

Review Series: Advances in Consensus, Pathogenesis and Treatment of Urticaria and Angioedema

Wheat-Dependent Exercise-Induced Anaphylaxis Sensitized with Hydrolyzed Wheat Protein in Soap

Yuko Chinuki¹ and Eishin Morita¹

ABSTRACT

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a specific form of wheat allergy typically induced by exercise after ingestion of wheat products. Wheat ω -5 gliadin is a major allergen associated with conventional WDEIA, and detection of serum immunoglobulin E (IgE) specific to recombinant ω -5 gliadin is a reliable method for its diagnosis. Recently, an increased incidence of a new subtype of WDEIA, which is likely to be sensitized via a percutaneous and/or rhinoconjunctival route to hydrolyzed wheat protein (HWP), has been observed. All of the patients with this new subtype had used the same brand of soap, which contained HWP. Approximately half of these patients developed contact allergy several months later and subsequently developed WDEIA. In each of these patients, contact allergy with soap exposure preceded food ingestion-induced reactions. Other patients directly developed generalized symptoms upon ingestion of wheat products. The predominant observed symptom of the new WDEIA subtype was angioedema of the eyelids; a number of patients developed anaphylaxis. This new subtype of WDEIA has little serum ω -5 gliadin-specific serum IgE.

KEY WORDS

angioedema, hydrolyzed wheat protein, percutaneous sensitization, wheat-dependent exercise-induced anaphylaxis, ω -5 gliadin

INTRODUCTION

Wheat protein derivatives are widely used in the composition of products worldwide. Industry uses gluten either with or without modifications. The main modification is hydrolysis, which is used to overcome its insolubility. Hydrolysis is performed either in acid conditions or increasingly with the use of enzymes. The procedure that is chosen and the degree of hydrolysis depend on the desired functionality and the manufacturer. Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a distinct form of wheat allergy induced by the combination of wheat ingestion and physical exercise.^{1,2} Aspirin intake is another well-known trigger for allergic symptoms.³ We identified wheat ω -5 gliadin, a component of water/salt-insoluble protein (gluten), as a major allergen in pa-

tients with WDEIA.^{1,4,7} When recombinant ω -5 gliadin was used in a fluorescent enzyme immunoassay combined with the CAP system (CAP-FEIA; Phadia, Uppsala, Sweden; detection range, 0.35-100 kUA/L), approximately 80% of patients with WDEIA tested positive.

Recently, increased incidence of a new subtype of WDEIA caused by hydrolyzed wheat protein (HWP) has been observed.⁸⁻¹⁰ Patients with this new subtype were likely to be sensitized to HWP primarily through percutaneous and/or rhinoconjunctival routes by using HWP (Glupearl 19S)-supplemented soap (Cha no shizuku). In Japan, this soap was very popular and more than 46 million soaps had been sold from March 2004 to September 2010. More than 1300 individuals who had used the soap developed allergic symptoms after ingesting natural wheat pro-

¹Department of Dermatology, Shimane University Faculty of Medicine, Shimane, Japan.

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Correspondence: Yuko Chinuki, MD, Department of Dermatology, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo,

Shimane 693-8501, Japan.

Email: ychinuki@med.shimane-u.ac.jp

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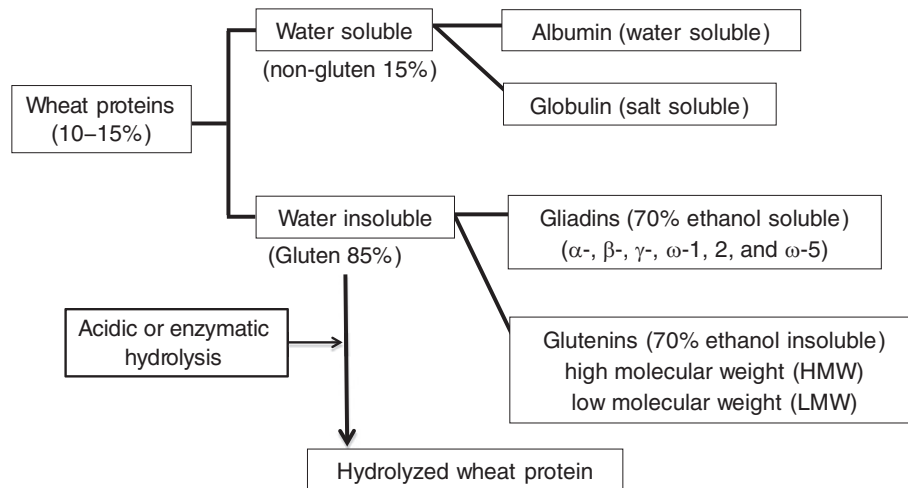


Fig. 1 Components of wheat protein.

teins to date.¹¹ Patients with this new WDEIA subtype tested positive for the HWP allergy by skin prick test (SPT) and had serum HWP-specific immunoglobulin E (IgE). In addition, the patients had characteristic features of facial angioedema distinct from the patients with conventional WDEIA (CO-WDEIA); they were designated as having WDEIA sensitized by HWP (HWP-WDEIA). We examined the sera of several patients with HWP-WDEIA by CAP-FEIA and found that these patients have no or low levels of ω-5 gliadin-specific IgE.

COMPONENTS OF WHEAT PROTEIN AND HWP¹²

Wheat flour contains 10-15% w/w protein. The wheat proteins are fractionated according to their solubility characteristics: albumins and globulins are soluble in salt solutions, while gluten proteins are precipitated by salt (Fig. 1). Among the gluten proteins, gliadins are soluble in 70% ethanol, while low and high molecular weight glutenin subunits are not. Wheat protein derivatives are used in a variety of products worldwide. HWPs are prepared either from insolubilized total flour proteins or more generally from gluten only. In order to increase the solubility of gluten, acidic or enzymatic hydrolysis is performed. Glupearl 19S, which was added to the soap (Cha no Shizuku), was produced by acid hydrolysis.

CASE REPORT⁹

We present our first patient who was given a diagnosis of HWP-WDEIA. The patient appeared to be sensitized to Glupearl 19S by using Glupearl 19S-supplemented soap.

A 49-year-old woman was referred to our clinic complaining of eyelid edema while working after ingesting bread. She had experienced similar episodes 3 times while working and 4 times while walking dur-



Fig. 2 Eyelid edema induced by challenge test with a combination of wheat and aspirin.

ing the past 11 months. Her wheat, gluten, and ω-5 gliadin antigen-specific serum IgE levels were 1.35, 1.78, and >0.34 kUA/L (CAP-FEIA, Phadia), respectively, whereas SPTs with wheat and bread were negative (Torii allergen extracts for scratch, Torii Pharmaceutical Co., Ltd., Tokyo, Japan). WDEIA was diagnosed based on a positive challenge test with the combination of wheat (120 g) and aspirin (500 mg). Eyelid edema induced by the challenge test is shown in Figure 2. No symptoms were observed with either the wheat challenge or aspirin intake alone.

A precise medical history revealed that the patient had used Glupearl 19S-supplemented soap for 1 year, and she had noticed facial wheals and nasal discharge occasionally while washing her face with the soap. She was not atopic. She was confirmed to have no other disease by blood and physical examinations. SPT showed a positive reaction to 0.1% soap solution and 0.01% Glupearl 19S solution. A face wash challenge test with the soap induced facial wheals. Sensitization to HWP was also confirmed by Western blotting. Coomassie blue staining of Glupearl 19S using sodium dodecyl sulfate polyacrylamide gel electro-

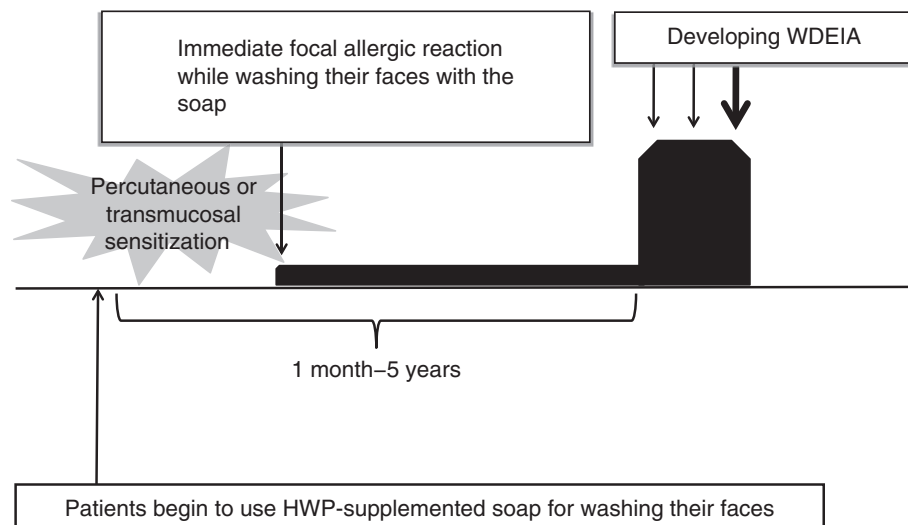


Fig. 3 Presumable time course of wheat-dependent exercise-induced anaphylaxis (WDEIA) patients sensitized to hydrolyzed wheat protein (HWP).

phoresis (SDS-PAGE) showed smears (ranging from 0-250 kDa) characteristic of random degradation and peptide rearrangement. The serum IgE of the patient reacted with the smear proteins ranging from 15-250 kDa, indicating that the IgE was specific to Glupearl 19S. In addition, the IgE of the patient reacted with both water-soluble and water-insoluble wheat proteins but not with ω -5 gliadin.

CLINICAL FEATURES OF THE PATIENTS WITH HWP-WDEIA

Almost all of the patients with HWP-WDEIA had used the same brand of soap (Cha no Shizuku), which included HWP (Glupearl 19S). Several patients developed contact urticaria on their faces several months later, and subsequently developed WDEIA. We summarized a presumable time course of patients with HWP-WDEIA (Fig. 3). After starting to use HWP-supplemented soap, patients were likely to be sensitized to HWP through the percutaneous and/or rhino-conjunctival route within 1 month to 5 years. Several patients had an immediate focal allergic reaction while washing their faces with the soap. Patients had systemic allergic symptoms when they were exposed to wheat products and subsequently exercised. In each of these patients, contact urticaria with soap preceded the food ingestion-induced reactions. The other patients directly developed generalized symptoms upon ingestion of wheat products. In some cases, allergies were triggered by mild exercise such as walking and bathing. The predominant observed symptom in all of the patients was angioedema of the eyelids; most of the patients developed anaphylaxis, and some of them developed anaphylactic shock. Several patients had pollinosis, and a few patients had atopic dermatitis.

IMMUNOLOGICAL STUDIES OF HWP-WDEIA PATIENTS

In our hospital, approximately half of the patients with HWP-WDEIA tested positive in the SPT to wheat and bread allergens. Almost all of the patients tested positive by SPT to 0.1% solution of soap supplemented with HWP in saline and 0.01% HWP solution diluted with saline. None of the patients with CO-WDEIA had positive reactions to diluted soap and HWP solution. None of the healthy subjects reacted to SPT with any of these allergens. Wheat protein-specific IgE was detected by CAP-FEIA. Wheat- and gluten-specific IgE were detected in almost all of the patients with HWP-WDEIA. A few patients with HWP-WDEIA had ω -5 gliadin-specific IgE. However, the level of IgE to ω -5 gliadin was significantly lower in patients with HWP-WDEIA than in those with CO-WDEIA. Challenge tests including exercise, wheat ingestion, aspirin intake, and/or a combination of these tests were performed for some patients.¹³ In almost all of the patients, a combination of aspirin/exercise and wheat challenge induced allergic symptoms such as angioedema, urticaria, and dyspnea.

SDS-PAGE AND WESTERN BLOTTING^{9,10}

To detect HWP-specific IgE in HWP-WDEIA patients, we performed SDS-PAGE and Western blotting. Coomassie blue staining of Glupearl 19S electrophoresed using SDS-PAGE showed smears characteristic of random degradation and rearrangement of peptides (Fig. 4). These smears spanned most of the gel, ranging from 0-250 kDa. In contrast, SDS-PAGE of wheat proteins showed characteristic bands mainly existing in the area of 25-100 kDa.

Representative blots are shown in Figure 4. The

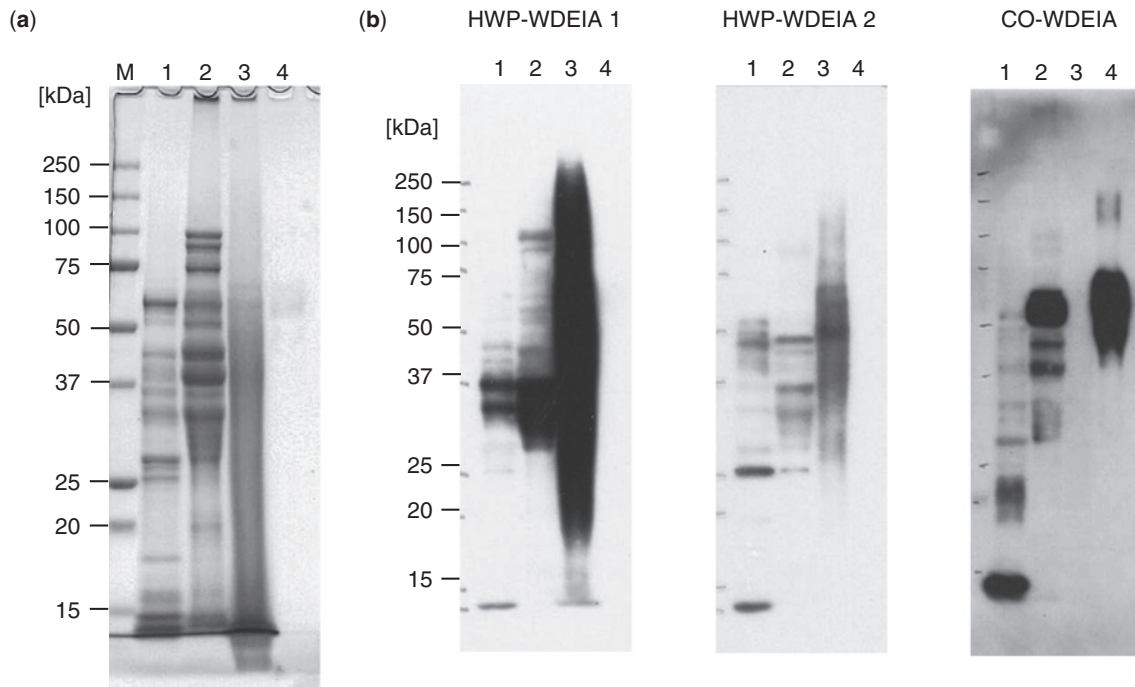


Fig. 4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting analyses of wheat protein fractions and hydrolyzed wheat protein (HWP [Glupearl 19S]). (a) Gel stained with Coomassie Brilliant Blue; (b) Immunoglobulin E (IgE) immunoblotting with the patients' sera (2 HWP-wheat-dependent exercise-induced anaphylaxis [WDEIA] patients and 1 conventional [CO]-WDEIA-patient). M, marker proteins; Lane 1, salt-soluble wheat proteins; Lane 2, salt-insoluble wheat proteins; Lane 3, Glupearl 19S; Lane 4, purified ω -5 gliadin.

IgE of all of the HWP-WDEIA patients reacted to Glupearl 19S with a smear pattern. The intensity of the reaction varied among the patients. The IgE of all of the patients also reacted with both water-soluble and water-insoluble wheat proteins, whereas they did not react with ω -5 gliadin. The IgE of the CO-WDEIA patients did not react with HWP, but reacted with water-soluble and water-insoluble wheat proteins, and ω -5 gliadin.

To perform immunoblotting inhibition assays for determining cross-reactivity of wheat proteins and Glupearl 19S, 100 μ L of serum was previously incubated with Glupearl 19S serially diluted from 100 to 5 μ g/mL. The reaction of the IgE to water-soluble and water-insoluble wheat proteins was inhibited by Glupearl 19S in a dose-dependent manner when the sera of HWP-WDEIA patients were preincubated with a series of Glupearl 19S concentrations (Fig. 5).

INDUCTION OF BASOPHILIC CD203c EXPRESSION BY GLUPEARL 19S AND ω -5 GLIADIN

Flow cytometry-based tests of basophil activation status have been used to diagnose or confirm sensitization in allergic patients.¹⁴⁻²⁵ CD203c is an ectoenzyme that belongs to a family of ectonucleotide pyrophosphatases and phosphodiesterases. It is ex-

pressed on the cell membrane of human peripheral basophils and mast cells, and cross-linking of the high-affinity IgE receptor upregulates membrane CD203c expression.

Glupearl 19S enhanced CD203c expression of basophils in a concentration-dependent manner in all of the HWP-WDEIA patients.¹⁰ In these patients, no significant enhancement of CD203c was observed with purified ω -5 gliadin. In contrast, purified ω -5 gliadin induces enhancement of CD203c expression in a concentration-dependent manner in CO-WDEIA patients, whereas no significant enhancement of CD203c is observed in the presence of Glupearl 19S. A representative reaction is shown in Figure 6.

INFORMATION FROM THE JAPANESE SOCIETY OF ALLERGOLOGY¹¹

Information concerning the wheat allergy associated with the use of soap bars containing Glupearl 19S provided in the Rheumatism & Allergy Information Center website includes "FAQs (for general consumers)," the "Definition of the disorder and diagnostic guidelines (for healthcare professionals)," and a "List of institutions offering treatment for wheat allergy associated with the use of 'Cha no Shizuku' soap." The "Diagnostic criteria for immediate wheat allergy to the hydrolyzed wheat (Glupearl 19S) contained in

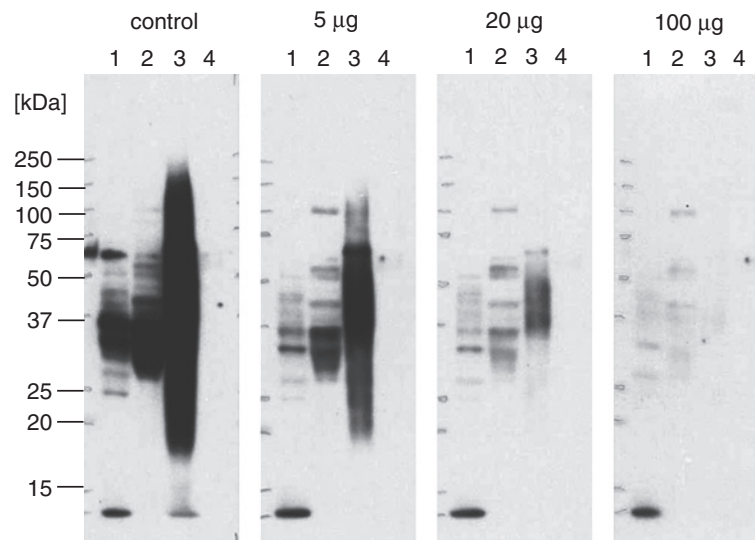


Fig. 5 Immunoblot inhibition assay for determining cross-reactivity of wheat proteins and Glupearl 19S. Lane 1, salt-soluble wheat proteins; Lane 2, salt-insoluble wheat proteins; Lane 3, Glupearl 19S; Lane 4, purified ω -5 gliadin. Electrophoresed membranes were blotted against patient sera (hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis [HWP-WDEIA]) without Glupearl 19S (control) or with increasing amounts of Glupearl 19S (5 μ g, 20 μ g, and 100 μ g).

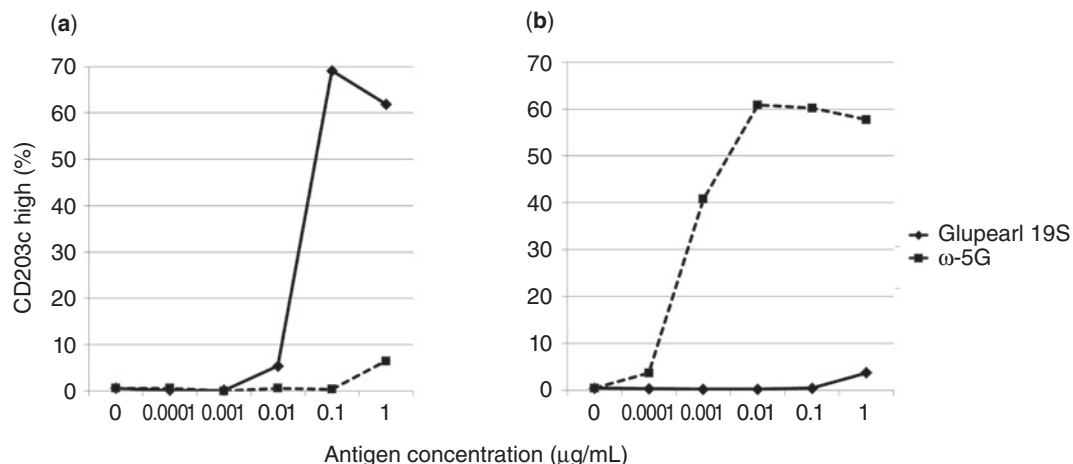


Fig. 6 Expression of CD203c on basophils induced by Glupearl 19S and ω -5 gliadin (ω -5G). (a) Results from a 44-year-old woman with hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis (HWP-WDEIA). Serum allergen-specific IgE to wheat, gluten, and ω -5G was 9.26 kUA/L, 15.8 kUA/L, and 1.16 kUA/L, respectively. (b) Results from a 39-year-old woman with conventional wheat-dependent exercise-induced anaphylaxis (CO-WDEIA). Serum allergen-specific IgE to wheat, gluten, and ω -5G was <0.34 kUA/L, 1.90 kUA/L, and 3.82 kUA/L, respectively.

‘Cha no Shizuku’ soap and some other products” have been published by the Special Committee for the Safety of Protein Hydrolysate in Cosmetics of the Japanese Society of Allergology (see below).

Diagnostic criteria for immediate wheat allergy

to the hydrolyzed wheat (Glupearl 19S) contained in “Cha no Shizuku” soap and some other products (Prepared by the Special Committee for the Safety of Protein Hydrolysate in Cosmetics on October 11, 2011)

[Definitive diagnosis]

Table 1 Comparison between CO-WDEIA and HWP-WDEIA

	CO-WDEIA	HWP-WDEIA
Age	School child age-old age	Adults in their 20s-60s
Gender	Both genders	Predominantly female
History of HWP-supplemented soap use	None	Essential
Symptoms occur while using the soap before developing WDEIA	None	Often
Predominant symptom of WDEIA	Urticaria (wheal)	Angioedema (especially on the eyelids)
Anaphylactic shock	Sometimes	Occasionally

Abbreviations: CO-WDEIA, conventional wheat-dependent exercise-induced anaphylaxis; HWP-WDEIA, hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis.

Satisfying all of the following criteria:

1. Have used “Cha no Shizuku” soap or other products containing hydrolyzed wheat (Glupearl 19S)
 2. Have had at least 1 of the following symptoms:
 - 2-1. Itching, eyelid edema, nasal discharge, and/or wheals within several to 30 minutes after using “Cha no Shizuku” soap or other products containing hydrolyzed wheat (Glupearl 19S)
 - 2-2. General symptoms such as itching, wheals, eyelid edema, nasal discharge, dyspnea, nausea, vomiting, abdominal pain, diarrhea, and decreased blood pressure within 4 hours after eating wheat products
 3. Have tested positive in at least 1 of the following tests:
 - 3-1. Prick test using $\leq 0.1\%$ Glupearl 19S solution
 - 3-2. Immunoassay such as dot blot, enzyme-linked immunosorbent assay (ELISA), and Western blot to identify specific IgE antibody to Glupearl 19S in the blood
 - 3-3. Basophil activation test using Glupearl 19S as the antigen
- [Exclusion criterion]
4. Tested negative in a prick test using 0.1% Glupearl 19S solution

[Suspected cases]

Satisfying Criteria 1 and 2 but not 3

*Wheat allergy is strongly suspected if sensitization to wheat or gluten is shown in a specific IgE antibody test or a prick test but there is no hypersensitivity to ω -5 gliadin or milder hypersensitivity to ω -5 gliadin compared with that to wheat and gluten.

COMPARISON BETWEEN CO-WDEIA AND HWP-WDEIA

Clinical features of CO-WDEIA and HWP-WDEIA observed in our hospital are summarized in Table 1. CO-WDEIA occurred more often in expanded age-group. HWP-WDEIA developed more commonly in women who use soap as cosmetics. In HWP-WDEIA, history of HWP-supplemented soap use is essential, and symptoms that occur while using the soap before

Table 2 Positivity rate of ω -5 gliadin-specific IgE and high molecular weight glutenin-specific IgE measurement in CO-WDEIA and HWP-WDEIA patients

	CAP-FEIA	CO-WDEIA (%, n = 54)	HWP-WDEIA (%, n = 30)
Wheat		31.4	70.0
Gluten		37.0	76.6
ω -5 gliadin		79.6	6.6
High molecular weight-glutenin		18.5	16.6
ω -5 gliadin and/or high molecular weight-glutenin		94.4	16.6

Abbreviations: CAP-FEIA, fluorescent enzyme immunoassay combined with the CAP system; CO-WDEIA, conventional wheat-dependent exercise-induced anaphylaxis; HWP-WDEIA, hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis.

the development of WDEIA often appear. A predominant symptom of CO-WDEIA is the development wheals on the entire body, whereas that of HWP-WDEIA is angioedema on the eyelids.

Anaphylactic shock sometimes developed in CO-WDEIA patients and occasionally developed in HWP-WDEIA patients.

Measurement of gluten-specific IgE as well as wheat-specific IgE is possible in the diagnosis of WDEIA using the CAP-FEIA; however, more than 60% of patients with definite CO-WDEIA are considered negative by these tests. Recently, recombinant food allergens, which are consistent in quality, have been produced and applied for the diagnosis of many food allergies. Measurement of IgE that is specific to ω -5 gliadin and high molecular weight-glutenin is highly useful in diagnosing CO-WDEIA when compared with the routine diagnostic CAP-FEIA for wheat and gluten.²⁶ As shown in Table 2, in our hospital, 79.6% of the patients with CO-WDEIA have IgE that reacted to recombinant ω -5 gliadin. Additionally, 94.4% of the CO-WDEIA patients were positive according to the combined recombinant ω -5 gliadin-specific IgE test and the recombinant high molecular weight-glutenin-specific IgE test, whereas gluten- and

wheat-specific IgE tests positively recognized only 31.4% and 37.0% of these patients, respectively. In contrast, the positive rate of ω -5 gliadin-specific IgE was only 6.6% in HWP-WDEIA patients, whereas gluten- and wheat-specific IgE tests positively recognized 70.0% and 76.6% of these patients, respectively.

PERCUTANEOUS SENSITIZATION TO FOOD ALLERGENS

The importance of percutaneous sensitization in the development of food allergies has been well recognized in the case of latex allergies in which fruits and vegetables are the causative agents.²⁷ Direct percutaneous sensitization by peanuts has recently been suggested by Fox *et al.*²⁸ who identified a dose-response relationship between environmental (non-oral) peanut exposure and the development of peanut allergies. HWP should now be considered a possible allergen for percutaneous sensitization, because they are currently used globally as ingredients of cosmetic products, and HWP cross-reacts to the wheat allergen in foods and may cause life-threatening anaphylaxis once sensitized. Several cases of contact urticaria due to HWP in cosmetics have been described.^{12,29-32} These findings indicate percutaneous and/or rhinoconjunctival penetration of HWP in patients. Some of these patients have developed generalized allergic symptoms upon ingestion of wheat products. Some of our HWP-WDEIA patients also experienced contact urticaria by using Glupearl 19S-supplemented soap and later developed generalized symptoms upon ingestion of wheat products. Another group of patients directly developed generalized symptoms upon ingestion of wheat products. Hydrophilic moieties of allergens might be necessary for percutaneous and/or rhinoconjunctival sensitization, as Glupearl 19S was hydrolyzed under acidic conditions and was highly allergenic. This process yields new terminal amino- and carboxyl-charged groups. Matsuo *et al.* reported that serum IgE of patients with wheat protein contact dermatitis reacted to water-soluble proteins rather than water-insoluble proteins.³³ They identified 3 water-soluble proteins, peroxidase, purple acid phosphatase, and wheat 27-kDa allergen, as candidate allergens for wheat protein contact dermatitis. These results suggest that glycan moieties in these proteins are involved in IgE binding.

When considering sensitization by the percutaneous and/or rhinoconjunctival route, a penetration of the molecule through the epithelia must occur. Large molecules such as proteins are generally not expected to cross the skin barrier, unless the skin has been damaged.³⁴ However, a few patients with HWP-WDEIA had atopic dermatitis, a condition arising from impaired function of the skin barrier. In addition to creation of new terminal amino- and carboxyl-charged groups due to the hydrolysis of peptide bonds, soap containing HWP might facilitate the

penetration of HWP into the epidermis, because soap contains surfactants that may cause destruction of skin barriers. It has been proposed by Lack that antigen exposure through inflamed skin might be involved in the establishment of allergy and tolerance.³⁵ He reported in his review that allergic sensitization to food could occur through low-dose cutaneous sensitization and that early consumption of food protein induced oral tolerance. He argued that low-dose exposure to environmental foods (on tabletops, hands, and dust) penetrates the skin barrier and is taken up by Langerhan's cells. This leads to T helper type 2 (TH2) responses and IgE production by B cells. In contrast, early high-dose oral consumption induces tolerance, and T helper type 1 (TH1) and regulatory T-cell responses occur in the gut-associated lymphoid tissue. The timing and balance of cutaneous and oral exposure determines whether a child has allergy or tolerance (dual-allergen-exposure hypothesis). Leduc *et al.* have suggested that acidic hydrolysis induces a conformational change in HWP and produces a conversion of a glutamine residue to glutamic acid and a conversion of an asparagine residue to aspartic acid.³⁶ As a result, new epitopes that differ from the epitopes of natural wheat proteins might be produced. It is conceivable that humans do not have sufficient tolerance to HWP, which are not natural proteins. Thus, humans appear to be easily sensitized to HWP once HWP penetrates into the skin or mucosa. As wheat proteins contain repetitive amino acid structures highly rich in glutamine and proline, it is likely that IgE produced against HWP cross-reacts to natural wheat proteins. In fact, preincubation of sera with HWP clearly revealed a decrease in the binding of IgE to natural wheat proteins (Fig. 5).

IgE produced against HWP in HWP-WDEIA patients do not react to ω -5 gliadin but instead to other undetermined protein components with specific epitopes. The patients with HWP-WDEIA had no or decreased levels of ω -5 gliadin-specific IgE.

HWPs are prepared either from insolubilized total flour proteins or more generally from gluten alone. The main modification of gluten is hydrolysis performed to overcome its insolubility. HWP, which includes large polypeptide aggregates, has a greater ability to induce sensitization than the HWP that is digested to lower molecular weight polypeptides.³⁷ This finding is supported by the previous observation by Palosuo *et al.*³⁸ that artificial polymerization of ω -5 gliadin increases its direct reactivity with IgE in an immunosorbent assay and in patients using SPT. They also hypothesized that large polymers have better IgE-bridging capacities.

CONCLUSIONS

We experienced an outbreak of wheat allergy with systemic symptoms, which may be due to percutaneous and/or a rhinoconjunctival sensitization following

the use of cosmetics. Such an event was almost completely unknown by healthcare professionals until recently. Thus, there is limited information regarding the clinical course. The serum IgE levels of many HWP-WDEIA patients against wheat and gluten decreased after the cessation of HWP-containing soap usage. In some patients, serum IgE against these antigens had disappeared. A remission case of HWP-WDEIA has been reported³⁹ and we also experienced some patients who had remission of WDEIA-symptoms (unpublished observation). However, many patients with HWP-WDEIA have not recovered from WDEIA-related symptoms. Large-scale studies are needed to clarify the prognosis of HWP-WDEIA patients.

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