A case of anaphylaxis after oats ingestion: Research for new allergens

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

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

Anaphylaxis due to oats ingestion is a rare clinical entity, with few cases of IgE-mediated hypersensitivity reported in the medical literature [1,5-8]. To date, *Avena sativa* allergens are not precisely characterized (oat proteins are not included into the WHO/IUIS allergen systemic nomenclature database). However, several proteins with suspicions of being involved in anaphylactic reaction due to oats ingestion have been described, including a 23 kDa 12S seed storage globulin and a serpin (serine protease inhibitor) of 48 kDa [1,5].
A 45-year-old man, with clinical history of bronchial asthma without sensitization to usual pneumoallergens in our environment, presented several episodes of acute generalized urticaria, facial angioedema and dyspnoea immediately after the ingestion of homemade oat floured crepes. The patient received urgent medical assistance in each episode, requiring treatment with parenteral adrenaline, corticosteroids and antihistamines, with progressive recovery after several hours of observation. Also, he refers to acute episodes of rhinoconjunctivitis after inhalation of flour.

An allergological study was carried out. Skin prick tests (SPTs) were positive for oats, corn, barley and wheat, with negative result for rice. SPTs for storage mites were negative. Determination of specific IgE using single-plex ImmunoCAP assay (Thermo Fisher Scientific, Uppsala, Sweden) for oats was positive (20.10 kIU/L), as well as for wheat, corn, barley, and rice, finding these values between 1.33 and 3.66 kIU/L, with a total IgE determination of 85.33 IU/mL. Allergy Explorer assay - ALEX (Macroarray Diagnostic, Vienna, Austria) detected specific IgE levels against oat (Ave s) of 4.20 kUA/L (moderate levels), and against barley (Hor v) of 0.23 kUA/L (negative or doubtful levels).

Oral challenge test with oats was not performed due to the risk of anaphylaxis. A cereal-free diet was prescribed, excepting wheat, corn, and rice, tolerated by the patient.

The allergen sensitization profile of the patient against oat proteins was studied by means of immunodetection of oat-specific blood IgE using the immunoblotting technique.

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

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

Chemical labelling of immune complexes was performed using streptavidin-HRP, previously carrying out signal amplification by means of biotin-streptavidin system. Through a chemiluminescence detector we obtained the protein bands showed in Figure 1. IgE-binding signals are observed against proteins of various molecular weights, ranked around 25 to 66 kDa.

An intense band stands out in the 25-26 kDa region, which could correspond to a protein band of approximately 25 kDa described by Ototake et al [6], as well as to a 26 kDa band that Tomás-Pérez et al observed [8]. A single band in this region was still being observed after decreasing the exposure time during the chemiluminescent development phase, causing the disappearance of the signals shown in the control and blocking reagent lanes.
IgE-binding bands of approximately 33 kDa, 35 kDa, 46 kDa, 50 kDa and 66 kDa were also observed, some of which could coincide with other protein bands described in the available scientific literature, not identified to date [5-8,10].

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

This is a case of oat allergy in a male with onset in adulthood, which associates sensitization to other cereals with no apparent clinical repercussion. An IgE-mediated hypersensitivity mechanism has been demonstrated through various molecular techniques.

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

The immunoblot assay allows us to detect the presence of IgE against proteins of various molecular weights with different signal strengths. It is remarkable that the band of approximately 25-26 kDa significantly stands out over the others, suggesting that this could be a protein to which the patient apparently could be most sensitized.

In short, the allergenic oat proteins, as well as the determining components in the cross-reactivity with other cereals, have not yet been characterized. This study provides additional information on oat allergy and could contribute to improving our understanding of this clinical entity.
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Ethical considerations

This case belongs to a clinical research study reviewed and approved by the local Research Ethics Committee (CEIm CHUC, code CHUNSC_2020_65). A written informed consent was obtained from the subject.

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Conflicts of interest

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

Previous presentation

Part of the results of this case were presented as a poster at the “European Academy of Allergy and Clinical Immunology (EAACI) Digital Congress 2020”, however the immunoblot analysis is totally original.
References


**Figure legend**

**Figure 1.** IgE-immunoblotting of oat flour extract under reducing conditions (with β-mercaptoethanol). M: molecular mass marker, P: patient blood serum, C: control blood serum, B: blocking reagent (gelatin from bovine skin).