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An oral challenge test with carmine red (E120) in skin prick test positive patients

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Key words

Carmine red (E120); food additive; IgE-mediated allergy; single-blind placebo-controlled oral challenge test; skin prick test.

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Summary

Background. *Positive skin prick test reactions to carmine red (E120) occur in approximately 3% of the patients studied for food allergy. Carmine ingestion associated systemic symptoms are occasionally suspected, but sufficient information of proven carmine allergy is not available.* ***Patients and methods.*** *To analyse carmine related symptoms in skin prick test positive patients a cohort of 23 patients with suspected allergy to carmine red was subjected to a single-blind placebo-controlled oral challenge test with carmine red.* ***Results.*** *Five patients developed clinical symptoms during the placebo-controlled oral challenge. As a result, the overall frequency of clinical carmine allergy is estimated to be 0.7% in general dermatology patients studied for food-associated symptoms.* ***Conclusions.*** *Oral challenge test provides a valuable in vivo tool to better inform patients with positive skin prick tests to additives to avoid false allergy diets.*

Introduction

Carmine red is a natural food additive (E120) and a cosmetic colorant (CI 75470) that is derived as an extraction product of the cochineal insect (Dactylopius coccus Costa) (1). Immediate IgE-mediated allergic reactions (urticaria, angio-oedema, anaphylaxis and asthma) have been reported to occur following oral carmine exposure (2-6). In a majority of cases positive skin prick test (SPT) reactions to carmine red seem to occur as immunologic cross-reactions concurrently with reactions to house dust and / or storage mites (7).

We have previously shown that approximately 25% of carmine red sensitive patients have no house dust or storage mite reactions in SPTs (7). Some patients thus seem to have developed primary carmine sensitization. Carmine ingestion associated symptoms seem to occur in approximately 10-20% of patients with positive SPT results (**table 1** and ref. 7). In addition, patients experience clinical symptoms at similar frequency regardless of their IgE reactivity to dust mites, and the nature of carmine associated symptoms seems to be comparable in both patient groups.

To better analyse the degree and also true individual susceptibility of carmine-related allergic symptoms, we have subjected 23 patients with positive SPT to oral challenge test (OCT) with carmine red.

Materials and Methods

Patients and skin prick tests

During 2007-2011, 2926 patients were tested at the Department of Dermatology Turku University Hospital with carmine red (5 mg/ml) E120 (Celego, Dr. Marcus) for suspected food

Table 1 *- Oral challenge test to patients with positive skin prick test to Carmine red (E120).*

Patient	Sex/age	Carmine SPT (mm)	Histamine SPT (mm)	D.pt. / D.far. SPT	Storage mites SPT	Active Carmine avoidance before oral challenge	Symptoms	Results of oral challenge to Carmine	S-IgE [kU/l]	Other SPT reactions (=/> than histamine)
1	F/61	3	5	neg	< hist	no	none[1]	neg	53	pollen & cross-reactions
2	M/30	4	5	neg	< hist	no	none	neg	NT	pollen & cross-reactions
3	M/39	4	5	< hist	NT	no	none	neg	NT	no other reactions
4	F/42	15	4	< hist	> hist	yes	probable	neg	300	nutmeg
5	F/39	10	5	> hist	= hist	yes	suspected	pos	62	pollen
6	F/48	5	5	neg	NT	yes	possible[1]	neg	1126	pollen, animals
7	F/67	4	4	neg	< hist	no	none	neg	548	pollen & cross-r., animals, soybean
8	F/21	4	4	neg	= hist	yes	none	neg	NT	no other reactions
9	F/50	5	5	< hist	= hist	no	none	neg	59	pollen
10	F/62	5	5	= hist	NT	yes	yes2[1]	pos	NT	pollen, animals
11	F/57	5	4	neg	> hist	yes	yes3[1]	pos	NT	pollen
12	M/26	7	6	neg	= hist	yes	none	neg	81	pollen & cross-reactions, soybean
13	M/46	5	5	< hist	< hist	no	none	neg	NT	gliadin, wheat
14	M/47	4	4	< hist	NT	no[2]	yes	neg	NT	pollen & cross-r., animals
15	F/33	8	6	neg	< hist	no	yes	pos	54	no other reactions
16	M/20	4	5	< hist	< hist	no	none	neg	231	soybean
17	M/33	5	5	< hist	= hist	no	none	neg	NT	pollen & cross-r., animals
18	F/57	6	5	= hist	NT	no	none	neg	348	pollen & cross-r., soybean, animals
19	M/19	5	5	neg	< hist	partially	probable	pos	NT	pollen & cross-r., animals
20	M/30	4	4	= hist	neg	no	none	neg	25	no other reactions
21	M/33	3	4	neg	< hist	no	none	neg	NT	pollen & cross-reactions
22	M/61	4	4	< hist	> hist	no	none	neg	419	rapeseed
23	M/20	9	5	< hist	> hist	yes	none	neg	NT	no other reactions

1) Three years after oral provocation: gastrointestinal symptoms associated with carmine red containing lipstick.
2) Facial flush, stomach pain, diarrhea and tachycardia.
3) Oral mucosa associated symptoms and general itch.
[1]No symptoms after carmine red avoidance.
[2]The patient had avoided one specific carmine containing yoghurt that had caused oral symptoms
SPT: skin prick test; NT: not tested; hist: histamine hydrochloride.

allergy. The patient records were retrospectively analysed and a cohort of 23 patients (11 females; average age 48.8 years and 12 males; average age 33.7 years) having carmine red SPT reaction 3 mm or more and suspected carmine allergy were invited for an oral challenge test with carmine red solution.

All these patients had been tested with the standard series of pollen (birch, alder, timothy, mugwort) and animal (cat and dog) as well as with Dermatophagoides pteronyssinus (D. pt.) and/or Dermatophagoides farinae (D. far.) allergens (Soluprick SQ allergens; ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark). Allergy to carmine red was studied using SPT panels containing various food allergens. 18/23 patients had also been tested with storage mites (Acarus siro, Tyrophagus putrescentiae and Lepidoglyphus destructor). SPTs were carried out using positive (histamine dihydrochloride 10 mg/ml, ALK) and negative (saline, Soluprick, ALK) controls. The two largest perpendicular diameters of the wheal were measured at 15 min to calculate the mean value representing the size of SPT reaction.

Single-blind placebo-controlled oral challenge with carmine red

Oral challenge tests were performed after patients' informed consent. The study protocol was approved by the ethics committee and was in line with the ethical standards of the Helsinki declaration. A placebo control solution with similar red colour as the carmine red test doses was produced using boiled beetroot extract dissolved in water. As a food additive carmine was found to be used in 5 mg / ml average concentration in food items. **Table 2** presents the protocol of the oral challenge test. Patients had avoided antihistamines 5 days before the challenge and they had no ongoing infections. SPT with carmine red was repeated before the challenge. The challenge was started with a placebo solution, and if any subjective or objective symptoms appeared, placebo doses were repeated. Blood pressure values and peak expiratory flow (PEF) rates were measured at each step. Patients were asked to report any symptoms experienced in the mouth, lip, tongue, nasopharynx, airways or in the skin. Patient-reported subjective symptoms and objective findings were registered during the challenge. Patients were followed 1-2 hours after the challenge and they were asked to contact the clinic if any later reactions occurred. OCT positive patients were also contacted 1/2 to 4 years later. Those, who were still motivated to avoid carmine, and had remained symptom free, were regarded as OCT positive cases.

Table 2 *- Protocol of oral challenge test with carmine red (E120). Intervals between challenge steps were 20 minutes, and a final 1-hour follow up was performed after the last dose. Oral doses were 1 ml and 2 ml and the 5 mg / ml concentration of carmine red was titrated from 1:100 to 1:2.*

1	Red placebo solution (boiled beetroot extract)
2	Red placebo solution (boiled beetroot extract)
3	1:100 (carmine solution 5 mg / ml) / vol. 2 ml
4	1:10 (carmine solution 5 mg / ml) / vol. 1 ml
5	1:10 (carmine solution 5 mg / ml) / vol. 2 ml
6	1:5 (carmine solution 5 mg / ml) / vol. 1 ml
7	1:5 (carmine solution 5 mg / ml) / vol. 2 ml
8	1:2 (carmine solution 5 mg / ml) / vol. 1 ml
9	1:2 (carmine solution 5 mg / ml) / vol. 2 ml

Results

The size of the carmine reaction reached or exceeded the positive control wheal size in 18/23 patients and 15/18 of them had equal or larger carmine reactions than any of the obtained mite reactions. After the previous SPT results 8/23 patients reported to have experienced carmine ingestion associated symptoms and in 5 of them the challenge test was interpreted as positive. Majority of patients (21/23) had SPT reactions to common IgE allergens, too. 13/23 patients reacted to D. far. or D. pt. and 17/18 to storage mites in SPTs.

Cases of positive challenge results

Patient no. 5 had been remitted for relapsing urticarial rash. She had experienced palmar itch and mouth tingling associated with food ingestion. She had not experienced any mucosal symptoms, although she had positive SPT reactions to birch and mites (**table 1**). This 39-year-old female worked with fresh food products in a grocery market. During the OCT she experienced facial and palmar itch for about half an hour. Symptoms disappeared following antihistamine intake. After a six-month follow-up she had remained symptom free when avoiding carmine red.

Patient no. 10 was a 62-year-old female and she was referred to the clinic with recurrent, occasional facial rash, gastrointestinal pain and diarrhoea following food ingestion. After the SPT with carmine red was positive, the patient started to avoid carmine red and no symptoms appeared during a 6-month follow-up. Once, by chance she ingested a piece of cake containing carmine red, which resulted in facial flush, stomach pain, diarrhoea and general discomfort. During the challenge she reported tingling in the lips followed by stomach pain in about 1 hour.

Three other patients reported mild symptoms during the test. Patient no. 11 was studied for hand eczema, but she had occasionally suffered from urticarial rash. Carmine red containing candies had caused mild oral symptoms, and she had actively avoided carmine. She reported tingling in the tongue after the third (1 mg) dose in the provocation, but the latter steps remained symptomless. Patient no. 15 reported that red coloured candies and red lip stick had caused swelling in lips and gastrointestinal symptoms earlier. She developed general itch during the OCT. Patient no. 19 had suffered from recurrent urticaria and stomach pain associated with food ingestion. She experienced upper body itch during the challenge. In addition to patients no. 5 and no. 10, also these three patients reported no carmine associated symptoms following an at least 2 years' follow-up.

The measured values of PEF and blood pressure remained unchanged in all the challenged patients. All the patients received a 12.1 mg cumulative dose of carmine red (starting dose 0.1 mg and maximum dose 5 mg) regardless of the symptoms during the provocation.

Discussion

Hypersensitivity reactions and subjective intolerance symptoms to food additives are commonly reported. True IgE-mediated

type I allergy to additives is far less common. It may however also appear as immunologic cross-reaction between conserved antigenic structures (8). The prevalence of intolerance to food additives seems not to exceed the level of 4% in western countries (9-12). Oral provocations or challenge tests can be used to differentiate true allergy from suspected reactions (13,14). Among urticaria patients previous oral challenge studies have shown 0.63% prevalence of allergy to food additives and carmine allergy seems to account for one half of those allergies (12). While the prevalence of positive SPTs to carmine has been reported to be 3% (7), the frequency of carmine allergy can be estimated to be approximately 0.7% among patients studied for suspected food allergy.

SPT with carmine red were done 2 to 24 months before the OCT. Still 9/23 patients, including the challenge positive cases, reported to have actively avoided carmine red containing food before the challenge, since ingestion associated symptoms were suspected. All these patients had carmine SPT reactions the size of which reached that of the positive control wheal. In OCT 5 patients developed positive symptoms and those patients had a history of corresponding symptoms. In the follow up they remained symptom free by partial avoidance. Following the negative OCT, 4/9 patients abandoned their carmine free diet. None of them reported symptoms, although they were encouraged to report if any carmine associated symptoms appeared. Our material suggests that challenge positive cases may have primary carmine sensitization, since mite reactions were smaller in all the cases with positive OCT. However, strong mite reactions may also lead to carmine ingestion related symptoms. Unfortunately, cross-inhibition studies, that may have helped to more accurately study the importance of immunologic cross-reaction between mite and carmine red epitopes, were not carried out in this study.

Like patient no. 15, patient no. 1 had used carmine (C.I. 75470) containing red lipstick at least three times preceding her symptoms. She had experienced local swelling and angioedema in the face, but in OCT she did not experience any symptoms. Only local symptoms after repeated applications are expected in sensitized subjects. According to the registry of the Helsinki Asthma and Allergy Association 40 lip sticks that are marketed in Finland contain carmine red as well as some make up creams. Immediate type symptoms in the face and lips are probably regarded as irritation in most cases.

There are no standardized methods to study allergy to food additives using oral challenges. If strong reactions are unlikely and broadening of the diet is desired, it is easier to start with higher concentrations and amounts of the allergen. Still, the interpretation of mainly subjective and often mild symptoms is difficult when provocation tests are being planned. In our previous report (7), carmine-associated symptoms were suspected in 8/94 patients at the time of initial skin testing. Two of them had developed anaphylaxis, while the others had suffered from urticaria or angioedema. The size of the carmine reaction in SPT was not less than histamine wheal in those who experienced carmine ingestion associated symptoms. The present report supports the importance of oral provocation test if suspected food allergy causes restrictions and constraint in normal life. As patients with history of anaphylaxis were not included this study, severe reactions were not found in the oral challenge.

Unspecific non-immunologic reactions are likely to generate food additive related symptoms via unknown mechanisms. Still, the amount of patients with challenge-proven clinical symptoms due to food additives appears to be rather low as earlier shown for e.g. tartrazine and sodium benzoate (15,16). Our patient cohort was chosen based on positive SPT results for carmine red. To our knowledge there is no regulation concerning the amount of E120 in food items. It can be argued that a proportion of our patients may have required additional steps with higher carmine concentrations or a larger cumulative dose (exceeding the used 12.1 mg) to reach the symptoms-eliciting allergen dose. On the other hand, false negative findings might partially be influenced by a specific oral tolerance induction (SOTI) that may appear during an oral provocation analysis with increasing concentrations of the orally administered antigen (17). The possibility of real and more gradual tolerance induction can neither be excluded between the initial SPTs and OCTs. Still, the total amount of carmine red used in our provocation test was almost 10-fold more compared to the amount (1.3 mg) that was enough to cause anaphylaxis in a patient reported earlier (2).

Potential concomitant intake of acetylsalicylic acid (ASA) has been reported to increase the risk of food-dependent severe allergic reactions following exercise (18). Also other non-steroidal anti-inflammatory drugs and alcohol intake increase the risk of anaphylaxis in patients prone to develop severe food-induced immediate allergic reactions (19). Although carmine ingestion associated exercise-induced anaphylaxis or urticaria has not been reported in the literature, a combination of ASA intake with carmine ingestion could improve the accuracy of carmine OCT or lower the reaction threshold in subjects who have no ASA hypersensitivity.

Conclusions

Oral challenge test combined to preceding SPT provides a useful tool to discriminate between true symptomatic allergy and other cases having mere SPT reactivity or milder reactions resulting from either immunologic cross-reaction or other unspecific hypersensitivity conditions. As a result, number of unnecessary or even false elimination diets can hopefully be reduced.

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Conflict of interest

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