

Anaphylactic Reaction to Inulin: First Identification of Specific IgEs to an Inulin Protein Compound

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Key Words

Inulin · Artichoke · Anaphylaxis · Specific IgE ·
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Abstract

Background: A woman with a past history of allergy to artichoke presented with two episodes of immediate allergic reactions, one of which was a severe anaphylactic shock after eating two types of health foods containing inulin. **Results:** Dot blot assay techniques identified specific IgEs to artichoke, to yoghurt F, and to a heated BSA + inulin product. Dot blot inhibition techniques confirmed the anti-inulin specificity of specific IgE. **Conclusions:** The absence of a positive reaction to an unheated milk-inulin mixture indicates the probability of protein-inulin binding. There is no cross-reactivity with the carbohydrates of the glycosylated allergens.

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Introduction

Only one case of anaphylaxis to inulin has been published. It was confirmed by double-blind placebo-controlled food challenge (DBPCFC), but specific IgE identi-

fication was not performed [1]. We report a second case supported by a positive DBPCFC at 900 mg. Specific IgE to an inulin-protein compound was identified using dot blot and dot blot inhibition techniques.

Material and Methods

Patient

A 50-year-old woman had a first episode of generalized urticaria after eating a health food biscuit in June 1999; then she had a severe prelethal anaphylactic shock with loss of consciousness after a health food yoghurt F in January 2002. Inulin (Raftiline® HP) was included in both products for its bifidogenic effect: 0.38 g in one biscuit and the yoghurt contained 2% of Raftiline (equivalent to 2.5 g).

The patient had no past history of atopic diseases. She had experienced several attacks of urticaria after eating artichoke. A daily ingestion of dairy products was well tolerated.

On a skin that was normally reactive to 9% codeine (3 mm), prick tests were negative to 12 common inhalants as well as to the marketed extracts of the 25 routine foods and the following natural foods: endive, lettuce, parsley, milk, various meats, spices, leguminous plants, sesame, mustard, and tomato. They were positive for raw and cooked artichoke (22 mm), salsify (6.5 mm), and yoghurt F (10 mm). The prick test to Raftiline was negative.

Total IgE were 364 kU/l. Results of the tryptase assay (Pharmacia®) were 5.23 µg/l. The radioallergosorbent test (RAST Cap System) to bromeline was carried out [2], and was negative.

The leukocyte histamine release test was carried out according to the Immunotech® method and was negative to artichoke heart and

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weakly positive to inulin: 5.5% of histamine release. The lymphocyte activation test and the basophil activation test by flow cytometry [3, 4] were negative to inulin (Raftiline).

Taking into account the concentration of Raftiline in the yoghurt F, the amount of inulin ingested is estimated at about 2.5 g. A DBPCFC was carried out with the yoghurt F. After ingestion of 45 ml (i.e. 900 mg inulin), the patient presented first with facial erythema and erythema with pruritis of the palms, then generalized erythema with urticaria spread over the whole body, with laryngeal pruritus and tachycardia without any drop in blood pressure. She was treated with injectable dexchlorpheniramine and methylprednisolone (120 mg) and then intramuscular epinephrine (250 µg). The reaction resolved within 2 h. There were no further reactions under strict avoidance of the agent within the following 2 years.

Samples

Artichoke Protein Extracts

Different protein extracts were prepared from cooked and raw artichoke bases and hearts (bought in two supermarkets). Five to 7 g of the sample were ground in 13 ml of buffer at pH 8 (0.1 M Tris, 0.14 M NaCl, 2 mM EDTA, 0.05% Triton, 0.05% deoxycholate and 0.1% Nonidet P40) cooled to +4°C. The mixture was left overnight at +4°C under stirring. It was then ultracentrifuged (17,000 rpm) and filtered. The protein concentration of the extracts was measured using the pyrogallol red method.

A cooked artichoke purée (20 min at 100°C) was prepared by grinding the base of leaves (250 mg) in distilled water (4 ml).

Inulin-Containing Products (Raftiline)

The following inulin-containing products (Raftiline) were used: yoghurt F containing 2% Raftiline, heated cow's milk (100°C for 10 min) in the presence of Raftiline (2%), aqueous solution of BSA (5%) heated for 15 min in the presence of Raftiline (2.5%), (BSA-inulin) vs. BSA (5 g/l).

Thin-Layer Chromatography

Sugars were identified using thin-layer chromatography (TLC). 5 µl of sample, demineralized by passing over ion exchange resin (Mixed Bed Resin, Biorad), were placed on an HTPLC plate (Merck) covered with a silica gel. After drying the deposits, the base of the plate was immersed in the migration solvent (methyl ethyl ketone: 30 vol, acetic acid: 10 vol, methanol: 10 vol). Migration lasted 20–30 min. The plate was then dried for 10 min at 80°C. The sugars were revealed by immersing the plate in a solution of 1–3 naphthoresorcinol (0.2 g naphthalene diol, 95 ml ethanol, 5 ml pure H₂SO₄ added extemporaneously). Sugars were identified by the migration distance and the coloration observed after revelation. The ketoses (fructose, saccharose) had a pink color, the aldoses (glucose, galactose, lactose) had a blue color.

Dot Blot

5 µl of different samples (yoghurt F, milk Raftiline, BSA, artichoke, BSA Raftiline) were placed on a nitrocellulose membrane. After drying in the atmosphere for 15 min, the membrane was saturated in 5% BSA and 0.05% Tween phosphate buffer solution (PBS) for 1 h. The membrane was then incubated overnight at +4°C in the serum diluted 1 in 10 in 1% BSA and 0.05% Tween solution. The control membrane was incubated in a 1% BSA solution. The membrane was then rinsed 4 times in 0.05% Tween PBS and incubated for 1 h 30 min at room temperature in a 1:1,500 dilution of secondary

anti-IgE human antibodies labeled with peroxidase (DAKO). The membrane was rinsed 4 times, then immersed in luminol in the presence of H₂O₂ directly onto the image analyzer (Kodak Digital Science 1D image analyzer). The image was acquired after an exposure time of 6.6 min (20 acquisitions).

Dot Blot Inhibition

The patient's serum (500 µl) was preincubated for 6 h at +4°C in the presence of 2 g of yoghurt F in 4 ml of PBS (i.e. 4% of proteins), or in the presence of 4 ml of a (4%) BSA solution heated to 100°C in the presence of inulin (2.5%), or in the presence of an artichoke purée. Two dot blot tests were carried out as described above, either in the presence of serum (500 µl in 4 ml PBS) or in the presence of inhibited serum. Inhibition by 2.5% Raftiline solution: the patient's serum (250 µl) was preincubated in the presence of 1.75 ml of 2.5% Raftiline solution. Two dot blot tests were carried out as described above, either in the presence of serum or in the presence of inhibited serum.

Results

Artichoke Protein Extracts

1% of the protein was recovered in the raw base extract. No quantifiable amount was obtained in the bases and leaves in preserves.

TLC of Sugars

Two spots were visible with the yoghurt F. One was pink and corresponded to inulin and the other corresponded to galactose. The artichoke extracts contained inulin and traces of fructose (fig. 1).

Dot Blot Analysis

The dots blots evidenced binding of specific IgEs to inulin-containing samples: yoghurt F, milk or BSA heated in the presence of Raftiline and the purée of artichoke leaves. There was no IgE binding to the control membrane, the BSA, the Raftiline or the nonheated milk and Raftiline mixture (fig. 2).

Dot Blot Inhibition

Inhibition of IgE binding to samples after preincubation of serum in the presence of yoghurt F or BSA-inulin is complete (fig. 3). Inhibition of IgE binding after incubation of serum with the Raftiline solution is incomplete in yoghurt F, BSA-Raftiline and the artichoke purée. Inhibition of IgE binding to BSA-Raftiline and to artichoke purée after incubation of serum with artichoke purée is complete. It is incomplete in yoghurt F.

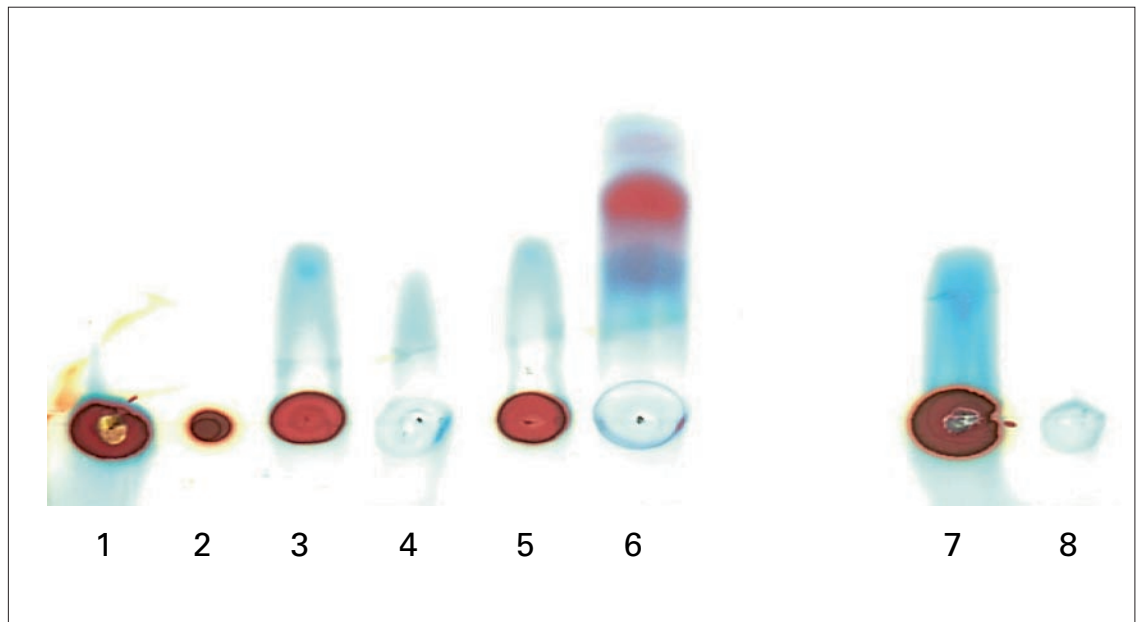


Fig. 1. TLC of sugars on silica plate (revelation by naphthoresorcinol). Inulin (oligofructoside) with a pink color (ketose) does not migrate because of its high molecular weight. 1 = Boiled milk in the presence of inulin; 2 = aqueous solution of inulin; 3 = artichoke solution; 4 = raw milk; 5 = yoghurt F; 6 = yoghurt Y; 7 = yoghurt F; 8 = raw milk.

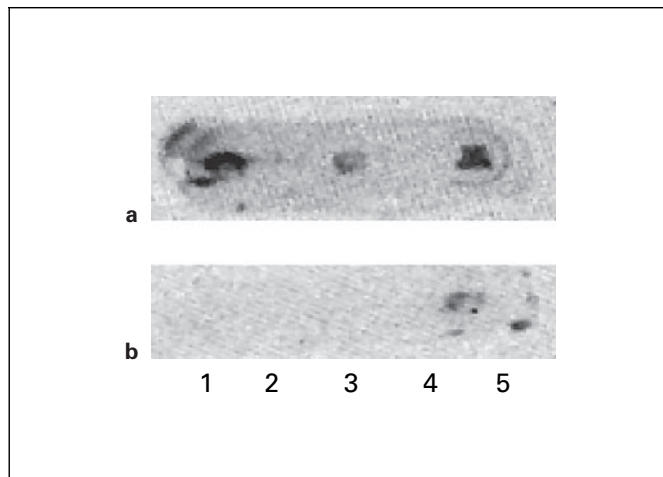


Fig. 2. Dot blot: Samples were placed on the membrane, then incubated in the presence of serum. Binding of specific IgE is revealed by a secondary antibody labelled with peroxidase. Revelation by chemiluminescence directly on the image analyzer. **a** In the presence of the patient's serum: 1 = yoghurt F; 2 = BSA; 3 = artichoke; 4 = BSA; 5 = BSA heated in the presence of inulin. **b** In the presence of a BSA solution: 1 = yoghurt F; 2 = BSA; 3 = artichoke; 4 = BSA; 5 = BSA heated in the presence of inulin.

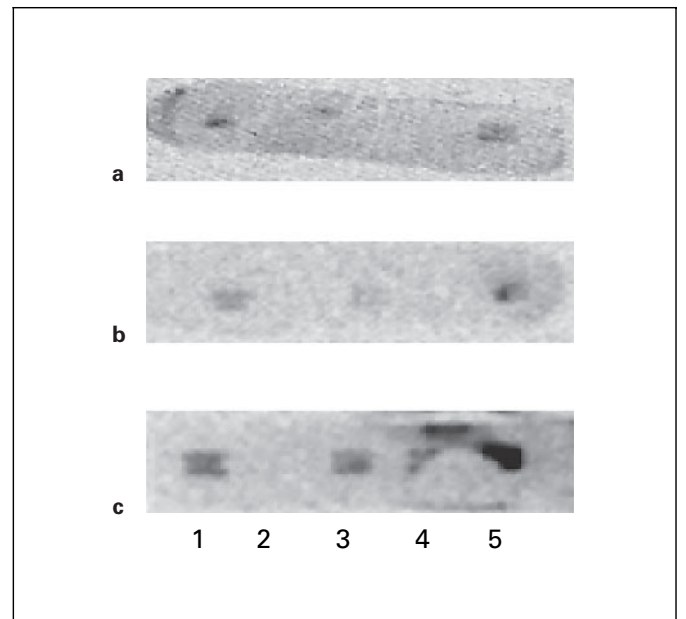


Fig. 3. Dot blot inhibition: The patient's serum is inhibited by yoghurt F (**a**), artichoke solution (**b**), and Raftiline solution (**c**). The dot blot analysis is then carried out as described above.

Discussion

Raftiline HP is a mixture of oligo- and polysaccharides which are composed of fructose units linked together by beta(2-1) linkages. The degree of polymerization ranges between 2 and 60 (average degree of polymerization: 23). It is extracted from chicory, a Compositae plant containing about 16% inulin located in vacuoles [5]. Allergy to closely related foods has been described, incriminating artichoke, Belgian endive, chicory and salsify. Specific IgEs to different proteins of endive, chicory and lettuce have been shown [6–8]. This patient was formerly allergic to artichoke and the positive prick test was impressive. The hypothesis of the allergenicity of chicory residual proteins in Raftiline cross-reacting with artichoke proteins was discarded given the absence of such proteins in Raftiline (Orafti Food Ingredients, Belgium) and the negative reaction to Raftiline during prick tests and dot blot tests. Moreover, the DBPCFC was positive to the yoghurt containing inulin, thus incriminating the inulin itself. As the RAST to bromelain was negative, it can be inferred that there were no specific IgEs binding to carbohydrate determinants [2, 9]. The positive reaction of the prick test to the yoghurt led to the hypothesis of the allergenicity to a product issuing from a manufacturing process. Heating together inulin and milk or inulin and BSA, we demonstrated specific IgEs to the product. The patient was not sensitized to milk proteins. The inhibition experiments

showed that specific IgEs were directed to inulin-protein products formed by heating and that they had specificity for inulin since there was incomplete inhibition of dot blot by inulin itself. The fact that dot blot inhibition to BSA-inulin was complete with the artichoke purée is an added argument in favor of this anti-inulin specificity. There was no cross-reaction with the carbohydrate determinants of glycosylated allergens (RAST to bromelain was negative) [6].

Allergy to inulin is extremely rare. A case of food allergy was reported by Gay-Crosier [1], and several cases have been reported after intravenous injection of inulin [10]. This case demonstrates the possibility of inulin binding to food proteins formed during heating. Yoghurt is processed by heating and the biscuit is a mixture of milk, soya, peas, flavoring and inulin heated to more than 200°C.

Currently, thousands of tons of Raftiline are used by the food industry every year. Health foods routinely contain Raftiline to replace fat and sugar and because of its bifidogenic effect. Consumers should be warned if they have any history of allergy to artichoke or endive.

Acknowledgment

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