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Cypress pollen does not cross-react to plant-derived foods

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Key words

Nasal polyps; severe asthma; asa-intolerance; cysteinil leukotrienes

Summary

Background and Objective: *Some studies hypothesize the existence of cross-reactivity between allergy to Cupressus sempervirens pollen and plant-derived foods. We aimed to assess whether this holds true.* ***Methods:*** *72 patients monosensitized to cypress pollen were investigated for food allergy to peach, apple, tomato and Juniperius oxycedrus berry.* ***Results:*** *No patient had a history of clinical allergy or showed in-vitro or in-vitro reactivity to peach, apple, and tomato. Two patients scored positive on SPT with Juniperius oxycedrus berry but in-vitro tests ruled out cross-reactivity with the corresponding pollen.* ***Conclusion:*** *Airborne allergy to Cupressaceae pollen is not associated with allergy to plant-derived foods.*

Some pollen allergens, such as the major birch allergen, Bet v 1, and the plant pan-allergen, profilin, are well known to cross-react to homologous allergens in vegetable foods causing the so-called oral allergy syndrome. In contrast, the existence of cross-reactivity between specific cypress pollen allergens and plant-derived foods is poorly defined. In recent years, some authors reported cross-sensitisation to tomato, banana and apple in patients allergic to *Juniperus ashei* or *Criptomeria japonica* pollen (1-5), and a French study observed potential cross-reactivity between Cypress pollen and peach (6). Further, in France the berry of *Juniperus oxycedrus* is present in several dishes, but data about potential cross-reactivity with the corresponding pollen are missing. We assessed whether cross-reactivity between *Cupresssus sempervirens* and peach and other fruits or between *Juniperus oxycedrus* pollen and *Juniperus oxycedrus* berry occur.

Seventy-two patients diagnosed as being monosensitized to Cupressus sempervirens at the outpatients allergy departments of Marseille, France, (n=39) and Bordighera, Italy, (n=33) were studied. The diagnosis was based on a clinical history of rhino-conjunctivitis with or without asthma from November to the end of March, confirmed both by positive SPT and increased levels of IgE specific for *Cupressus sempervirens*. All patients were thoroughly interviewed about adverse food reactions (including oral allergy syndrome, urticaria/angioedema, gastrointestinal symptoms immediately after the ingestion of specific foods). All patients underwent skin prick tests with our routine panel of aeroallergens (house dust mites, pellitory, grass, olive, *Cupressus sempervirens, Betula alba,* Alternaria, Cockroaches, Cat and Dog). Further, *Juniperus oxycedrus* fruit was tested by the prick-prick technique. IgE to peach, tomato and apple were measured by Immuno-CAP (Padia, Uppsala, Sweden) in all subjects. Thirty patients allergic to pollens other than cypress were used as controls. All skin tests were carried out using Lofarma extracts (Milan, Italy; 1/20 w/v), and were performed and read following established methods.

Juniperus oxycedrus pollen (Jo) and desiccated Jo berries underwent 5% (w/v) aqueous extraction in 0.125 M NH_4HCO_3 for 4 h at 4°C under stirring at 4°C. The suspensions were centrifuged at 20,000 g for 1 hour at 20°C and supernatants were extensively dialyzed against distilled water. Protein content of samples was assessed by BioRad assay.

Specific IgE to *Juniperus oxycedrus* pollen or berry extracts were measured by ELISA using a pool of sera from patients allergic to *Cupressus sempervirens* pollen. A pool of 5 sera from non-atopic individual was used as control. To this purpose, 5μg of Jo pollen and berry extracts in 100 μl of buffer (15 mmol/L Na_2CO_3 and 35 mmol/L $NaHCO_3$, pH 9.6) per well of 96-microtitre plates (Maxisorp Nunc, Roskilde, Den-

Table 1 - ELISA inhibition of Jo pollen extract using pollen or fruit Jo extracts or *J. Oxycedrus* pollen extract as inhibitors

	inhibitor			
	Juniperus oxycedrus pollen extract		Juniperus fruit extract	
Inhibitor dilution	UA	% inhibition	UA	% inhibition
1:1	0.05	100	1.101	0
1:4	0.046	100	1.008	2
1:16	0.128	88	1.034	0
no inhibition	1.024	0	1.024	0

mark) were used in the coating phase. After washings with 0.15 M phosphate-buffered saline, pH 7.4 (PBS) and 0.05% Tween 20 (Sigma, Milan, Italy), wells were saturated with 2% bovine serum albumin (BSA) in PBS (saturation and diluition buffer) for 2 hours at room temperature. Subsequently, after further washing, 100 µl of positive or negative pool diluited 1:2 in diluition buffer were added to the wells and incubated for 2 hours at room temperature. Wells were washed, and bound specific IgE was detected by peroxidase-conjugated anti-human IgE from goat (diluited 1:1500, Biospacific, Emeryville, CA, USA); a colorimetric reaction was induced using tetramethyl-benzidine/H_2O_2 as substrate. The enzyme reaction was stopped after 20 minutes by the addition of 1 mol/L HCl. Absorbance values were read at 450 nm by spectrophotometer. In inhibition studies, patients' sera positive to both pollen and berry extracts were pooled before pre-absorption for 2 hours at room temperature with different concentrations of Jo pollen or berry extracts (40 µl of sera and 80 µl of inhibiting extract diluted 1.1, 1:4 or 1:16). Subsequently, 100 ul of such solutions were added to Jo-coated wells and ELISA performed as before performing ELISA as described before. IgE levels were expressed as optical density units (OD). Based on the mean + 2SD of IgE levels found in normal controls, values less < 0.100 OD were considered negative.

No patient allergic to *Cupressus sempervirens* reported clinical allergy or showed either in-vivo or in-vitro hypersensitivity to peach. Similarly, none out of 33 patients reported clinical allergy or immunological sensitivity to tomato or apple. Only 2/72 patients scored positive on SPT with Jo berry. The pool of sera used in the study showed strong IgE reactivity to *Juniperus oxicedrus* (O.D.: 1.024 UA) (Tab. 1). As a difference from patients, 25% of 30 control subjects with pollen allergy reported oral allergy syndrome following the ingestion of vegetable foods. SPT with *Juniperus oxicedrus* pollen extract scored positive in 90% of patients, thus confirming the cross-reactivity between Cupressus and Juniperus pollen. No cross-reactivity between Juniperus pollen and berry was observed as shown by the lack of any inhibition of IgE reactivity to pollen pre-adsorption sera with Jo berry extract (Tab. 1). Altogether, our findings clearly rule out an association between *Cupressus sempervirens* pollen allergy and hypersensitivity to plant-derived foods. The two cases of skin reactivity to Juniperus berry in French subjects are probably the result of alimentary habits, and we weren't able to find any cross-reactivity between *Juniperus oxycedrus* pollen and berry extracts.

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