

Anaphylaxis to Buckwheat in an Atopic Child: A Risk Factor for Severe Allergy to Nuts and Seeds?

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Established Facts

- Proteins in buckwheat are potent allergens and can induce anaphylaxis.
- Characterization of some buckwheat allergens at the molecular level has identified 2S albumins, 11S globulins and proteins with homology to vicilin-like allergens in some nuts.

Novel Insights

- Apart from causing anaphylaxis to buckwheat, 11S globulins in buckwheat can induce IgE responses to 11S globulins in other, botanically unrelated foods, like hazelnuts and poppy seeds, with subsequent severe allergic reactions.
- Knowledge of cross-reactive proteins in foods is essential to implement advice on allergen avoidance and help prevent future anaphylaxis.

Key Words

Anaphylaxis • Buckwheat • Hazelnut • Poppy seed •
Cross-reactivity

Abstract

Common buckwheat (*Fagopyrum esculentum*) is known to cause severe anaphylactic reactions in adult individuals. However, type I allergy to buckwheat is rarely seen in children. We report on a 7-year-old boy who developed a grade III anaphylactic reaction after consumption of a cake containing buckwheat flour. Prior to this incident, the boy had

developed severe allergic reactions to hazelnuts and suffered from an oral allergy syndrome to poppy seed. Analysis of the patient's IgE reactivity by immunoblotting experiments revealed that he was sensitized to members of the 2S albumin and 11S globulin protein families in buckwheat. Additionally, cross-reactivity was found between the 11S globulins in buckwheat, poppy and hazelnut. IgE inhibition experiments indicated that the 11S globulin in buckwheat was the initial sensitizing protein. We conclude that 11S globulins in buckwheat have the potential to induce IgE antibodies cross-reactive with 11S globulins in other, botanically unrelated foods and may induce anaphylactic reactions.

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Introduction

Flour from common buckwheat (*Fagopyrum esculentum*) is frequently used as a gluten-free source of diet in bread, pasta and cakes for patients with celiac disease. It can also be found as an ingredient in national specialties. Buckwheat allergy is an IgE-mediated immediate reaction, sometimes causing severe reactions similar to those caused by soybean or peanuts [1]. Recent epidemiological data from South Korea have identified buckwheat as the leading cause of food-induced anaphylaxis during an observation period of 6 years [2]. Allergy symptoms to buckwheat are mostly observed in adult individuals and rarely reported in children.

Buckwheat contains several proteins with molecular weights of 8, 9, 16, 19 and 24 kDa that have been reported as major allergens [3, 4]. Some of these allergens have been characterized at the molecular level. The buckwheat 16-kDa protein (BWp16) has been cloned, mutated and analyzed for IgE-binding epitopes [5–7]. This allergen and the 10-kDa allergen belong to the 2S albumin family. 2S albumins have also been identified in Brazil nuts (Ber e 1), English walnuts (Jug r 1), cashew nuts, sesame seeds (Ses i 2), yellow mustard (Sin a 1) and oriental mustard (Bra j 1) [5, 8]. The 19-kDa buckwheat allergen shows weak homology to the vicilin-like allergens of cashew (Ana o 1), English walnut (Jug r 2) and 7S globulin from sesame seeds [9]. Buckwheat also contains 11S globulins, which are oligomeric storage proteins in legumes, nuts and seeds composed of two polypeptides, a 30- to 40-kDa α -subunit and a 20-kDa β -subunit, linked by a disulfide bond. Under nonreducing conditions, 11S globulins have an apparent molecular weight of 50–60 kDa in the polyacrylamide gel electrophoresis.

This case report demonstrates that 11S globulins in buckwheat are capable of sensitizing children with the potential of inducing severe allergic reactions. IgE cross-reactivity with 11S globulins in other foods may also cause anaphylactic reactions.

Case Report

Clinical Findings

A 7-year-old boy was admitted to our department. After tasting a buckwheat cake, a traditional Austrian pastry, he had immediately complained of an itching sensation in the mouth, followed by angioedema, sneezing, a dry cough, vomiting and a generalized urticaria. At the age of 4, he had developed a grade II allergic reaction after eating hazelnuts [10] and a generalized urticaria, emesis and dyspnea following the consumption of a buckwheat flour-containing meal. The child had had atopic eczema in

infancy with complete resolution at school age. He was neither asthmatic nor did he suffer from allergic rhinitis to pollen, animal dander or house dust mites. In his allergy history, the boy also reported an itching and burning sensation in the mouth when eating a roll covered with poppy seeds. No other clinically relevant food allergies were detected.

Determination of IgE Antibodies

Total serum IgE was measured by ImmunoCAP (Phadia AB, Uppsala, Sweden). Allergen-specific IgE antibodies were determined by ImmunoCAP and ISAC (both from Phadia AB). For immunoblotting experiments, hazelnuts, buckwheat or poppy seeds (3 g each) were pounded in liquid nitrogen and incubated with 30 ml of phosphate-buffered saline overnight at 4°C. The mixture was centrifuged at 15,000 rpm for 30 min. The protein concentration of food extracts was determined by BCA assay (BioRad Inc., Richmond, Calif., USA). Then, extracts (25 μ g) were separated by 17% sodium dodecyl sulphate polyacrylamide gel electrophoresis under nonreducing conditions and blotted onto a nitrocellulose membrane. The membrane was incubated with sera overnight at 4°C. Bound IgE was detected with ^{125} I-labeled anti-human IgE antibody (Demeditec Diagnostics, Kiel-Wellsee, Germany) and visualized by autoradiography. A serum pool from nonallergic donors served as negative control. For inhibition experiments, the patient's serum was pre-incubated with different concentrations (100, 50 and 25 μ g/ml) of the respective extracts over night at 4°C before immunoblots were performed.

IgE Levels to Different Allergen Sources

IgE responses to different allergen sources and single allergens in our patient serum were determined (table 1). His serum contained 491 kU/l of total IgE comprising specific IgE for buckwheat, hazelnut, peanut, poppy seed, sesame, soybean and walnut as well as for inhalant allergens from house dust mite, birch and timothy grass pollen. Using the ISAC system, IgE reactivity to 12 different allergens out of 101 allergens derived from 28 plant and 17 nonplant allergen sources was found (table 1). The boy showed IgE reactivity to Cor a 9 in hazelnut, a member of the 11S globulin protein family [11]. In addition, IgE specific for Cor a 1, an allergen structurally related to the major birch pollen allergen Bet v 1, was found [12]. Matching the ImmunoCAP data, IgE antibodies specific for different allergens from house dust and storage mites as well as timothy grass pollen were detected (table 1). In addition, the boy showed IgE reactivity to Bet v 1. Of note, sensitization to any inhalant allergen was not clinically relevant.

IgE Reactivity to Allergens in Buckwheat, Hazelnut and Poppy

To analyze the patient's reactivity pattern to individual allergens contained in buckwheat, hazelnut and poppy seed, we performed immunoblot experiments. For buckwheat extract the patient displayed strong IgE reactivity to several proteins in the size of 8–16 kDa, presumably 2S albumins, and to two proteins of approximately 60–70 kDa, presumably 11S globulins (fig. 1a). For poppy seed, IgE reactivity to several proteins ranging from 34 to 55 kDa was detected (fig. 1b). Immunoblots using hazelnut extract revealed IgE reactivity to Cor a 1 (18 kDa) and Cor a 9 (59 kDa) (fig. 1c).

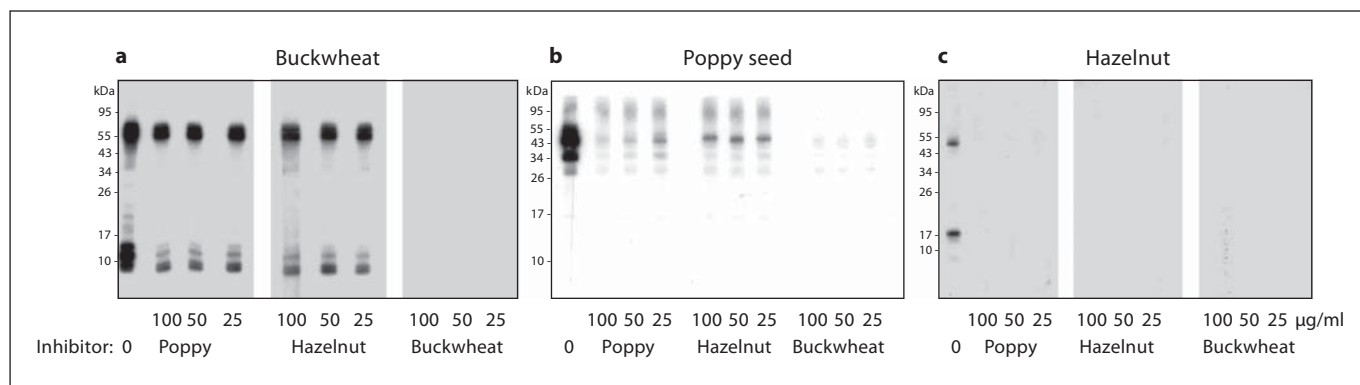


Fig. 1. IgE inhibition experiments. The serum was pre-incubated with different concentrations (100, 50 and 25 µg/ml) of extracts from buckwheat, poppy seed or hazelnut prior to analysis of IgE reactivity to blotted extracts from buckwheat (a), poppy seed (b) and hazelnut (c).

Table 1. IgE response of the patient

ImmunoCAP		ISAC	
allergen source	kU/l	allergen	ISU
Birch pollen	<0.35	rBet v 1	2.1
Buckwheat	51.9	–	
House dust mite	45.2	nDer p 1	54.9
		nDer p 2	36.7
Storage mite	83.4	nDer f 1	52.1
		nDer f 2	41.1
		rEur m 2	5.2
Hazelnut	4.87	rCor a 1.0401	0.7
		nCor a 9	0.5
Peanut	1.62	–	
Poppy seed	7.05	–	
Sesame	5.02	nSes i 1	0
Soy bean	4.65	nGly m β conglycinin	0
		nGly m glycinin	0
Timothy grass	8.3	rPhl p 1	0.8
		rPhl p 11	11.1
		rPhl p 2	0
		nPhl p 4	0.8
		rPhl p 5	18.9
		rPhl p 6	0
Walnut	2.47	–	

Cross-Reactivity of Allergens in Buckwheat, Poppy and Hazelnut

Potential cross-reactivity of the patient's IgE antibodies among the different plant food allergens was investigated in IgE inhibition experiments. As shown in figure 1a, pre-incubation of the serum with different concentrations of poppy seed extract or hazelnut extract reduced the IgE-reactivity to the 2S albumins (8–15 kDa) in buckwheat. In contrast, the reactivity to the 11S globulins

(50–60 kDa) was only marginally reduced. As expected, pre-incubation with buckwheat extract totally abolished IgE binding to all buckwheat allergens. Figure 1b shows that pre-incubation of the serum with poppy seed or hazelnut extract reduced IgE binding to the poppy allergens ranging from 34 to 55 kDa. Interestingly, pre-incubation with buckwheat extract totally abolished any IgE reactivity to allergens in poppy seed. Figure 1c shows that IgE binding to Cor a 1 and Cor a 9 in hazelnut extract was totally abolished after incubating the serum with either poppy, hazelnut or buckwheat extract.

Discussion

A 7-year-old boy was admitted to our allergy clinic following a grade III anaphylactic reaction to buckwheat. In the past, the boy had already experienced a severe systemic reaction to a buckwheat-containing meal, not requiring hospital admission, and an anaphylactic reaction to hazelnuts. In addition, he suffered from an oral allergy syndrome to poppy seeds. We sought to identify the allergens causing these allergic reactions and by immunoblot experiments found that the boy displayed IgE specific for 11S globulins in buckwheat, hazelnut and poppy.

Inhibition experiments revealed that the 11S globulin in buckwheat was able to completely inhibit IgE binding to its homologs in hazelnut and poppy seed (fig. 1). In contrast, Cor a 9 and the 11S globulin in poppy seed only marginally reduced IgE binding to the 11S globulin in buckwheat (fig. 1). This observation provides evidence that the 11S globulin in buckwheat was the initial sensitizer and induced IgE antibodies that cross-reacted with their homologs in hazelnut and poppy. This cross-reactivity even resulted in severe allergic reactions. As seed

storage proteins, 11S globulins are very abundant and several members of this protein family have been identified as allergens associated with systemic allergic reactions to the respective foods, for example, Ara h 3 in peanut [13], Gly m 6 in soybean [14] and Ses i 6 in sesame seed [15, 16].

In addition to buckwheat, hazelnut and poppy, our patient showed IgE reactivity to peanut, soybean, walnut and sesame seed (table 1). Immunoblot analyses of his IgE reactivity to sesame extract revealed the recognition of a single protein of approximately 55 kDa, presumably Ses i 6, and IgE reactivity to this allergen was inhibited by pre-incubation with buckwheat extract (data not shown). We conclude that the buckwheat-specific IgE antibodies of our patient cross-reacted with 11S globulins in all these foods. Until now, the boy has not experienced any allergic symptoms to sesame seeds, peanuts or walnut. However, he has already reported an oral allergy syndrome to poppy seeds, and poppy seed-induced anaphylaxis in a 17-year-old girl with concomitant sensitization to buckwheat has previously been observed [17]. Therefore, we cannot rule out the possibility that our patient may develop mild or more severe allergic reactions to the above-mentioned foods in the future. However, despite our findings, strict avoidance of potentially cross-reacting foods in the absence of an allergic reaction in the past may not be justified, as regular exposure to foods may be necessary for the induction and persistence of oral tolerance. Therefore, we advised the patient to strictly avoid only buckwheat, hazelnuts and poppy seeds in the future.

Of note, the boy showed IgE reactivity to Cor a 1, the Bet v 1 homolog in hazelnut. IgE reactivity to Cor a 1 mostly results from cross-reactivity with the major birch pollen allergen [18]. In line with this observation, the ISAC assay detected Bet v 1-specific IgE antibodies, whereas ImmunoCAP to birch pollen was negative. These differences may result from the use of the recombinant protein in the ISAC system and whole birch pollen extract in ImmunoCAP. Presumably, the concentration of Bet v 1 in the pollen extract was below the detection limit for Bet v 1-specific IgE antibodies in the serum of our patient. Until now, the child has not shown any sign of respiratory allergy during the birch pollen season. Because Cor a 1 is rarely associated with systemic reactions to hazelnuts, we conclude that Cor a 9 caused the systemic reaction to hazelnuts [19].

In conclusion, our findings emphasize the importance of taking cross-reactivity between botanically unrelated foods into account, to implement avoidance measures where appropriate and thereby help prevent future anaphylaxis.

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