyzed by enzyme-linked immunosorbent assay (ELISA) [10]. Briefly, microtiter wells (Maxisorp; Nunc, Roskilde, Denmark) were coated with eggplant extract (25 g protein/well; pH 9.6) at 4°C overnight. This was followed by incubation with 100 L allergic or normal serum (1:3 dilution). Alkaline phosphatase-conjugated mouse anti-human IgE (Sigma-Aldrich, St Louis, MO, USA) was used as the secondary antibody (1:1000 dilution). Serum allergen-specific IgE values in the allergic subject were found to be negative based on a less than 2-fold difference in absorbance compared with healthy control subjects (data not shown). The ELISA values for the acetone supernatant, precipitate, 10 kd retained fraction, and filtrate were also found to be similar in the allergic and normal subjects. These results indicate the absence of allergen-specific IgE to eggplant or its components in this sensitized subject.

The concentrated eggplant extract was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (12%). Despite a low protein content of 1.1% in eggplant [1], the gel revealed several protein bands in the 10 to 100 kd range (data not shown). IgE immunoblotting [10] was carried out using electrophoretic transfer of protein bands to a nitrocellulose membrane followed by blocking, incubation with allergic or normal serum (1:3 dilution), secondary antibody (alkaline phosphatase-conjugated murine anti-human IgE, 1:1000 dilution), and color development. The IgE immunoblot developed using serum from the eggplant-allergic subject and

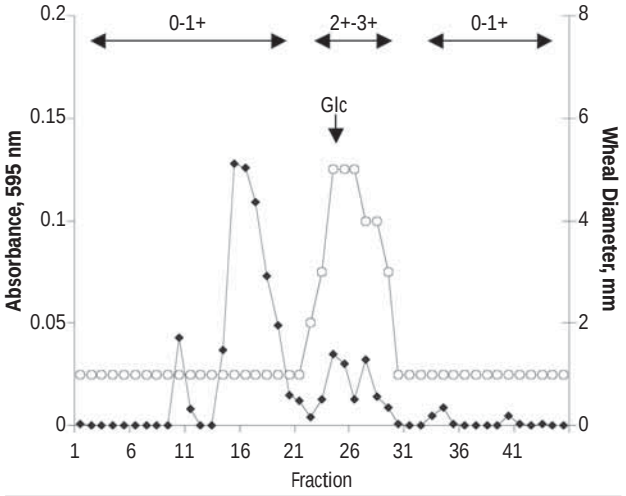

Figure. Gel filtration profile of 10 kd filtrate of eggplant (green long) extract on Sephadex G-25 (0.5 × 46 cm). Sample volume, 0.5 mL; flow rate, 4 mL/h; elution buffer, phosphate buffered saline; fraction volume, 0.3 mL. Absorbance at 595 nm (♦) represents the protein profile based on Coomassie blue binding assay. Wheal diameter in mm (o) represents the results of skin prick testing. The color change obtained after keeping the fractions at 4°C for 48 hours is indicated in the top portion of the figure: 0 to 1+ indicates light brown color and 2+ to 3+ indicates dark brown color. The elution position of D-glucose (Glc) under identical conditions is indicated by an arrow; glucose was detected by phenol-sulfuric acid assay [12].

control subjects did not reveal any protein bands, indicating the absence of specific IgE against proteins in the molecular weight range of 10-100 kd (data not shown).

In order to determine the nature of the allergen (whether a low molecular weight polypeptide of <10 kd or a nonprotein component), the concentrated 10 kd filtrate was subjected to size exclusion chromatography on Sephadex G-25 (fractionation range: 1-5 kd for peptides and globular proteins [11]). The elution profile based on protein assay [9] and SPT results of column fractions is shown in the figure. Fractions 1-20, containing low molecular weight proteins and peptides in the 1 to 10 kd range, were negative by SPT, and the subsequent fractions (21-30), representing small molecules of <1 kd (as standardized by the elution of glucose using colorimetric assay [12]; molecular mass, 0.18 kd) were found to be positive by SPT. This clearly indicated that the suspected allergen for the sensitized subject was a low molecular weight compound of <1 kd. Interestingly, fractions 22-30 turned strong brown following storage of samples at 4°C for 2 days, indicating that there was nonenzymatic browning of polyphenol compounds at neutral pH as a result of their oxidation to form colored adducts.

Some known nonprotein compounds from eggplant (serotonin, indole, caffeic acid, chlorogenic acid, L-tryptophan, tryptamine, and tyramine) selected from Duke's Phytochemical and Ethnobotanical Databases [13], tested at 1 mg/mL, were found to be negative by SPT (wheal/flare diameter of 1 × 1/0 mm in all cases), thus ruling out these secondary metabolites as allergens.

Discussion

We present a case of allergy to eggplant (both green and purple varieties) identified based on patient history and positive SPT. Eggplant-specific IgE was found to be negative by both ELISA and IgE immunoblot, a result which is surprisingly different from other cases of eggplant allergy, where allergenic proteins have been described [4-6]. It is possible that the negative result with ELISA is not due to the absence of allergen-specific IgE per se, but rather to the absence of binding of the low molecular weight allergen in eggplant extract (or the acetone supernatant or the 10 kd filtrate) to the polystyrene surface of the ELISA plate. Based on biochemical fractionation studies, the putative allergen appears to be a secondary metabolite or peptide of <1 kd, as indicated by positive SPT. However, the allergic subject was unavailable for further follow-up studies including double-blind placebo-controlled food challenge. Although small molecules are rarely identified as allergens from food sources, mannitol was identified as a low molecular weight allergen in an unusual case of anaphylaxis to pomegranate [14] and cultivated mushroom [15]. Ethanol in overripe rock melon has been shown to cause anaphylaxis [16], and it has also been reported that ethanol can cause urticarial reactions following consumption of alcoholic beverages [17].

In our earlier study of 3 eggplant-allergic subjects, we detected 3 protein allergens (60, 64, and 71 kd) in eggplant [4]. Three different allergens (a protein band between 22 and 36 kd, a band near 36 kd, and a band between 36 and 50 kd) were identified as IgE-binding proteins in eggplant in a case of anaphylaxis with cross-reactivity to latex [5]. In the present study, possible nonprotein allergenic compounds in eggplant include pigments (cyanidin, delphinidin, lycoxanthin, and nasunin), alkaloids (solamargine, solanidine, solanine, solasodine, solasonine, and trigonelline), and phytosterols [13]. It is noteworthy that the alkaloid properties of eggplant have been suggested as the cause of occasional allergies to eggplant [18], though direct evidence is still lacking. To our knowledge, this is the first report of allergy caused by ingestion of eggplant in which a nonprotein secondary metabolite has been detected as a putative allergen.

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