
Case report

Systemic urticaria in an infant after ingestion of processed food that contained a trace quantity of wheat

Tomoaki Matsumoto, MD,* and Tohru Miyazaki, MD†

Background: Wheat is a food allergen that occasionally causes a systemic allergic reaction; however, little is known about the quantities of wheat allergen required to evoke allergic symptoms.

Objective: To report the case of a wheat allergic boy who experienced systemic urticaria and angioedema within 40 minutes after the ingestion of 9 g of packed rice crackers.

Methods: A skin prick test and IgE immunoblotting with wheat proteins were performed. Contamination of wheat protein in the offending rice cracker and other processed rice crackers from local food retail outlets, with labels that did not mention wheat, was examined with enzyme-linked immunosorbent assaying.

Results: A skin reaction to wheat was positive. IgE-bound bands were observed with water- and salt-soluble wheat protein and ethanol-soluble wheat gliadin in immunoblotting. A trace quantity of wheat protein, 1.50 $\mu\text{g/g}$, was determined in the offending rice cracker. In addition, 3 of 8 other kinds of processed rice crackers were contaminated by wheat protein, with levels ranging from 0.26 to 1.13 $\mu\text{g/g}$.

Conclusions: Approximately 13.5 μg of wheat protein can elicit a systemic adverse reaction in highly sensitive, wheat allergic individuals. The present study confirms the need for control of contamination of food by nondeclared proteins.

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INTRODUCTION

Wheat protein is an occasional cause of immediate allergic reactions in sensitive individuals, and some individuals claim to be so highly sensitive to trace amounts of wheat that reactions such as urticaria and angioedema have occurred in the presence of or on handling wheat.^{1,2} However, there are few quantitative data regarding the minimal amounts of wheat protein required to elicit clinical symptoms. Herein, we report the case of a wheat allergic boy who experienced systemic urticaria and angioedema within 40 minutes after the ingestion of 9 g of packed rice crackers.

CASE REPORT

A 7-month-old boy developed a swollen face and generalized hives minutes after he had eaten a food product containing wheat, chicken, skimmed milk, butter, and potato. He was treated successfully with intravenous hydrocortisone and oral diphenhydramine at the emergency department of a local

hospital. He had been fed breast milk since birth. The only foods he had been fed before this episode were rice, potato, radish, carrot, onion, tomato, apple, and orange. He had not been given any foods containing wheat, milk, or chicken before. Although no allergy evaluation was performed, he was advised to avoid the foods eaten just before this episode, namely, wheat, milk, and chicken. Three months later, an identical skin reaction occurred within 40 minutes after he had eaten two 4.5-g packs of rice crackers stated to contain “rice, maize starch, ‘wakame’ seaweed, sugar, and salt.” His symptoms subsided within 90 minutes after the oral administration of diphenhydramine. A sample of the remaining rice crackers was brought to our clinic by the parents for analysis. He had been thriving well (body weight, 8 kg), and he did not exhibit pruritus, skin lesions, or a respiratory disorder.

SKIN TESTS, SERUM SPECIFIC IgE DETERMINATIONS, AND ORAL PROVOCATION TESTS

A skin prick test with food extracts (Torii, Tokyo, Japan), including wheat, buckwheat, milk, egg-white, chicken, beef, pork, peanuts, mackerel, and salmon, was performed. A prick-by-prick skin test with maize starch and “wakame” seaweed was also performed according to the methods of Dreborg and Foucard.³ Skin tests with a commercial rice extract or with an extract made from the particular crackers implicated in this reaction were not performed. Histamine

* Department of Child Development, Graduate School of Medical Sciences, School of Medicine, Kumamoto University, Kumamoto, Japan.

† Private practice, Sadowara, Kumamoto, Japan.

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dihydrochloride (1 mg/mL; Nakalai Tesque Inc, Kyoto, Japan) was used as a positive control, and physiologic saline solution was used as a negative control. A wheal diameter at least half that of the histamine reaction was considered positive. The serum levels of specific IgEs were determined by CAP-FEIA (Pharmacia, Uppsala, Sweden). An oral provocation test was performed using increasing doses of maize starch, "wakame" seaweed, milk, and boiled chicken.

Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis and Immunoblotting

Sodium dodecylsulfate-polyacrylamide gel electrophoresis with water- and salt-soluble wheat protein (Torii) and 70% ethanol-soluble wheat gliadin (ICN Biochemicals, Aurora, OH) was performed by the method described previously.⁴ In brief, 10- μ L aliquots of the samples were loaded on a slab gel (12.5% acrylamide), and then a constant current of 20 mA was applied. The migration buffer was composed of 25-mmol/L Tris hydrochloride, 192-mmol/L glycine, 15% methanol, and 0.1% sodium dodecylsulfate. The separated proteins were fixed by incubation of the gel in 12% trichloroacetic acid for 90 minutes and visualized with rapid Coomassie stain. The gels were then probed with serum samples at 37° C overnight. After washing 3 times with phosphate-buffered saline with 0.05% Tween 20, the strips of the gel were incubated with peroxidase-conjugated goat IgG antibodies against human IgE (1:20,000; Kirkegaard & Perry Laboratories, Gaithersburg, MD) at room temperature for 2 hours. After 3 washes, the IgE binding sites were detected using the enhanced chemiluminescence system (Amersham Bioscience, Piscataway, NJ) according to the manufacturer's instructions. The duration of exposure to photographic film was 3 minutes in this study. Protein markers (Pharmacia Biotech, Tokyo, Japan) were used for estimating the molecular weights of the electrophoresed proteins. The distance traveled by each protein was compared with those by protein markers to determine the approximate molecular weight of the protein. Control serums were obtained from 2 healthy children without any allergic diseases and 1 baker with generalized chronic dermatitis, which improved to a great extent within 2 weeks after suspension of work followed by aggravation again after resumption of work. The baker met the diagnostic criteria for atopic dermatitis by Hanifin and Rajka.⁵

Enzyme-linked Immunosorbent Assay

The presence of wheat, milk, or egg in the offending rice crackers was examined with enzyme-linked immunosorbent assay (ELISA) kits (Morinaga Institute of Biological Science, Yokohama, Japan) according to the manufacturer's instructions. At first, 2 g of the rice crackers was ground into a fine powder with a blender (Labo-mill-LM-2; Osaka Chemicals, Osaka, Japan) and mixed with 38 mL of the diluent buffer using a rotator. The ELISA kits included polyclonal antibodies to wheat gliadin, β -lactoglobulin, or ovomucoid. Each kit gave an almost linear curve for the standard proteins from 1 to 64 ng/mL. From local food retail outlets, we purchased 8 kinds of rice crackers packed by 5 different manufacturers

with labels that did not mention wheat and then examined them for the presence of wheat protein.

RESULTS

Positive wheal and flare responses were observed with wheat, egg-white, beef, and peanuts. The diameter results of the wheal/flare responses were 15/36 mm for wheat, 20/35 mm for egg-white, 10/22 mm for beef, and 18/37 mm for peanuts compared with 8/26 mm for histamine dihydrochloride and 2/5 mm for physiologic saline. The skin reactions to buckwheat, milk, chicken, pork, mackerel, salmon, maize starch, and "wakame" seaweed were considered negative. The specific IgE levels in the serum were 16.4 kU/L for wheat, 2.5 kU/L for gluten, 59.3 kU/L for egg-white, 17.9 kU/L for ovomucoid, 1.3 kU/L for beef, 8.9 kU/L for peanuts, 0.12 kU/L for milk, and 0.15 kU/L for chicken. The ingestion of maize starch, "wakame" seaweed, and boiled chicken up to 20 g and milk up to 100 mL did not evoke any allergic reaction.

As shown in Figure 1, the patient's serum gave major IgE-bound bands corresponding to 16 kDa in the lanes with both kinds of wheat protein, and 7 more bands were observed in the lane containing water- and salt-soluble wheat protein. The serum from a baker with generalized chronic dermatitis gave many IgE-bound bands in the lanes with both kinds of wheat protein, including the bands corresponding to 16 kDa. The serum from 2 healthy children without any allergic diseases gave no IgE-bound band for either kind of wheat protein (data not shown).

The ELISA result indicated the presence of a small quantity of wheat protein (1.50 μ g/g) in the offending rice crackers. The results for milk protein and egg protein were under the respective detection limits (0.04 μ g/g). Rice, maize starch, and "wakame" seaweed did not affect the absorbance

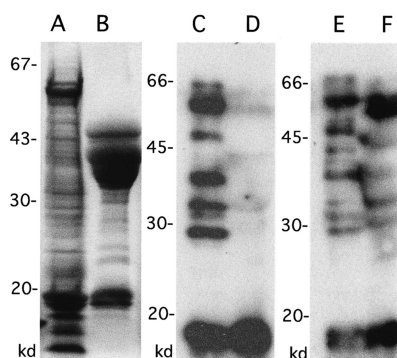


Figure 1. Sodium dodecylsulfate-polyacrylamide gel electrophoresis fractions of water- and salt-soluble wheat protein (lanes A, C, and E) and 70% ethanol-soluble wheat gliadin (lanes B, D, and F). The separated bands were stained with Coomassie blue (A and B) or probed with serum from the patient (C and D) or a baker with dermatitis (E and F) and then incubated with peroxidase-conjugated anti-IgE antibodies. IgE was detected with the enhanced chemiluminescence system. Although the strips were probed with the serum from 2 healthy children without any allergic diseases, no IgE-bound band was observed (not shown).

Table 1. Determination of Wheat Protein in Processed Rice Cracker Samples with Labels That Did Not Mention Wheat

Labeled contents	Wheat protein, $\mu\text{g/g}$
Rice, rice starch, vegetable	<0.04
Rice, rice starch	0.26
Rice, starch (wheat, soybean)*	<0.04
Rice, starch, vegetable	<0.04
Glutinous rice, maize starch, scallop (milk, soybean)	<0.04
Rice, maize starch, "wakame" seaweed†	0.78
Rice, maize starch, rice starch, vegetable, malt	1.13
Rice, rice starch, seaweed (soybean)	<0.04

* The label warns of cross-contact with these foods from the use of shared equipment in processing facilities.

† This cracker is implicated in this case.

on the respective assay plates. Trace quantities of wheat protein, ranging from 0.26 to 1.13 $\mu\text{g/g}$, were also detected in 3 of 8 rice cracker samples obtained from local food retail outlets (Table 1). We contacted the manufacturers of these products and discussed our results with them to identify any possible sources of wheat contamination. Although no specific errors in processing were identified, they informed us that the equipment used to produce and pack the rice crackers was also used to produce and pack wheat crackers.

DISCUSSION

Several clinical groups have obtained potentially useful information relating to the threshold doses of common allergenic foods through double-blind, placebo-controlled food challenges or open challenges used for diagnostic purposes. The most useful data available are for peanuts, hen's eggs, and cow's milk, with the provoking doses ranging from 0.1 to 1,000 mg of peanut protein, 2 to 7,110 mg of crude hen's egg white, and 0.1 to 200 mL of cow's milk.^{6,7} There is very little information relating to the threshold doses for wheat allergic patients. Malmheden Yman et al⁸ reported a series of incidents related to inadvertent or unexpected exposure to allergenic foods that resulted in allergic reactions in sensitive individuals, including wheat gluten in 2 incidents at levels ranging from 0.3% to 1.3% (wt/wt).

The present patient ingested 9 g of crackers containing 1.5 $\mu\text{g/g}$ of wheat protein, and thus, the total amount of wheat protein ingested was presumably 13.5 μg . Although the nature of the food materials is an important factor, creating some uncertainty, the estimated provoking dose of wheat protein in the present case was much less than those from the allergic patients with peanuts, hen's eggs, or cow's milk. There is the question, however, of whether the patients selected for double-blind, placebo-controlled food challenges or open challenges in their study were representative of the entire population of individuals with allergies to those specific foods. Most physicians acknowledge excluding some of

the most seriously affected patients (ie, those with histories of anaphylactic shock) from challenge trials. It could be argued that these patients may be among the most sensitive.

The age and body weight of the present patient should also be taken into consideration. Some of the challenges were conducted on adults. If calculated on a body weight basis, the minimum provoking doses determined in the other studies might be closer to the dose for the presented patient. In addition, the immature gastrointestinal barrier may permit increased penetration of wheat protein into the body. Infants may also have some defect in food protection due to the immature gastrointestinal immune system for oral tolerance.

This particular case demonstrates that a tiny amount of ingested wheat protein can cause an allergic reaction in susceptible individuals, requiring accurate declaration of the wheat contents of consumer products. Because of various practical limitations in food production and processing, foods may occasionally contain trace residues of an offending protein. The results of this study confirm the need for sensitive and specific methods for the control of unintentional or deliberate addition of nondeclared proteins, and the methods described in this article are recommended for this purpose.

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Requests for reprints should be addressed to:

Tomoaki Matsumoto, MD

Department of Child Development

Graduate School of Medical Sciences

School of Medicine

Kumamoto University

1-1-1 Honjo

Kumamoto 860-0811, Japan

E-mail: mac@kaiju.medic.kumamoto-u.ac.jp