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Clinical relevance of sensitization to hydrolyzed wheat protein in wheat-sensitized individuals

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Title:
Clinical relevance of sensitization to hydrolyzed wheat protein in wheat-sensitized individuals

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Capsule summary:
Patients with allergy to hydrolyzed wheat protein (HWP) are mono-sensitized and tolerate ingestion of non-hydrolyzed wheat products. Some patients with other wheat allergy phenotypes recognize HWP in sIgE, BaHR and SPT, but apparently without clinical relevance.

Key words: Hydrolyzed wheat protein, anaphylaxis, wheat allergy, wheat dependent exercise induced anaphylaxis, oral challenge, specific IgE, basophil histamine release, skin prick test.


Word count: 1288
To the Editor:

Wheat is the source of allergens for different type-I allergies comprising classical food allergy, wheat-dependent, exercise-induced anaphylaxis (WDEIA) and occupational allergic rhinoconjunctivitis or asthma (1).

Hydrolyzed wheat proteins (HWP) are used in a variety of products ranging from food to cosmetics. Wheat hydrolysates are produced by either a chemical process by heating gluten at acidic pH or by enzymatic treatment. This provides the food industry a wider range of use, as the hydrolyzed product is soluble in water. Although, the general opinion is, that hydrolysis reduces allergenicity the number of reported cases and studies on sensitization and allergy to HWP is limited (2–7).

The aim of this study was to characterize patients with a case-history of anaphylaxis related to ingestion of a product containing HWP and to investigate patients with other phenotypes of wheat allergy for co-sensitization to HWP.

Details of the methods can be found in this article’s Methods section in the Online Repository at www.jacionline.org. In this study we looked at a single hydrolyzed wheat product, Meripro 711 (Tate and Lyle, Syral, Aalst, Belgium). In brief, we assessed 9 patients with a history of anaphylaxis to HWP and for comparison we determined the frequency and assessed the potential relevance of HWP-sensitization in an existing cohort of 45 patients with suspicion of WIA and WDEIA (see Figure 1).

All patients were examined with detailed case-history, skin prick test (SPT), specific IgE (sIgE) and basophil histamine release (BaHR) followed, by an open food challenge (OFC) with the cake mix containing HWP to the extent where it was available since the product (AMO Letbagt®) was withdrawn from the market after the first public reports on allergic reactions. The WIA and WDEIA patients were challenged with wheat or gluten (+exercise).

The total positive rate of sIgE to Meripro 711 was 83.9% (47/56), SPT 83.3% (35/42) and BaHR 57.1% (24/42). Fourteen patients were positive to Meripro 711 in both sIgE, SPT and BaHR, of whom 7 patients were found in the HWP group. In total 9 (16.0%) patients (female/male ratio 9:0, median age 31.1 years, [16.8-68.3]) were identified with a case-history of anaphylaxis related to a HWP containing product (see Table 1). The same cake-mix product (AMO Letbagt®, containing Meripro 711) was identified as culprit for patient 1-7. Patient 8 and 9 reacted to different foods containing HWP but with the same pattern in sIgE, BaHR and SPT as for patient 1-7. Whether their products contained Meripro 711 or the reactions were due to a different HWP is unknown due to unavailability of recipes of culprit foods for patient 8-9.

All 9 patients tolerated ingestion of regular non-hydrolyzed wheat products (such as bread or pasta) on regular basis, and none had any previous episodes of food allergy.

The serum level of Meripro 711 specific IgE was higher in patients with a case-history of HWP (median 5.3 kU/L, [2.12;21.9]) compared to the WIA (median 2.1 kU/L [0.02;56.6]) and WDEIA (median 0.8 kU/L [0.0;19.2]) groups (p<0.05)(see Figure 2), with no significant difference in sensitization pattern between men and women. Same picture was found for SPT for Meripro 711 (median 6.0 mm, [5.5;16.5]) (p<0.05) and BaHR for Meripro 711 (median threshold level 0.003 µg/mL [0.0;1]) (p<0.05). The commercially available test for wheat-specific IgE (f4) was only positive in one patient and wheat components normally associated with other wheat allergy phenotypes showed no response (8). Weak reactivity in BaHR to wheat (between 300-100 µg/mL, ie. a factor $10^5$ less sensitive) was found in 6/9 patients (see Table 1).

Forty-seven patients were co-sensitized to Meripro 711 either by sIgE 85.1% (40/47) or SPT 79.4% (27/34), with 47.0% (16/44) being positive in both.
In the WIA group (female: male ratio 10.9, median age 10.3 years, [1.5; 77.2]) the positive rate of sIgE to wheat was 100% (19/19) and Meripro 711 84.2% (16/19) with a predominance of children <15 years of age (n=11). SPT and BaHR with Meripro 711 was not routinely performed in children, therefore only 6, two of whom were positive, had this performed. In the WDEIA group (female: male ratio 12:16, median age 47.5 years, [21.4; 72.2]) the positive rate of sIgE to omega-5 gliadin was 82.1% (23/28) and to Meripro 711 78.6% (22/28). Among 71 patients suspected for WDEIA 30.1% (22/71) had an elevated sIgE to omega-5 gliadin. Positive BaHR to Meripro 711 (51.9% (14/27)) showed a wide range of reactivity (median threshold level 0.03 µg/mL [0;300]). SPT to Meripro 711 was positive in 79.0% (22/28)(median 3.0 mm, [3.0;7.5]). No significant difference in sensitization (sIgE or SPT) to Meripro 711 was found between the challenge positive and negative patients in the WIA or WDEIA group.

There are three important findings in this study. First, hydrolyzed wheat protein should be considered as a causative allergen in cases of severe systemic reactions after food intake, where a culprit food cannot be pinpointed and where the patient regularly ingest wheat. Labelling of HWP is mandatory in many countries but often omitted, where the products already contains wheat. Second, HWP patients are mono-sensitized to HWP and tolerate ingestion of non-hydrolyzed wheat products. Third, some patients with other wheat allergy phenotypes recognize Meripro 711 in sIgE, SPT and BaHR but apparently without clinical relevance.

Meripro 711 is a distinct example of a HWP-containing allergenic product and has only been described in one previous case report (6). All mono-sensitized to HWP were women and probably being more likely to have used cosmetics and potentially have become sensitized through the skin. However, none of the patients in the HWP group had experienced any form of contact urticaria to cosmetics containing HWP. The route of sensitization remains elusive in all three groups.

Recent studies hypothesize primary sensitization through the skin or mucosa (9): These patients had used a HWP (Glupearl 19S) in facial soap, and developed contact urticaria at the application site and subsequently anaphylaxis upon ingestion of unmodified wheat in foods. A different pattern is described by Laurière et al, describing contact urticaria to cosmetics containing HWP and subsequent anaphylaxis to food containing HWP in patients tolerating unmodified wheat products.

It is expected that sIgE of patients with other wheat allergies (WIA and WDEIA) recognize Meripro 711 since it originates from wheat and therefore must have retained some of the original allergenicity. It may be speculated, that hydrolysis rendering the molecules water soluble, unveil allergens, normally hidden in the intact gluten. However, the clinical relevance of co-sensitization to Meripro 711 is questionable and likely irrelevant, but due to the absence of obtainable purified Meripro 711 this could neither be confirmed nor ruled out by challenge.

A weakness of this study is that only two patients were challenged with the cake-mix and not a purified product. However, the specific cake-mix containing Meripro 711 was withdrawn from the Danish market in 2010, and we failed to obtain further purified product from the manufacturer. Furthermore, very severe reactions to the cake-mix were seen to minute amounts in the first 2 patients. Meripro is still available from the manufacturer and other food products thus still contain HWP. None of the patients in the WIA or WDEIA groups were challenged with Meripro 711, but all had a negative case-history to HWP. A reaction to Meripro 711 in the WIA and WDEIA patients cannot be ruled out especially in the challenge negative patients in the cohorts of WIA and WDEIA, but could not be elucidated due to the aforementioned unavailability of purified challenge material.

In conclusion, reactivity to HWP seems to be restricted to patients specifically sensitized to this heterogeneous group of products without concomitant allergy to non-hydrolyzed wheat.
Further investigations into HWP is needed, in order to evaluate the allergenicity of different wheat hydrolysates created by different processes of hydrolysis and the possible cross-reactivity between the resulting mixtures of epitopes.

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Conflict of interest

CGM has been a board member and received honorarium for lectures from Novartis. CBJ has received honorarium for an expert report on wheat hydrolysates from Lantmännen, Cerealia A/S. LKP has received consulting fees from Novozymes A/S. PSS is a research consultant for Reflab ApS. MJC has no conflict of interest to disclose.
References


Figure legends

Figure 1. Flowchart of patients sensitized to hydrolyzed wheat protein
P: Positive, N: Negative, n.d.: not done, HWP: Hydrolyzed wheat protein (n=9),
WIA: Wheat induced anaphylaxis (n=19), WDEIA: Wheat-dependent exercise-induced anaphylaxis (n=28).
*Sensitized either with sIgE and/or SPT.
** The seven patients in the HWP group were not challenged due to very severe initial reactions to the cake
mix and due to lack of purified culprit material.
*** The challenge negative patients were subsequently diagnosed with other allergies: WIA; egg (n=5),
peanut (n=2), cashew (n=1), milk (n=1). WDEIA; lost to follow-up (n=2), celery (n=1), unknown (n=1).

Figure 2. Comparison of specific IgE in the HWP, WIA and WDEIA group
HWP: Hydrolyzed wheat protein (n=9), WIA: Wheat induced anaphylaxis (n=19), WDEIA: Wheat-dependent
exercise-induced anaphylaxis (n=28).
*p<0.05 between meripro711 and omega-5 (f416) and grass (g6).

Table 1. Test results and clinical features of the patients with a HWP case-history
F: Female, Ang: Angioedema, Diar: Diarrhea, Dysp: Dyspnoea, OAS: Oral allergy syndrome, Urt: Urticaria
HWP: Hydrolyzed wheat protein, WDEIA: Wheat-dependent exercise-induced anaphylaxis
sIgE: Specific IgE (kU/L), SPT: Skin prick test (mm), BaHR: Basophil histamine release (µg/mL).
* were subsequently also diagnosed with WDEIA.
Table 1: Test results and clinical features of the patients with a HWP case-history

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
<th>Patient 8</th>
<th>Patient 9</th>
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</thead>
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<tr>
<td>Age/Gender</td>
<td>63/F</td>
<td>61/F</td>
<td>31/F</td>
<td>43/F</td>
<td>24/F</td>
<td>49/F</td>
<td>29/F</td>
<td>31/F</td>
<td>17/F</td>
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<tr>
<td>Symptoms on intake of HWP product</td>
<td>Urt, Dysp</td>
<td>Ang, Dysp</td>
<td>Urt, Diar, Dysp</td>
<td>OAS</td>
<td>Urt</td>
<td>Urt</td>
<td>OAS, urt</td>
<td>Urt</td>
<td>Ang</td>
</tr>
<tr>
<td>Food type</td>
<td>Cake mix</td>
<td>Cake mix</td>
<td>Cake mix</td>
<td>Cake mix</td>
<td>Cake mix</td>
<td>Cake mix</td>
<td>Cake mix</td>
<td>Bread roll</td>
<td>Cake Mix</td>
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<tr>
<td>Tolerates wheat</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>(Yes)*</td>
<td>Yes</td>
<td>(Yes)*</td>
<td>Yes</td>
<td>(Yes)*</td>
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<tr>
<td>Other diseases</td>
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<td>Thyroiditis</td>
<td>Atopic dermatitis</td>
<td>none</td>
<td>none</td>
<td>Hand eczema</td>
<td>none</td>
</tr>
<tr>
<td>slgE Meripro 711</td>
<td>2.12</td>
<td>12.4</td>
<td>9.1</td>
<td>5.2</td>
<td>17.6</td>
<td>5.2</td>
<td>4.1</td>
<td>5.3</td>
<td>21.9</td>
</tr>
<tr>
<td>slgE Wheat</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
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<td>2.2</td>
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<td>slgE Omega-5 gliadin</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
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<td>&lt; 0.35</td>
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<tr>
<td>slgE Gliadin</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
<td>0.56</td>
<td>&lt; 0.35</td>
<td>&lt; 0.35</td>
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<td>&lt; 0.35</td>
</tr>
<tr>
<td>SPT Meripro 711</td>
<td>5.5</td>
<td>5.0</td>
<td>n.d.</td>
<td>12.0</td>
<td>6.5</td>
<td>16.5</td>
<td>5.5</td>
<td>5.5</td>
<td>7.0</td>
</tr>
<tr>
<td>SPT Wheat</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n.d.</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
<td>3.5</td>
<td>7.5</td>
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<tr>
<td>BaHR Meripro 711</td>
<td>0.01</td>
<td>0.003</td>
<td>0.01</td>
<td>0.01</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>Negative</td>
<td>0.1</td>
</tr>
<tr>
<td>BaHR Wheat</td>
<td>100</td>
<td>100</td>
<td>300</td>
<td>100</td>
<td>Negative</td>
<td>Negative</td>
<td>100</td>
<td>Negative</td>
<td>3</td>
</tr>
</tbody>
</table>
Figure 1

- **HWP sensitized**
  - (n=56)
  - HWP case-history
    - (n=9)
  - WIA
    - (n=19)
  - WDEIA
    - (n=28)
  - Children
    - (n=11)
  - Adults
    - (n=8)
  - Challenge outcome
    - (wheat)
      - P=4, N=7
    - (gluten ± exercise)
      - P=24, N=4
  - Challenge outcome
    - (cake mix)
      - P=2, n.d.=7
  - Co-sensitized
Specific IgE

Log (kU/L)

- **HWP**
  - p<0.05
- **WIA**
  - p>0.05
- **WDEIA**
  - p>0.05*

Meripro 711 (u777)
Wheat (f4)
Omega-5 gliadin (f416)
Grass (g6)

Meripro 711 (u777)
Wheat (f4)
Omega-5 gliadin (f416)
Grass (g6)

Meripro 711 (u777)
Wheat (f4)
Omega-5 gliadin (f416)
Grass (g6)

- p<0.05
- p>0.05
- p>0.05*
Methods

Study population

In May 2010 the first patient (index patient #1) was identified at the Allergy center, Odense University Hospital, Denmark, based on a case-history of anaphylaxis immediately after intake of a cake mix product (AMO Letbagt, Lantmännen Cerealia A/S, Stockholm, Sweden). One of the components in the cake mix was palm oil in which partially hydrolyzed gluten (Meripro 711) was identified. Meripro 711 is a chemically modified wheat gluten emulsifier supplied by Tate and Lyle (Syral, Aalst, Belgium) developed for use in fat concentrates and creamers.

From May 2010 to August 2015 we investigated 56 patients (31 female, 25 male, mean age 39.0 years [1.1 – 78.6] years) sensitized to Meripro 711, either by skin prick test (SPT) and/or specific immunoglobulin E (sIgE) or basophil histamine release (BaHR). Serological tests and SPT for Meripro 711 were routinely performed in all adults with suspicion of wheat allergy. However, SPT and BaHR for Meripro 711 were initially only performed in selected children with a case-history to an easy-to-bake product while sIgE to Meripro 711 was performed in all children with suspicion of wheat allergy. Serological testing for celiac disease did not identify any positive candidates for further intestinal biopsy.

The population sensitized to Meripro 711 was divided into three groups based on case-history:

1. Case history of an allergic reaction to ingestion of a HWP product (group 1, n=9)
2. Case history of an allergic reaction to ingestion of a wheat product; WIA (group 2, n=19).
3. Case history of an allergic reaction to ingestion of a wheat product in combination with physical exercise; WDEIA (group 3, n=28) (see figure 1)

A detailed case history and clinical examination were performed in all patients together with SPT, BaHR and measurement of specific IgE. Challenges were performed in HWP, WIA and WDEIA groups according to EAACI guidelines. Two patients (patient #1 and patient #2), were challenge positive with the cake mix. Since patient #2 developed a severe anaphylactic reaction to a minute amount of the cake mix (1.2 g) which corresponds to approximately 7.5 mg Meripro 711, challenges were omitted in the remaining 7 patients for safety reasons. No challenges with purified Meripro 711 were performed, due to the reaction of the mixed product and lack of availability.

All data were extracted anonymously from the Allergy Centre database (AC-Base, an approved clinical database, No. 15/18486). The study was approved by The Danish Data Protection Agency, No. 16/13456 and not supported by any funding. CBJ was commissioned by the manufacturer of the ready-to-bake cake mix to write a report on the findings for their interaction consultations with their insurance company.

SPT, sIgE and BaHR

SPT was performed on the volar surface of the forearm according to EAACI guidelines. A positive SPT was defined as a mean wheal diameter of ≥ 3 mm. Histamine dihydrochloride (10 mg/ml, ALK, Copenhagen, Denmark) and physiological saline were used as positive and negative controls. Meripro 711 was diluted to 10 mg/ml in sterile physiologic saline and reactions read after 15 minutes. Prick to prick with other cereals were performed if relevant to case-history. Antihistamines and other drugs interfering with SPT were discontinued at least three days prior to testing.

Specific IgE to regular wheat (f4), rye (f5), barley (f6), omega-5 gliadin (f416), gliadin (f98), and inhalant allergens was analyzed by using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Experimental ImmunoCAP tests to Meripro 711 (U977) were developed after initial ELISA measurements were performed and they confirmed each other. The cut-off for positive sIgE levels was ≥ 0.35 kU/L.

BaHR was performed according to the method described by Stahl Skov P. et al. Briefly, whole blood collected in heparinized tubes from patients and controls were centrifuged and plasma was removed and
replaced by the same volume of PIPES buffer (10 mM piperazine-N,N(bis-ethane sulfonic acid, 140 mM sodium acetate, 5 mM potassium acetate, 0.6 mM CaCl$_2$, 1.1 mM MgCl$_2$, 1 mg/mL glucose, 0.3 mg/mL HSA, 15 IE/mL heparin). Afterwards, aliquots of 25 µL washed blood in the presence of IL-3 (2 ng/mL blood) were incubated in the glass fibre-prepared microtiter plates, HR-Test plates from RefLab ApS, Copenhagen, Denmark) for 60 min at 37°C with 25 µl of test allergens in twelve dilutions from ranging from 10 µg/mL to 0.0003 µg/mL. As a positive control of cell reactivity 25 µl of anti-IgE were included in other wells as well as histamine in a concentration of 50ng/mL. After incubation the microtiter plate was washed with H$_2$O for 1 min to remove cells and interfering substances. Thereafter 75 µl NaOH/ortho-phtaldialdehyde (OPA) mixture was added to each well allowing glass fiber bound histamine to be released and coupled to OPA. After 10 min the coupling reaction was stopped and histamine-OPA complexes stabilised by adding 75 µl of 0.59% HClO$_4$. Histamine was determined fluorometrically in the Histareader™ 501 (RefLab, Copenhagen, Denmark). The threshold concentration of Meripro 711 inducing a positive histamine release was translated to a titre ranging from 1 to 12 where 1 is the strongest Meripro threshold concentration and 12 the lowest concentration. The analytical detection limit of HR-Test is ≥10 ng histamine/mL corresponding to 3 x SD of background values.

The highest Meripro 711 concentration chosen for this test (10 µg/mL) corresponding to titer 1 was the highest concentration producing no unspecific histamine release in five non-allergic subjects tested in the range of 1000; 300; 100; 30; 10; 3; 1; 0.3; 0.1; 0.03; 0.01; 0.003 µg/mL.

Statistical analysis
For the purpose of statistical analysis, sIgE values <0.35 kU/L were assigned a value of 0.01 kU/L. Results are given as medians and 25-75 percentiles. Statistical significance was calculated using the Mann-Whitney U-test and defined for all comparisons by p values <0.05.

Statistical analyses were performed using statistical software (STATA version 14.0, StataCorp, College Station, TX, USA).