

Systemic allergic dermatitis caused by Apiaceae root vegetables

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doi:10.1111/cod.12122

Summary

Background. Immediate hypersensitivity reactions to root vegetables of the Umbelliferae plant family (Apiaceae) is well known. Delayed-type hypersensitivity is rarely reported.

Objective. To report the first case of systemic contact dermatitis caused by root vegetables and some chemical implications.

Materials and methods. Prick and patch testing were performed with fresh vegetables and selected allergens, and this was followed by high-performance liquid chromatography–mass spectrometry (MS)/MS analysis of the falcarinol syringe.

Results. The patient was contact-sensitive to celeriac, parsnip, and carrot, but tested negative to falcarinol. Subsequent analysis showed that the syringe contained falcarinol.

Conclusion. The non-occupational sensitization resulting from both direct and systemic contact with Apiaceae root vegetables was apparently not caused by falcarinol.

Key words: allergic contact dermatitis; Apiaceae; *Apium graveolens* var. *rapaceum*; carrot; celeriac; *Daucus carota*; parsnip; *Pastinaca sativa*; patch testing; systemic allergic dermatitis.

Immediate-type allergy to vegetables of the Umbelliferae family of plants (Apiaceae) is among the most well-known food allergies. Thus, celeriac (*Apium graveolens* var. *rapaceum*), carrot (*Daucus carota* ssp. *sativus*) and parsnip (*Pastinaca sativa*) of the Apiaceae are all reported to be allergenic, and allegedly share some antigens (1). Furthermore, cross-reactivity between birch pollen allergens and celeriac and carrot, respectively, means

that reactions may occur in persons who are not primarily sensitized by food items, and, because of the high prevalence of birch pollen allergy, they occur occasionally.

Contact allergy to these vegetables, on the other hand, is less frequently reported, although many plants of the Apiaceae and Araliaceae cause allergic contact dermatitis, primarily through occupational exposure (1, 2). It has been shown that falcarinol (Fig. 1) is a potent contact allergen, being responsible for many allergic skin reactions caused by plants of the above-mentioned plant families (1–5). However, allergic contact dermatitis caused by common vegetables of the Apiaceae is rare (6, 7), probably because of the relatively low concentrations of falcarinol in food plants as compared with ornamental and wild plant species (1, 8).

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Conflicts of interest: Evy Paulsen is an investigator and Klaus E. Andersen is an advisor for SmartPractice™ (Phoenix, Arizona).

Accepted for publication 26 June 2013

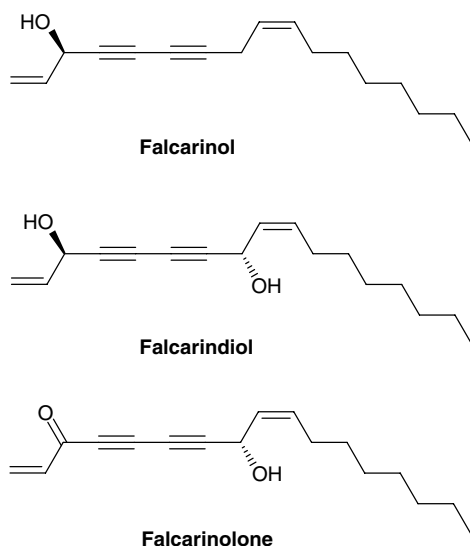


Fig. 1. Polyacetylenes occurring in carrot, celeriac, and parsnip.

The aims of this study were to describe the first case of systemic allergic dermatitis caused by root vegetables, and to show that falcarinol was probably not the sensitizing allergen.

Materials and Methods

Clinical study

The common inhalant allergens included in our baseline prick test series were: pollens of birch (*Betula verrucosa*), grass (*Phleum pratense*), and mugwort (*Artemisia vulgaris*), horse, dog and cat dander, house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), moulds (*Alternaria alternata* and *Cladosporium herbarum*), Soluprick[®] positive and negative controls, and natural rubber latex at 500 µg/ml (Soluprick[®]; Alk-Abelló, Hørsholm, Denmark). The food baseline series included the following fresh food items: wheat flour, egg (pasteurized), low-fat milk, peanuts, hazel nuts, soy milk, codfish, shrimp, celeriac, Brazil nuts, sesame seeds, and poppy seeds. Likewise, the supplementary prick tests with suspected vegetables were performed with fresh specimens (unspecified varieties), with prick-by-prick tests. Histamine release tests were performed at RefLab ApS (Copenhagen, Denmark). A wheal with a diameter of at least 3 mm was considered to be positive.

The patch test baseline series included TRUE Test[®] Panels 1–3 and varying petrolatum-based allergens. The patch test material was applied to the back for 2 days with 8-mm Finn Chambers[®] on Scanpor[®] tape

(SmartPractice[™], Phoenix, AZ, USA). Readings were performed on D3/D4 and possibly D7, according to the criteria of the International Contact Dermatitis Research Group (9).

Chemical study

Chemicals. High-performance liquid chromatography (HPLC)-grade acetonitrile (MeCN), methanol (MeOH), ethyl acetate (EtOAc) and *n*-hexane were obtained from Fisher Scientific (Loughborough, UK). Formic acid 99% was purchased from Sigma-Aldrich (Steinheim, Barsbüttel, Germany). Deionized water was prepared with an SG Ultra-Pure Water system (Siemens, Germany).

Determination of falcarinol in the syringe for patch testing. A standard of falcarinol (purity > 98%; Fig. 1) was isolated from an EtOAc extract of carrot roots (*D. carota* cv. Bolero) by flash chromatography and semi-preparative HPLC, and characterized by spectroscopic and spectrometric methods as described previously (10, 11). The purified falcarinol standard was stored in 96% ethanol at –20°C until the preparation of a freshly prepared syringe for patch testing. A sample from the syringe (1.14 g) used for patch testing was extracted with EtOAc (10 ml) for 1 hr, and the solvent phase was then filtered through a Q-Max syringe filter (Ø, 13 mm; polytetrafluoroethylene membrane pore size, 0.22 µm; Frisette APS, Knebel, Denmark) directly into an HPLC vial, and analysed by HPLC–mass spectrometry (MS)/MS. HPLC–MS/MS data were obtained with an LTQ XL (Linear Quadrupole 2D Ion Trap Mass Spectrometer; LTQ20992; Thermo Scientific, San Jose, CA, USA) operated in APCI positive mode, and attached to an Accela HPLC pump and a photodiode array detector. Settings for the mass spectrometer were 50, 5 and 5 (arbitrary units) for sheath, auxiliary and sweep gas flow rates, respectively, a spray voltage of 6 kV, a vaporizer temperature of 450°C, a discharge current of 5 µA, and a capillary temperature of 275°C. The settings for capillary voltage and tube lens voltage were 15 V and 30 V, respectively. Auto gain control target settings were 3×10^4 and 1×10^4 for full MS and MS/MS, respectively. Separations were obtained with a linear-programmed solvent gradient with A = 0.1% formic acid in H₂O and B = 0.1% formic acid in MeCN on a Hypersil Gold column (1.9 µm; 50 × 2.1 mm; Thermo Scientific); 0 min, 1% B; 20 min, 100% B; 29 min, 100% B; 30 min, 1% B; and 40 min, 1% B. The flow rate was 800 µl/min, the column temperature was 35°C, and the injection volume was 10 µl.

Falcarinol was determined by extracting the main adduct ion (base peak) generated in the APCI

positive mode, m/z 268 $[M + H - H_2O + MeCN]^+$, from the LC-MS chromatogram. Falcarinol showed adduct ions at m/z (relative abundance in parentheses) 309 $[M + H - H_2O + 2MeCN]^+$ (4), 268 $[M + H - H_2O + MeCN]^+$ (100), 245 $[M + H]^+$ (6), and 227 $[M + H - H_2O]^+$ (80). MS/MS analysis of the adduct ion peak at m/z 268 confirmed that this peak originated from falcarinol. Quantification of falcarinol in pet. from the syringe used for patch testing was performed by using a calibration curve of the purified falcarinol from a stock solution (1 mg/ml) and comparing the area value of falcarinol in pet. with that of the calibration curve. Based on the area values, the quantity of falcarinol in the syringe was calculated to be $0.031\% \pm 0.002\%$ (wt/wt). Although falcarinol is unstable, being sensitive to heat, light, and oxidation, no degradation products were observed in the pet. or in the standard.

Case history

A 21-year-old female nurse student was referred to the Allergy Centre because of suspicion of allergy to root vegetables. She had a family history of atopy, and her past medical history included type 1 diabetes for 11 years, perioral dermatitis as a teenager, recurrent rhinitis, especially in spring, and flexural dermatitis (popliteal fossae) in the previous winter.

Previously, the patient had worked at a supermarket, where she handled celeriac without gloves. Carrots and parsnips were usually handled in plastic bags. She had some itching of the hands when taking stock in dusty surroundings, but did not remember having hand eczema. She stopped her part-time work at the supermarket some months before the first symptoms appeared.

At the initial visit, the patient described some itching on the hands after handling root vegetables, and some itching and burning of the oral mucosa and lips as well as rhinitis and conjunctivitis occurring hours after the ingestion of root vegetables. On one occasion, she felt slightly ill after eating root vegetable soup, but without developing manifest anaphylactic symptoms. The symptoms had been present for ~4 months. Prick tests gave negative results with standard inhalant allergens and the baseline food items supplemented with carrot, and the patient was discharged.

Three months later, the patient was referred again after having developed hand eczema when peeling parsnips. She also described localized erythema of the left palm corresponding to the area of contact with a carrot during peeling. On a later occasion, the patient felt ill and developed palmar erythema and swelling a few hours after the ingestion of celeriac.

Results

Supplementary prick tests and histamine release tests for parsnip, Hamburg parsley, celeriac, carrot (Apiaceae), beetroot (Amaranthaceae) and Jerusalem artichoke (Compositae = Asteraceae) gave negative results. The levels of specific IgE for birch, grass and mugwort pollen were not elevated.

Open oral challenge with a mixture of cooked root vegetables (altogether, ~79 g of parsnip, Hamburg parsley, carrot, and celeriac) at the Allergy Centre, followed by 12 hr of observation, failed to elicit any symptoms. The patient was discharged but, later that night, she developed severe erythematous hand eczema, and a systemic allergy was therefore suspected.

The results of patch testing with the baseline series, celeriac, parsnip, carrot and selected allergens are shown in Table 1: the reactions to celeriac and parsnip were strongly positive, mostly to the water-wetted plant material, at least for celeriac, whereas the results with carrot were negative (Fig. 2). The patient noticed erythema of the hands during the test period. As the syringe of falcarinol used for the initial testing was old, the patient was re-tested with a recently made batch of falcarinol that was isolated from carrots and identified by spectroscopic and spectrometric methods. Furthermore, the patient was tested with organically and conventionally grown carrots, as shown in Table 1. The negative results with falcarinol were unexpected, and led to chemical analysis of the new syringe. Analysis of the syringe was performed by HPLC-APCI-MS/MS in positive mode, using the adduct ion peak at m/z 268 $[M + H - H_2O + MeCN]^+$, for identification and quantification of falcarinol (see Materials and Methods). Quantification of falcarinol in the syringe was based on a calibration curve of the falcarinol standard, and confirmed the expected concentration of falcarinol in the syringe, which was ~0.03% (wt/wt). In addition, no degradation products of falcarinol were observed in the standard or in the syringe.

For the previous 2 years, the patient had prepared and eaten root vegetable dishes at least twice a month, and possibly once a week for shorter periods. The sensitization was thus considered to be non-occupational.

Ten consecutive eczema patients were tested with celeriac wetted with water, and all had negative results. Another 10 consecutive patients were tested with celeriac wetted with ethanol as well as parsnip wetted with water and ethanol: 1 patient had transient doubtful positive reactions to all plant materials on D3, and another tested doubtful positive to celeriac and water-wetted parsnip on both D4 and D7. Organically grown carrot (same specimen as tested in the patient) was tested in another

Table 1. Patch test reactions to vegetables and allergens in a patient suspected of having contact allergy to root vegetables

August 2012				October 2012	
Allergen	D2	D3/D4	D7	Allergen	D3
Raw carrot + water	—	? + — foll.	—	Raw organic carrot peel + water	? + — foll.
Raw carrot + ethanol	—	—	—	Raw organic carrot peel + ethanol	? + — foll.
Raw beetroot + water	—	—	—	Raw organic carrot inner side + water	+
Raw beetroot + ethanol	—	—	—	Raw organic carrot inner side + ethanol	+
Raw celeriac + water	++	++	+	Raw carrot peel + water	? + — foll.
Raw celeriac + ethanol	++	++	+	Raw carrot peel + ethanol	? + — foll.
Raw parsnip + water	++	++	+	Raw carrot inner side + water	+
Raw parsnip + ethanol	++	++	+	Raw carrot inner side + ethanol	+
Raw Jerusalem artichoke + water	—	—	—	Falcarinol 0.03% pet. (new syringe)	—
Raw Jerusalem artichoke + ethanol	—	—	—		
D-Limonene 10% pet.		? + —			
Hydroperoxides of limonene 0.3% pet.		? + —			
Falcarinol 0.03% pet.		? + —			

foll., follicular reaction.

**Fig. 2.** Positive patch test reactions to celeriac ('rodselleri') and parsnip ('pastinak').

10 consecutive eczema patients, and doubtful positive reactions were recorded in 2 patients on D4 and to a lesser degree on D7.

Discussion

Parsnip is an ancient cultivated plant, and dermatitis caused by parsnip has been known since the end of the 19th century, including in occupational settings (12). The phototoxic properties of the plant were recognized relatively early on, but cases are still being reported, elicited both by natural sunlight in, for instance, farmhands, and by artificial ultraviolet (UV)A light in people using sun beds (12–14). However, parsnip may also cause photoallergic reactions through

8-methoxypsoralen and possibly 5-methoxypsoralen (15). Furthermore, Picardo et al. reported a photoallergic reaction to a fraction of a parsnip MeOH extract (*P. sativa* ssp. *urens*) activated only by UVB irradiation (16). They also suspected an irritant response to the leaves, and diagnosed allergic contact dermatitis caused by this particular subspecies of parsnip (16). Allergic contact dermatitis caused by parsnip was later reported by Poniecka (17) and Poljacki et al. (18).

Like parsnip, carrot is also an ancient cultivated plant. The first report on contact dermatitis is probably that of Henry, who described occupational celery dermatitis in workers at three vegetable canning factories in the autumn of 1932 (19). One of the affected workers had had dermatitis 9 months previously when she scraped 'large and old' carrots, and another 4 developed vesicular hand eczema after handling carrots (and turnips) (19). Vickers also reported on occupational dermatitis caused by carrots in 7 female cannery workers (20). Five of the 7 women developed symptoms after handling diced carrots for 2–4 days, and all 7 tested positive to raw and blanched carrots after 24 hr (20). However, 32 of 205 (15.6%) controls tested positive after up to 3 days of application, indicating an irritant potential (20). Occupational sensitization was also reported in two other case series from a canning factory and a food-processing factory, in a caterer, and in a female chef and a female school cook (6, 21–24). Mainly non-occupational sensitization was reported in a case series by Sinha et al. (25), and sporadic cases were reported by Agrup (26), Foulds and Sadhra (27), and Murdoch and Dempster (7). Although polyacetylenes were isolated from the plant in 1969 (28), falcarinol was not recognized as the main allergen until 1986 (1). However, Hausen

emphasized that the majority of cases were either irritant or phototoxic, and that allergic reactions were rare (1). This may be true of the early reports of Henry and Vickers, but more recent findings obtained with extracts and/or falcarinol seem to represent true contact allergy (6, 27).

Like parsnip and carrot, celery has been cultivated since ancient times. Some of the early reports on celery dermatitis concerned workers at vegetable canning factories (19, 29). Henry reported prevalence rates between 4% and 30%, and suspected limonene or derivatives of limonene from celery oil to be the main culprits, but patch testing was not performed (19, 29). Later reports focused on the phototoxic properties of celery and the aggravation of these in plants infected with the fungus *Sclerotinia sclerotiorum* (12).

Contact dermatitis caused by the closely related celeriac, on the other hand, has rarely been reported. Henry quoted a French case of a kitchen gardener with celery dermatitis who had a flare when he scraped roots of celery, and Agathos reported occupational sensitization to celeriac in a female vegetable cannery worker (19, 30). Klauder and Kimmich, in their survey of 13 workers with occupational carrot dermatitis, tested 3 workers for cross-reactivity with raw celeriac and 4 for cross-reactivity with parsnip: all 3 had positive reactions to celeriac, and 3 of 4 had positive reactions to parsnip (22). According to Klauber and Kimmich, these cross-reactions had not been recorded before, although Henry observed a case of celery dermatitis in a woman who had previously reacted to carrot (19, 22). More recent case reports support cross-reactivity between these root vegetables of the umbelliferous family of plants: a male sensitized to carrot (and common ivy) also tested positive to celery, and a female cook had positive patch test reactions to carrot, celery (stem), parsley (stem), and falcarinol (6, 7). The last-mentioned allergen has been detected in carrot, celery, celeriac, parsnip, and parsley (8), but the results of the falcarinol patch testing and subsequent chemical analysis suggest that the patient in the present case report was sensitized to another allergen. Of course, uneven distribution of the pet.-based falcarinol test material may result in lower reactivity, but it seems unlikely that a lower concentration of this strong contact allergen would not elicit at least a doubtful positive reaction if the patient was sensitized to falcarinol. Falcarinol is relatively unstable, being sensitive to, for example, oxidation, and therefore another explanation for the negative patch test reactions of the patient could be that falcarinol had been degraded before or during the preparation of the test syringe. Chemical analysis of the test syringe by HPLC-MS/MS confirmed that the patient was tested with falcarinol and not an oxidized/degraded derivative of falcarinol, and that

the test concentration of falcarinol in the syringe was as expected. Therefore, falcarinol is most likely not the main source of the patient's allergic reactions.

In some of the first reports on carrot allergy, positive reactions were recorded to, among other things, carrot juice, water, alcohol, acetone, and ether extracts of carrots (20, 21). Even though some of these reactions may have been irritant, the variety of possible solvents suggests more than one allergen (1). Besides falcarinol and the monoterpene D-limonene, other obvious candidate allergens in the above-mentioned vegetables include other falcarinol-type polyacetylenes (Fig. 1), which, like falcarinol and D-limonene, are largely insoluble or slightly soluble in water, but soluble in ethanol. Positive patch test reactions to both raw and blanched/cooked carrots were described in the first reports, implying that the allergen was relatively heat-stable (20, 21). Although falcarinol-type polyacetylenes are heat-labile, it has been shown that thermally processed carrot pieces still contain falcarinol after blanching and boiling, although the amount is reduced by ~70% after 15 min of boiling (31). The three vegetables have another two falcarinol-type polyacetylenes in common, namely falcariindiol and falcariinolone (Fig. 1) (8), but neither has been described as a contact allergen (3).

R-limonene and S-limonene have been detected in carrot, but they are weak contact allergens unless oxidized (32, 33). The doubtful positive reactions in our patient do not support the possibility that this allergen is responsible for her symptoms.

Provided that the allergen in carrot also caused the reactions to celeriac and parsnip, the presence of another allergen different from polyacetylenes and monoterpenoids is still supported by the fact that the reaction to water-wetted celeriac was stronger than that to the corresponding alcohol-wetted plant material in our patient.

The patient was not tested with psoralens, which have been reported as contact allergens (34, 35). However, sensitization has mainly occurred through iatrogenic exposure, and is thus less likely in our patient.

Oral challenge with carrot in carrot-sensitive patients has been reported: Vickers described negative oral challenge results with raw carrot in 2 severely affected persons, and, likewise, Foulds and Sadhra did not observe any reactions to the ingestion of raw and cooked carrot in their 2 cases (20, 27). Peck et al. observed 1 case of oedema of the lips and perioral dermatitis after the ingestion of cooked carrot in 15 patients, and soreness of the mouth and lips after the ingestion of raw carrot was noted in the case reported by Murdoch and Dempster (7, 21). Thus, systemic allergic dermatitis caused by carrot or any

other root vegetable of the Apiaceae family has not been reported before. The clinical symptoms, with dermatitis of the palms and lateral aspects of fingers, as well as malaise, are consistent with systemic contact dermatitis, whereas the mucosal symptoms are unusual (36). Although type I allergy was ruled out, the atopic diathesis of the patient may have modified her reactions.

In conclusion, the case report shows topical and systemic allergic dermatitis caused by raw and cooked root vegetables of the Apiaceae family of plants, acquired non-occupationally, and the clinical and chemical analyses point to an allergen other than the polyacetylene falcarinol, which is considered to be the main allergen in carrot.

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