

Food-dependent exercise-induced anaphylaxis: a report of two cases and determination of wheat- γ -gliadin as the presumptive allergen

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Summary

Water/salt-insoluble wheat proteins have been identified as the most frequent allergenic foodstuffs in patients with food-dependent exercise-induced anaphylaxis (FDEIA) in Japan. However, the specific allergenic proteins in wheat-dependent exercise-induced anaphylaxis have not been well defined. Challenge testing, skin testing and a fluoroenzyme immunoassay were used for diagnosis in two patients suspected by history of having wheat-dependent exercise-induced anaphylaxis. Gel chromatography and IgE immunoblotting followed by N-terminal amino acid sequencing were used to identify the allergenic wheat protein. The challenge test revealed that both patients had FDEIA. The skin tests and the immunoassay results suggested that wheat gluten was the allergen in both patients. Gel chromatography of wheat gluten revealed that the antigens had molecular weights ranging from 40 to 250 kDa. IgE immunoblotting and subsequent N-terminal amino acid sequencing revealed that wheat- γ -gliadin was the antigen predominantly bound by IgE in the two patients.

Key words: food-dependent exercise-induced anaphylaxis, gluten, wheat, wheat- γ -gliadin

Food-dependent exercise-induced anaphylaxis (FDEIA) is a relatively rare but clinically distinct form of anaphylaxis occurring in association with exercise, after ingestion of an allergenic food. This clinical entity was first described by Kidd *et al.*¹ and more than 200 cases have been reported. Exercise challenge tests combined with ingestion of an allergenic food successfully induce symptoms in approximately 60% of patients.² Although various foods such as shellfish, hazel nuts, celery and maize are reported in association with FDEIA, wheat is reported to be the most frequent allergenic food, especially in Japan.³ Wheat is composed of four classes of proteins: albumins and globulins, both of which are water/salt-soluble proteins, and gliadins and glutenins, both of which are water/salt-insoluble proteins. Wheat allergens have mainly been analysed in baker's asthma, a hypersensitivity to inhaled wheat flour, and several antigens have been identified in both the water/salt-soluble and insoluble proteins. However, wheat allergens in FDEIA remain largely uninvestigated. In skin testing and

immunoblot studies, Varjonen *et al.* found that gliadins of cereals were the allergens in cereal-dependent exercise-induced anaphylaxis.⁴ They recently identified a novel wheat gliadin as a cause of wheat-dependent exercise-induced anaphylaxis.⁵ In this report we describe two cases of FDEIA in which wheat- γ -gliadin was found to be the common allergen.

Case reports

Patient 1

A 43-year-old Japanese man had generalized urticaria and lost consciousness while playing golf after lunch. The symptoms resolved within 2 h. He had a similar episode 20 days later. The meal consisted of Chinese noodles and beer on both occasions. He was evaluated at the Department of Dermatology, Hiroshima University Hospital. No significant abnormalities were found on physical examination or laboratory examination including urinalysis, full blood count, serum electrolytes, liver function tests, creatinine, blood urea and nitrogen and total serum IgE.

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Intracutaneous injection of various allergens revealed weal and flare reactions to bread, *Dermatophagoides farinae* and gluten, but reaction to wheat was negative. *D. farinae* extract was obtained from Bayer Corporation, Pharmaceutical Division (Elkhart, IN, U.S.A.). Wheat gluten was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan), and wheat gluten solution was prepared according to the method described by Kushimoto and Aoki.⁶ Other allergens were obtained from Torii Pharmaceutical Co. Ltd (Tokyo, Japan).

Specific IgE antibodies in serum were 0.37 KU mL⁻¹ (class 1 reactivity) for wheat and 0.65 KU mL⁻¹ (class 1 reactivity) for gluten, when determined by a fluoro-enzyme immunoassay (CAP system; Pharmacia Upjohn, Uppsala, Sweden).⁷ Specific IgE antibodies to beer yeast, wheat germ and barley were under the detection limit of 0.34 KU mL⁻¹.

Patient 2

A 44-year-old Japanese man presented with an 8-year history of urticaria associated with exercise occurring 1 h after meals. Symptoms occurred irregularly, five to seven times per year. He was evaluated at the Department of Dermatology, Hiroshima University Hospital following an episode of generalized urticaria and syncope while jogging after breakfast. The breakfast consisted of an egg, bread and milk. No significant abnormalities were found on physical examination or laboratory examinations as already described for patient 1.

An intracutaneous injection test revealed positive reactions to bread and gluten. Specific IgE antibodies in serum were 1.76 UA mL⁻¹ (class 2 reactivity) for wheat, 0.58 UA mL⁻¹ (class 1 reactivity) for gluten, 0.52 UA mL⁻¹ (class 1 reactivity) for soya bean and 0.42 UA mL⁻¹ (class 1 reactivity) for sesame. Specific IgE antibodies to egg and meats were under the detection limit.

Materials and methods

Challenge test

Exercise challenge was undertaken using the standard Bruce protocol as described previously.⁸ Initially, after a 6-h fast a treadmill exercise challenge was carried out. Next, the same exercise challenge was carried out 10 min after ingesting foods, with a 1-day interval between the tests. Heart rate, blood pressure and

electrocardiogram were monitored. Venous blood was taken serially during the tests to measure plasma histamine levels and total blood histamine content.

Preparation of wheat gluten solution

One gram of wheat gluten was defatted with diethyl-ether, air dried, dissolved in 10 mL of 1% acetic acid, and centrifuged at 12 000 g for 30 min. The supernatant of the sample was sterile-filtered through a 0.22 µm Millipore filter, and stored at -20 °C.

Gel chromatography

The sample was applied to a TSK gel G2000SWXL column (7.8 × 300 mm; Tosoh Corporation, Tokyo, Japan) previously equilibrated with phosphate-buffered saline (pH 7.2). Chromatography was carried out with the same buffer at a flow rate of 0.7 mL min⁻¹. The eluent was monitored under ultraviolet at 280 nm, and fractions of 1 mL were collected. All fractions were prick-tested on the back of the patients. The column was calibrated with molecular weight markers (Gel filtration standard; Bio-Rad Laboratories, Hercules, CA, U.S.A.).

Immunoblotting

Immunoblotting was performed using peroxidase-labelled antihuman IgE antibodies as a second antibody, as previously described.⁴ Briefly, wheat gluten proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis,⁹ and the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Sequi-BlotTM; Bio-Rad) in a transfer cell (Bio-Rad). The transferred PVDF membrane was incubated with 10% patient serum at 4 °C overnight after blocking with skimmed milk, and IgE bound to antigen was visualized using peroxidase-labelled anti-human IgE antibodies.

N-terminal amino acid sequencing

N-terminal amino acid sequencing of the samples was performed with an automated amino acid sequencer (Procise491; PE Applied Biosystems, CT, U.S.A.).

Results

Challenge test

In patient 1, four kinds of exercise challenge were done: a fasting treadmill exercise test, a treadmill exercise test

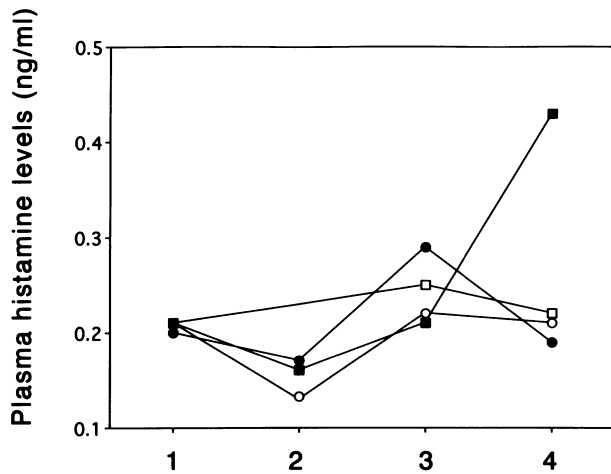


Figure 1. Patient 1: plasma histamine levels during the fasting treadmill exercise test (open squares), the treadmill exercise test after ingestion of 180 g Chinese noodles (open circles), the exercise test after drinking 500 mL beer (closed circles), and the exercise test after ingestion of a combination of 180 g Chinese noodles and 500 mL beer (closed squares). (1) Before tests; (2) 10 min after ingesting foods; (3) immediately after exercise; (4) 10 min after exercise.

10 min after ingestion of 180 g of Chinese noodles, an exercise test 10 min after drinking 500 mL beer, and finally an exercise test after ingestion of a combination of 180 g Chinese noodles and 500 mL beer. Patient 1 exhibited no unusual symptoms during the fasting treadmill exercise test, the exercise test after the ingestion of Chinese noodles, or the exercise test after drinking beer. During the final treadmill challenge after taking noodles and beer, the patient developed weals on his neck, and weals developed on his trunk and extremities after the exercise. Blood pressure and electrocardiogram were normal during and after the exercise. Plasma histamine levels were significantly elevated in association with the weal reaction (Fig. 1), whereas the total histamine content remained constant during the challenge test (data not shown).

In patient 2, four kinds of exercise challenge test were done: a fasting treadmill exercise test, a treadmill exercise test 10 min after ingestion of 80 g bread, an exercise test 10 min after ingestion of 150 g udon (a Japanese-style noodle made from wheat), and finally an exercise test after taking a combination of *okonomiyaki* (a Japanese-style pizza made from flour, noodles, cabbage, bean sprouts, pork and egg) and 80 g bread. The first three challenges were well tolerated with no development of symptoms. During the final treadmill challenge test, the patient developed weals on his neck, and weals developed on his trunk and extremities after exercise. Blood pressure decreased to a minimum of

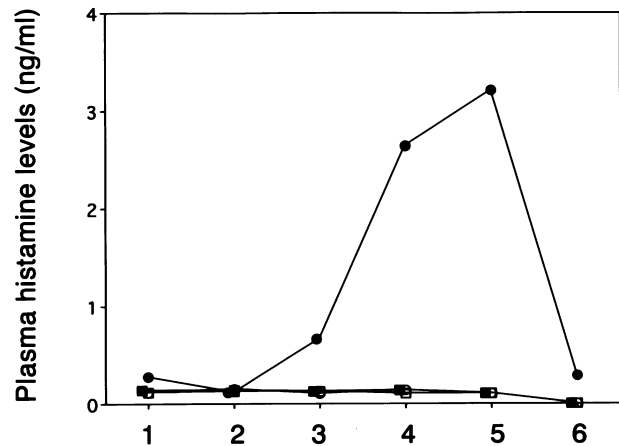


Figure 2. Patient 2: plasma histamine levels during the fasting treadmill exercise test (open squares), the treadmill exercise test after ingestion of 80 g bread (open circles), the exercise test after ingestion of 150 g udon (closed squares), and the exercise test after ingestion of a combination of *okonomiyaki* and 80 g bread (closed circles). (1) Before tests; (2) 10 min after ingesting foods; (3) after exercise; (4) 10 min after exercise; (5) 40 min after exercise; (6) 60 min after exercise.

90/60 mmHg after the challenge, but the electrocardiogram was normal during and after the exercise. Plasma histamine levels were significantly elevated in association with symptoms (Fig. 2), and the total histamine content in the blood decreased from 74.4 ng mL^{-1} (basal level) to a nadir of 50.5 ng mL^{-1} , in association with the decrease in blood pressure.

Analysis of wheat antigen

Challenge testing revealed that both patients had FDEIA. The skin tests and the immunoassay results suggested that wheat gluten was the allergen in both patients. We therefore further fractionated the gluten solution by gel chromatography, and all fractions were used in the skin test. Positive skin reactions were found at a molecular range from 40 to 150 kDa for patient 1, and from 40 to 250 kDa for patient 2 (Fig. 3).

An acetic acid solution of gluten was analysed by immunoblotting with the patients' sera: proteins of 40, 36 and 30 kDa were found to bind IgE from serum of patient 1, and proteins of 120 and 36 kDa bound IgE from serum of patient 2 (Fig. 4). N-terminal amino acid sequencing of the 36-kDa protein revealed the sequence NMQVDPSRQVQWPQQQ, identical to that of wheat- γ -gliadin.¹⁰

Discussion

We successfully precipitated the symptoms by treadmill

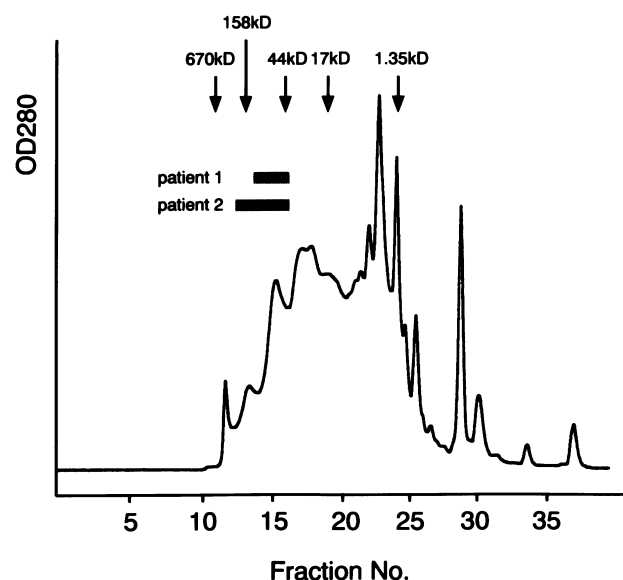


Figure 3. Gel chromatography of the wheat gluten solution. Bars indicate fractions giving immediate positive skin reactions.

challenge testing in the present two patients with FDEIA, although patient 1 needed a combination of wheat noodles and beer, and patient 2 needed to ingest large amounts of wheat-containing foods. The results in patient 2 support the findings of Hanakawa *et al.*² that the induction of an allergic response is dependent on the amount of wheat ingested. In patient 1, the combination of wheat noodles and beer successfully

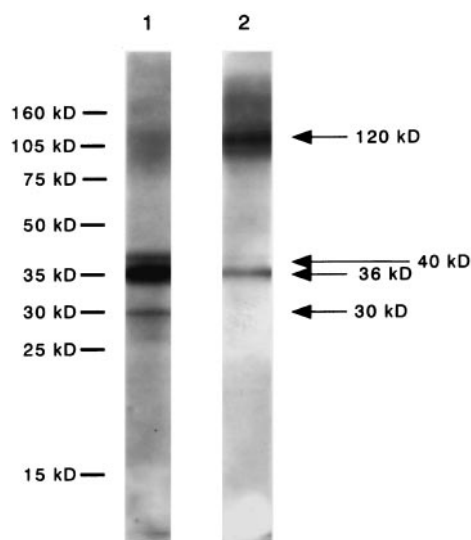


Figure 4. IgE immunoblots with sera of the two patients. Lane 1, patient 1; lane 2, patient 2. A 40-kDa protein, a 36-kDa protein and a 30-kDa protein were found to be positive in patient 1, and a 120-kDa protein and a 36-kDa protein were found to be positive in patient 2.

induced weals, suggesting that alcohol accelerates the absorption of wheat antigen from the gut. Wheat, especially wheat gluten, was suspected as an antigen in these patients, because both reacted strongly to wheat gluten on skin testing and showed positive immunoassay scores to wheat and wheat gluten.

Gluten is a major component of wheat protein, and consists of several proteins such as gliadins and glutenins.¹¹ Palosuo *et al.*⁵ reported previously undescribed γ -like wheat gliadin and α -gliadin as the allergens in wheat-dependent exercise-induced anaphylaxis in studies using skin-prick tests, enzyme-linked immunosorbent assay and immunoblotting. Our studies provide additional information in that wheat- γ -gliadin might also be an important allergen in wheat-dependent exercise-induced anaphylaxis. Recently, γ -gliadin has also been reported to be an important wheat antigen in coeliac disease, because gut-derived T cells from patients with coeliac disease recognize an epitope in γ -gliadin.¹²

The mechanisms of exercise-induced anaphylaxis have not been well defined. Although specific IgE against certain foods is usually detected in patients with FDEIA, food ingestion alone does not induce an anaphylactic reaction in these patients when not followed by exercise challenge. In addition, food-exercise challenge is not necessarily sufficient to develop an anaphylactic reaction. As gliadin is essentially a water-insoluble protein, it is speculated that digestion and absorption of the protein is inefficient, and that ingestion of a large amount of food, as shown in patient 2, or another facilitating factor, is needed for absorption of a sufficient amount of allergen to induce an anaphylactic reaction. Alcohol might serve as a facilitating factor in patient 1, because gliadin is soluble in ethanol. In addition, as gliadin is soluble in acidic solution, a decrease of pH in association with exercise might result in absorption of enough gliadin. Katsunuma *et al.*⁸ reported that pretreatment with sodium bicarbonate prevented the recurrence of symptoms and the elevation of plasma histamine levels in a patient with wheat-dependent exercise-induced anaphylaxis, supporting a role for pH in the body in the allergic response. The reaction seems to occur not only in skin mast cells, but also in circulating basophils, because in patient 2 total histamine content of circulating blood markedly decreased, suggesting degranulation of basophils.

In the present study, we provide evidence that IgE antibodies against wheat- γ -gliadin play an important part in the induction of anaphylaxis in patients with

wheat-dependent exercise-induced anaphylaxis. It is thus speculated that skin testing with gliadins could serve as a screening method for FDEIA.

Acknowledgment

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