Identification of Lipoproteins From Sunflower Seeds in 2 Monosensitized Anaphylaxis Patients

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Sunflower (*Helianthus annuus*) seeds are widely eaten in Spain and normally well tolerated by patients who are allergic to nuts [1]. Sunflower seeds contain diverse proteins, some of which are known allergens [2]. We report 2 cases of anaphylaxis involving lipoproteins from sunflower seeds.

The first patient was a 41-year-old homemaker who had been diagnosed with mild rhinoconjunctivitis induced by spring pollen. In 2012, she experienced palmoplantar pruritus, generalized urticaria, dysphagia, lip angioedema, vomiting, and dizziness 20 minutes after eating a small amount of roasted sunflower seeds. The patient had previously experienced episodes of palmar pruritus after eating sunflower seeds. The second patient was a 42-year-old nonatopic woman who worked as a clerk. In 2016, she crushed some roasted sunflower seed shells with her teeth to release the seeds for her son, although she did not ingest them herself. She immediately experienced lingual edema, rhinitis, lip edema, dysphagia, dysphonia, and dyspnea and required emergency treatment at home. One week earlier, she had developed intense oral pruritus and palatal edema 5 minutes after eating 1 sunflower seed. At present, both patients avoid sunflower seeds, although they tolerate sesame seed, peanut, and other nuts. Neither patient keeps birds at home. An allergy work-up was carried out.

Skin-prick-testing (SPT) was performed using a subset of indoor and outdoor aeroallergens (pollens, house dust mites, dander, and fungi), together with a nut series (sunflower seed, almond, hazelnut, chestnut, pistachio, cashew, walnut, and pine nut), peanut, sesame, polcalcin, profilin, and lipid transfer protein (LTP, Pru p 3) (ALK-Abelló). Skin prick-by-prick (SPP) tests were performed with fresh and roasted nuts (eg, peanut, hazelnut, walnut, chestnut, pistachio, cashew, almond, pine nut, and sunflower seed) and a negative control (50% glycerinated saline) and a positive control (histamine, 10 mg/mL). Serum tryptase, total IgE, and specific IgE against the SPT and SPP

allergens were measured using ImmunoCAP (Thermo Fisher Scientific), following the manufacturer's instructions. SDS-PAGE followed by immunoblotting with the patients' sera was performed. Proteins from the hydro- and liposoluble fractions of roasted sunflower seeds (Supreme Sunflower Seeds Facundo, Facundo Blanco S.A.) were obtained as previously described for sesame seeds [3]. Samples were separated by SDS-PAGE according to the Laemmli method [4] and electrotransferred onto polyvinylidene fluoride (PVDF) membranes as previously described [5]. After blocking with 0.5% PBS Tween-20 buffer, the membranes were incubated overnight with the patients' sera (dilution 1:5). PVDF membranes containing the same extract were incubated with 0.5% PBS Tween-20 buffer and with serum from a nonatopic individual as a negative control. PVDF membranes were incubated with mouse antihuman IgE Fc-HRP (Southern Biotech) at a dilution of 1:1000. Reactive bands were detected using enhanced chemiluminescence following the manufacturer's instructions (Western Lightning Plus-ECL, Perkin Elmer).

In the case of patient #1, the result of SPT was positive (wheal ≥3 mm than the negative control) to *Olea europaea, Cupressus arizonica, Dactylis glomerata, Lolium perenne, Artemisia vulgaris*, and *Salsola kali*. SPT with nuts was only positive for sunflower seed. SPP with nuts was positive (wheal ≥3 mm than negative control) for raw and roasted sunflower seeds. Serum total IgE was 66 IU/mL. Specific IgE to *Artemisia* was 2.3 kU_A/L; specific IgE to sunflower seed, nuts, peanut, sesame, profilin, and Pru p 3 was <0.10 kU_A/L. Baseline serum tryptase was 1.9 µg/L. In the case of patient #2, the result of SPT was positive to *Olea europaea, Dactylis glomerata, Lolium perenne, Artemisia vulgaris*, and *Salsola kali*. SPT with nuts yielded negative results. SPP with nuts displayed a positive result only for raw and roasted sunflower seeds. Serum total IgE was 267 IU/mL. Specific IgE to *Artemisia* was 4.80 kU_A/L; specific IgE to sunflower seed, nuts, peanut, sesame, profilin, and Pru p 3 was <0.10 kU_A/L. Baseline serum tryptase was 6.7 µg/L.

SDS-PAGE showed distinct hydro- and liposoluble bands (Figure, A1 and A2). IgE-immunoblotting assays were performed under reducing and nonreducing conditions. IgE reactivity in both hydrophilic and lipophilic fractions could only be revealed under reducing conditions (Figure, B, P1 and P2). In patient #1, 4 proteins (37, 21.5, 20, and 12.5 kDa) were recognized in the hydrosoluble fraction and 3 proteins (23.5, 20, and 17 kDa) in the lipophilic fraction. In patient #2, 2 proteins (22.5 and 20 kDa) were recognized only in the lipophilic fraction. No signal was obtained for the negative controls. These lipoproteins are not in the main allergen databases but are compatible by molecular mass with some of those already referenced in Uniprot (http://www.uniprot.org). Specifically, the 17-kDa protein could be compatible with 6 proteins described, the 20-kDa protein with 4, the 22.5-kDa protein with Casparian strip membrane protein 1, and the 23.5-kDa protein with 30S ribosomal protein S4, chloroplastic.

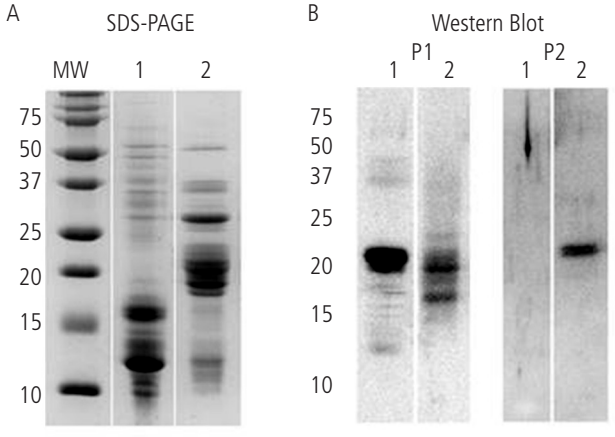

Figure. A, SDS-PAGE from roasted sunflower seeds. Lane 1, Hydrosoluble fraction. Lane 2, Liposoluble fraction. B, Western blot from patients' sera. P1, Patient #1. P2, Patient #2. Lane 1, Hydrosoluble fraction. Lane 2, Liposoluble fraction. MW indicates molecular weight marker.

We report 2 cases of anaphylaxis by sunflower seeds. The novelty of our results lies in the identification of several lipoproteins as allergenic. These allergens are probably major allergens and could be relevant in clinical practice. Sunflower seeds induce various types of allergic reactions, including anaphylaxis [6]. They contain several allergenic proteins: Hel a 1 (34 kDa, a major allergen), Hel a 2 (14.7 kDa, profilin), Hel a 3 (9 kDa, LTP), Hel a 2S albumin (16 kDa), and a 12-kDa storage protein [1,2]; sunflower pollen contains allergens weighing 55, 42.8, 32, and 24 kDa [2]. Hel a 1 and Hel a 2 are inhalant allergens [2]; Hel a 3 is a food allergen [2], and Hel a 2S albumin does not seem to be a relevant allergen [7]. Liposoluble proteins other than seed oleosins have been reported, although the authors were not able to identify the peptide sequence [8]. Furthermore, oil body proteins have reported to be responsible for severe allergy symptoms [9,10], such as those experienced by the 2 patients we discuss here. Therefore, we propose that some or all of the proteins found in the lipophilic fractions from the 2 patients (from 17 to 23.5 kDa) are lipophilic allergens and, therefore, the real culprits of the anaphylactic reactions. However, although the lipophilic fractions seem to be responsible for the severe reactions of patient #1, clinical involvement of the hydrophilic proteins cannot be ruled out completely. We suggest that the IgE-binding bands in the hydrophilic fraction from patient #1 were aggregation states and monomeric states of several proteins. We also highlight the absence of cross-reactivity between *Artemisia* pollen (another *Compositae* family member containing LTPs such as Art v 3) and sunflower seed in both patients. In fact, neither patient developed symptoms after taking LTP-containing foods, and only patient #1 experienced mild pollen symptoms in the spring but not in the summer. Nevertheless, because no cross-inhibition studies have been carried out, whether there was any cross-reactivity between *Artemisia* pollen and sunflower seed, this would not be caused by Pru p 3 owing to the lack of recognition in immunoblotting and the negative result in determination of specific IgE.

In conclusion, to our knowledge, we report the first cases of monosensitization to sunflower seeds. Our findings reveal several lipophilic proteins that had not been previously described in the literature as allergens. We state that the liposoluble protein fraction could be used for diagnostic purposes, especially in patients with a convincing history and whose results were negative in some skin tests and/or specific IgE measurements.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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