A Case of Anaphylaxis After Ingestion of Oats: Research Into New Allergens

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J Investig Allergol Clin Immunol 2022; Vol. 32(6): 506-508
doi: 10.18176/jiaci.0798

Key words: Oat allergy. Oat hypersensitivity. Cereal allergy. Avena sativa. Immunoblotting.

Palabras clave: Alergia a avena. Hipersensibilidad a avena. Alergia a cereales. *Avena sativa*. *Immunoblotting*.

Common oat (*Avena sativa* L) is a species of cereal belonging to the Poaceae family (Poaceae or grasses), which includes wheat, rice, barley, rye, and corn [1]. Oat grain derives from a herbaceous plant cultivated mainly in Europe, North America, and Western Asia [2,3]. It is consumed worldwide for its numerous properties and nutritional benefits.

The molecular composition and physicochemical characteristics of oats have been studied. The main part of the protein fraction corresponds to globulins (50-80%), followed by albumins (1-12%), prolamins (avenins) (4-15%), and glutenins (<10%) [4]. However, it remains uncertain which oat proteins are involved in hypersensitivity reactions after intake.

Anaphylaxis due to ingestion of oats is a rare clinical entity, with few cases of IgE-mediated hypersensitivity reported in the medical literature [1,5-8]. To date, *A sativa* allergens are not precisely characterized (oat proteins are not included in the WHO/IUIS allergen nomenclature database). However, several proteins suspected of being involved in anaphylactic reactions due to ingestion of oats have been described, including a 23-kDa 12S seed storage globulin and a 48-kDa serpin (serine protease inhibitor) [1,5].

We report the case of a 45-year-old man with a clinical history of bronchial asthma without sensitization to the usual aeroallergens in our environment who experienced several episodes of acute generalized urticaria, facial angioedema, and dyspnea immediately after the ingestion of homemade oat flour crepes. The patient received emergency medical care for each episode (parenteral adrenaline, corticosteroids, and antihistamines) and gradually recovered after several

hours of observation. He also reported acute episodes of rhinoconjunctivitis after inhalation of flour.

This case is included in a clinical research study that was reviewed and approved by the local Research Ethics Committee (CEIm CHUC, code CHUNSC_2020_65). The patient gave his written informed consent for his medical data to be used in the present report.

An allergological study was carried out. Skin prick tests (SPTs) were positive for oats, corn, barley, and wheat, with a negative result for rice. SPTs for storage mites were negative. Determination of specific IgE using singleplex ImmunoCAP assay (Thermo Fisher Scientific) was positive for oats (20.10 kIU/L), as well as for wheat, corn, barley, and rice (values between 1.33 and 3.66 kIU/L). Total IgE was 85.33 IU/mL. The Allergy Explorer assay (ALEX, MacroArray Diagnostics) revealed specific IgE of 4.20 kU_A/L to oat (Ave s) (moderate levels) and of 0.23 kU_A/L to barley (Hor v) (negative or doubtful levels).

An oral challenge test with oats was not performed owing to the risk of anaphylaxis. A cereal-free diet was prescribed, excepting wheat, corn, and rice, which the patient tolerated.

The patient's allergen sensitization profile for oat proteins was studied by means of immunodetection of oat-specific blood IgE based on the immunoblotting technique.

Oat extract was produced from organically grown pure oatmeal flour of Spanish origin, requiring homogenization in 1X phosphate-buffered saline with magnetic stirring (1:10 dilution), centrifugation at 12 000 rpm for 70 minutes, dialyzation by diffusion (3.5-kDa molecular weight cut-off membrane), filtration through a membrane filter (0.22 µm pore size), and lyophilization. Colorimetric quantification of total protein concentration was carried out using the bicinchoninic acid assay (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific) and a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out according to Laemmli [9] using β-mercaptoethanol as a reducing agent. The process was followed by electrophoretic transfer of proteins to a nitrocellulose membrane. Immunoblotting with the patient's blood serum and oat flour extract was performed using blood serum from a healthy (asymptomatic) individual as a negative control sample. Gelatin from bovine skin at 0.25% was utilized as a reagent to block nonspecific protein-binding sites on the membrane.

Immune complexes were chemically labeled with streptavidin-HRP after previous amplification of the signal using the biotin-streptavidin system. The protein bands shown in the Figure were obtained using a chemiluminescence detector. IgE-binding signals were observed against proteins of various molecular weights (around 25 to 66 kDa).

An intense band stood out in the 25- to 26-kDa region. This could correspond to the protein band of approximately 25 kDa described by Ototake et al [6], as well as to the 26-kDa band reported by Tomás-Pérez et al [8]. A single band in this region could still be observed after the exposure time was decreased during the chemiluminescence assay, causing the disappearance of the signals shown in the control and blocking reagent lanes.

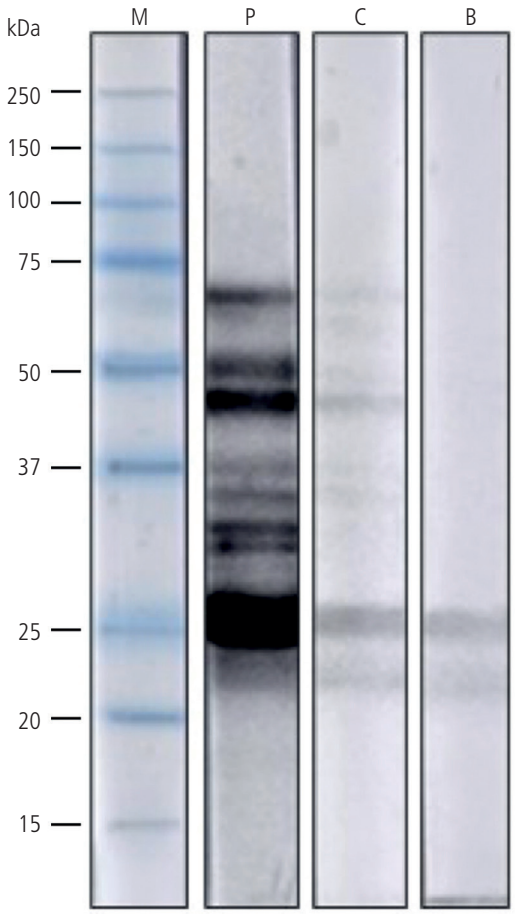

Figure. IgE immunoblotting of oat flour extract under reducing conditions (with ß-mercaptoethanol). M indicates molecular mass marker, P, patient blood serum, C, control blood serum, B, blocking reagent (gelatin from bovine skin).

IgE-binding bands of approximately 33 kDa, 35 kDa, 46 kDa, 50 kDa, and 66 kDa were also observed. Some of these could coincide with other protein bands described in the available scientific literature but not identified to date [5-8,10].

In addition, IgE-binding bands of approximately 31 kDa and 37 kDa were detected. To our knowledge, these have not been mentioned in reported cases of oat allergy, although they may correspond to protein bands of similar molecular weights described elsewhere [5,6].

We report a case of adult-onset oat allergy in a male that was associated with sensitization to other cereals with no apparent clinical repercussions. Using various molecular techniques, we demonstrated an IgE-mediated hypersensitivity mechanism.

Given that oatmeal skin care products can be a route of sensitization to oat proteins, we assumed that the patient was sensitized through the digestive tract, as there is no evidence of previous application of oat-based skin products [7].

The immunoblot assay enabled us to detect the presence of IgE against proteins of various molecular weights with different signal strengths. The approximately 25- to 26-kDa

band stood out over the others, suggesting that it is the protein to which the patient is most sensitized.

In conclusion, allergenic oat proteins and the determinants of cross-reactivity with other cereals have yet to be characterized. Our study provides additional information on oat allergy and could help to improve our understanding of this clinical entity.

Acknowledgments

We thank the Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD, UAM) for their contribution to this study.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The transport costs of the biological samples were covered by ROXALL Medicina España SA, as reflected in the study protocol. ROXALL was not involved in any way in the study design, data interpretation, or writing of the manuscript.

Previous Presentations

The results of this case were presented in part as a poster at the "European Academy of Allergy and Clinical Immunology (EAACI) Digital Congress 2020". The immunoblot analysis is totally original.

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Manuscript received December 6, 2021; accepted for publication February 24, 2022.

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