

Case report

Occupational asthma and food allergy due to carmine

Acero S, Tabar AI, Alvarez MJ, Garcia BE, Olaguibel JM, Moneo I.
Occupational asthma and food allergy due to carmine.
Allergy 1998; 53: 897-901. © Munksgaard 1998.

**S. Acero¹, A. I. Tabar¹,
M. J. Alvarez², B. E. Garcia¹,
J. M. Olaguibel¹, I. Moneo²**

¹Seccion Alergología, Hospital Virgen del Camino, Pamplona; ²Instituto Carlos III, Madrid, Spain

Carmine (E120), a natural red dye extracted from the dried females of the insect *Dactylopius coccus* var. *Costa* (cochineal), has been reported to cause hypersensitivity reactions. We report a case of occupational asthma and food allergy due to carmine in a worker not engaged in dye manufacturing. A 35-year-old nonatopic man, who had worked for 4 years in a spice warehouse, reported asthma and rhinoconjunctivitis for 5 months, related to carmine handling in his work. Two weeks before the visit, he reported one similar episode after the ingestion of a red-colored sweet containing carmine. Peak flow showed drops higher than 25% related to carmine exposure. Prick tests with the cochineal insect and carmine were positive, but negative to common aeroallergens, several mites, foods, and spices. The methacholine test was positive. Specific bronchial challenge test with a cochineal extract was positive with a dual pattern (20% and 24% fall in FEV₁). Double-blind oral challenge with E120 was positive. The patient's sera contained specific IgE for various high-molecular-weight proteins from the cochineal extract, as shown by immunoblotting. Carmine proteins can induce IgE-mediated food allergy and occupational asthma in workers using products where its presence could be easily overlooked, as well as in dye manufacture workers.

Key words: carmine; cochineal; dyes; food allergy; IgE; occupational asthma.

S. Acero
CS Conde Oliveto
Alergología, 3ª planta
Plaza de la Paz SN
31002 Pamplona
Spain

Accepted for publication 7 April 1998

Carmine is a natural dye derived from the dried bodies of females of the insect *Dactylopius coccus* var. *Costa*. Carmine is widely used in the cosmetic, pharmaceutical, food, and dyeing industries. A few cases of occupational asthma, extrinsic allergic alveolitis, and food allergy to carmine have been described before (1-6). Occupational asthma due to carmine has been reported to affect workers in dye manufacturing (4, 5) and other jobs involving carmine handling (3). Nevertheless, food allergy and asthma have not been described in the same patient. Carmine-specific IgE has not been demonstrated, except for one report in which specific positive RAST values were obtained (5).

he began repeatedly to experience rhinoconjunctivitis and shortness of breath when he was occasionally exposed to carmine in his job, as he soon noticed. Although symptoms were present immediately after carmine handling, they were worse at night, and sometimes lasted more than 1 day. Fifteen days before the visit, he developed a systemic reaction with rhinoconjunctivitis and dyspnea within minutes after ingestion at home of a strawberry sweet containing E120 dye. He reported not having allergic symptoms when handling various other spices or dyes.

Symptoms improved with inhaled budesonide and bronchodilators when needed, and disappeared when he was not at work.

Case report

A nonsmoking 37-year-old man, without personal or family history of atopic diseases, had been working for 4 years in a spices warehouse, packing spices and sometimes mixing them for use as food additives. Five or six months before the first visit,

Material and methods

Allergen extracts preparation

In vivo tests. For skin prick tests (SPT), commercial extracts were used when available (ALK-Abelló,

Spain). Spices, carmine, and minced dried bodies of female cochineal insects were extracted at 20% w/v in phosphate-buffered saline (PBS) solution and mixed 1/1 with glycerol. For the specific bronchial challenge test, minced dried cochineal insects extracted at 10% w/v in PBS were used.

In vitro tests. Dried cochineal insects were minced and diluted in sample buffer (SB) at 100 mg/ml. After centrifugation (4000 g for 15 min), supernatants were used as antigen before (A extract) or after boiling (B extract), and precipitates were redissolved in 1 ml SB and centrifuged (C and D extracts, respectively). Pounded dried cochineal insects (0.5 g) were washed once with methanol (20 ml) and three times with saturated ammonium sulfate (20 ml). PBS (20 ml) was added to the precipitate and dialyzed against saline with a 3.5-kDa cutoff membrane. Then it was diluted 1/2 in SB, boiled for 10 min, and centrifuged at 3200 g for 10 min. The supernatant was used as antigen (E extract).

Clinical investigation

Skin prick tests (SPT) were performed as described (7) with 14 common inhalants and foods. Several spices and carmine extracts were also tested. Histamine chlorhydrate (10 mg/ml) and normal saline solution were used as positive and negative controls, respectively. Serum total IgE and specific IgE against house-dust mite (CAP System RAST-FEIA; Pharmacia, Uppsala, Sweden) were measured according to the manufacturer's instructions.

Peak expiratory flow (PEF) recordings

PEF was registered three times a day for 1.5 months during working periods and holidays. A Mini-Wright peak flow meter (Clement Clark International Ltd, London, UK) was used.

Bronchial provocation test (BPT)

Tests were done when the patient was absent from work, free of symptoms, and not receiving medication that could affect the results.

Methacholine inhalation test. Nonspecific bronchial responsiveness was assessed by abbreviated methacholine inhalation challenge test, as described by Chatham et al. (8). A forced expiratory volume in 1 s (FEV₁) higher than 70% of predicted normal was required to start the BPT. Methacholine (Provocholine, Roche Laboratory, Nutley, NJ, USA) was administered with a nebulizer, model DeVilbiss 646 (DeVilbiss Co, Somerset, PA, USA)

connected to a Rossental French dosimeter (Laboratory for Applied Immunology, Baltimore, MD, USA), powered by an oxygen tank with a pressure of 20 psi and calibrated to deliver 15 µl/breath. The result was expressed in terms of the provocation dose necessary to decrease FEV₁ by 20% (PD₂₀) given in µM methacholine.

Specific BPT. The BPT was performed with freshly prepared cochineal insect extract. The aerosolized particles were generated by continuous pressurized DeVilbiss 646 nebulizer with a fixed output of 0.28 ml/min, and inhaled by tidal breathing for 2 min. The SPT with twofold dilutions of allergen extract was performed to enable selection of a safe initial dose for the BPT (concentration of allergen that produces a 3×3 mm wheal); this concentration was 3.125 mg/ml. No response to the PBS challenge was required to start the allergen challenge. The allergen extract was inhaled every 10 min with a twofold increasing allergen concentration at each step (FEV₁ was measured at 10 min after each inhalation), until the highest dose of allergen was attained or an early asthmatic reaction occurred. When the last dose of allergen was administered, FEV₁ measurements were performed at 20, 30, 60, and 90 min. PEF measurements were done hourly until 10 h after challenge to record any late asthmatic reaction. Finally, a new spirometry was performed 24 h after BPT.

Oral challenge test

The double-blind, placebo-controlled oral challenge test (DBPC) was performed on two separated days, enteric opaque capsules being used. PEF and arterial pressure were monitored during the challenge. Carmine was administered in increasing doses (5, 10, 20, 50, 100, and 150 mg), with 1-h intervals between doses, until a positive reaction occurred or until the highest accumulated allowed dose per kilogram body weight of carmine (5 mg/kg body weight) was reached.

SDS-PAGE

SDS-PAGE was carried out on a 4% acrylamide stacking gel and 16% separating gel (9). Electrophoresis was performed at 150 V until the bromophenol blue reached the bottom of the gel. After electrophoresis, proteins were either stained with Coomassie brilliant blue or transferred to a nitrocellulose membrane for reaction with IgE.

Immunoblotting

Proteins in the SDS-PAGE gel were blotted onto a 0.45-µm nitrocellulose membrane (Trans-Blot®

Transfer Medium, Bio-Rad) in PBS-25% methanol by passive diffusion. Then the membrane was incubated overnight at room temperature with the patient's serum, with a monoclonal, biotin-labeled anti-IgE, and finally with streptavidine-alkaline phosphatase, as previously described (10). Between each step, 0.5 M NaCl, 2 mM Tris, 0.1% Tween 20 solution was used for washing.

Bands were visualized by incubation with the BCIP-NBT substrate for 1 h.

Results

SPT and serum IgE

SPT were all negative to common allergens. The hydrosoluble carmine and cochineal insect extract induced 9×4 and 11×5 mm diameter wheals, respectively.

Total IgE was 1590 IU/ml. Specific IgE against house-dust mites was below 0.35 IU/ml.

PEF measurements

PEF monitoring during working periods showed falls between 17% and 30% on the days carmine was used. PEF during holidays or outbreaks was normal and stable.

BPT

In the BPT, methacholine was positive with a PD_{20} of 6.06 μ M methacholine.

Specific bronchial challenge test with the newly prepared cochineal extract achieved an immediate FEV_1 fall and a late (6 h) fall in PEF of 20% and 24%, respectively (PC_{20} 6.25 mg/ml) (Fig. 1).

Oral challenge test

The DBPC with carmine achieved a positive result on the day carmine was administered. The patient reported shortness of breath, pharyngeal pruritus, and rhinitis, and a fall of 50% in PEF was assessed 45 min after the last dose of carmine was administered. Bronchial symptoms spontaneously recovered in 30 min, but rhinoconjunctivitis persisted for 5 h.

On the day placebo was administered, subjective or objective signs or symptoms were not registered.

SDS-PAGE

As shown in Fig. 2 for the cochineal insect, a Coomassie brilliant blue-stained SDS-PAGE gel revealed two main bands of 50 and 28 kDa, but several minor bands were also shown (A, B, C, and

SPECIFIC BRONCHIAL CHALLENGE TEST

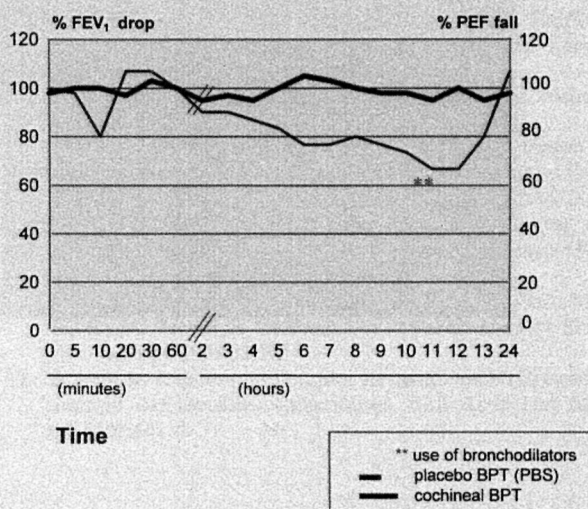


Fig. 1. Results of specific bronchial inhalative challenge test with cochineal insect extract (6.25 mg/ml in PBS) performed 1 month after last occupational exposure. First hour was monitored by FEV_1 recordings, and following hours (separated by // sign) by PEF.

D extracts). Bands were better shown with boiled extracts weakened in SB (B and D extracts). Electrophoretic separation and immunoblotting improved after processing the antigen as in method E.

Immunoblotting

Immunoblot made with the E extract was of higher quality and allergen characterization much better than with the other extracts. The patient's serum reacted strongly to a great number of bands of the E cochineal extract, all of them of high molecular weight. It showed more bands than SDS-PAGE (Fig. 2).

Discussion

A few cases of hypersensitivity to carmine in dye manufacture workers, as well as asthma in occupationally exposed patients, have been reported (3–5), but an IgE mechanism could not always be demonstrated, and immunoblotting has never been done. Carmine was identified by our patient as the origin of his symptoms because it was only infrequently used in his work. SPT with carmine and cochineal insect extract were clearly positive, contrary to other reports (4), and negative to other substances, in agreement with the subject's history. The clearly carmine-related significant changes in PEF obtained while the subject was working confirmed the diagnosis of occupational asthma, and

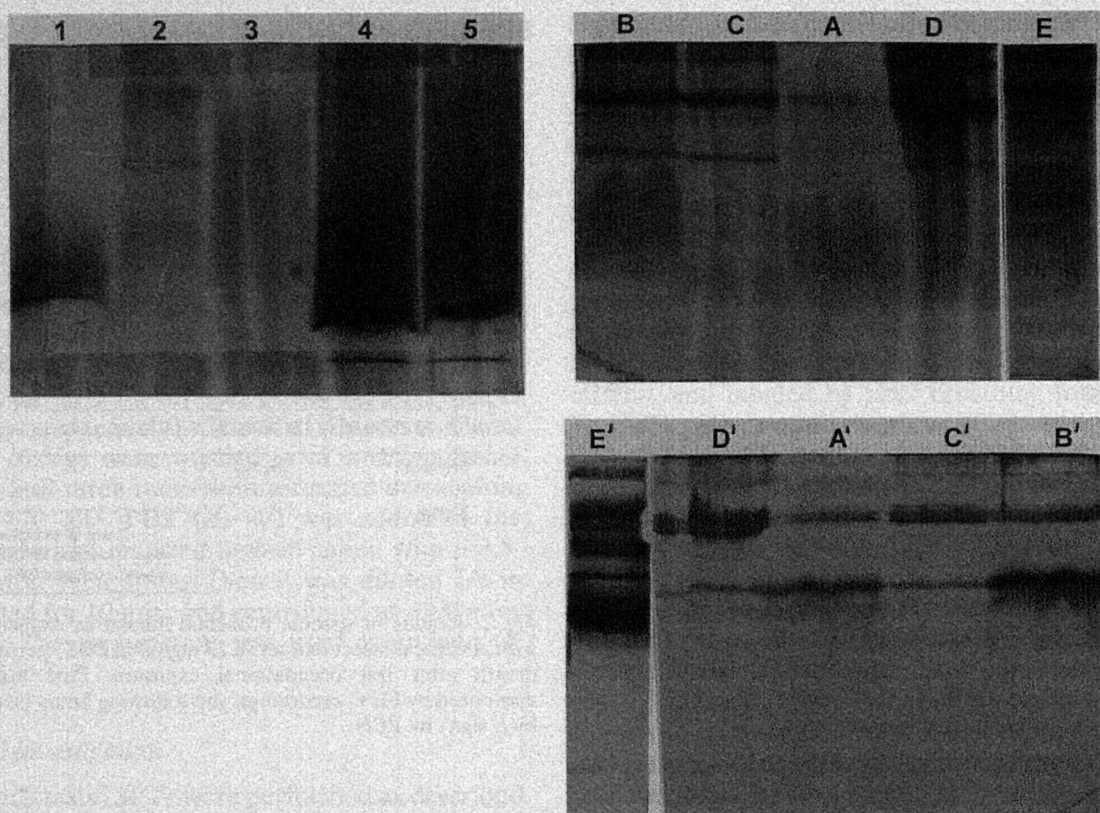


Fig. 2. SDS-PAGE and immunoblotting results. 1) Carminic acid, 2) cochineal insect in PBS, 3) cochineal insect in SB, 4) hydrosoluble carmine in PBS, 5) hydrosoluble carmine in SB; A) dried minced cochineal insect in SB, B) boiled A extract, C) precipitate from A extract preparation redissolved in SB, D) precipitate from B extract preparation redissolved in SB, E) pounded dried cochineal insect washed with methanol and saturated ammonium sulfate; A', B', C', D', and E') immunoblotting results of A, B, C, D, and E extracts, respectively.

carmine-induced asthma was also confirmed by a dual positive specific bronchial challenge test with the cochineal extract (Fig. 1). Specific IgE to cochineal insect was shown by the strongly positive immunoblot (Fig. 2).

All recognized bands were of high molecular weight, indicating that the responsible agent for the symptoms is not a hapten, as previously suggested (2), but a true allergen or a hapten bound to high-molecular-weight carriers present in cochineal extracts.

The negative SPT or specific IgE results of other groups may be due to the use of a carminic acid extract or to a not optimally processed cochineal insect or carmine extract, or one not freshly used. We got much better characterized immunoblotting when we slightly processed our cochineal extract (Fig. 2). We used cochineal insect extracts and not carmine due to the results of the SDS-PAGE in which many more protein bands were shown with the cochineal insect extract with better electrophoretic separation and less stain background. It is assumed that protein residues are present in commercial dye (11), and this seems to be the allergen

source in carmine handlers. Nonetheless, we are aware that working with dried insects can lead to problems that remain to be solved. Thus, it is reasonable to say that the carmine allergens are still not completely characterized, and more studies are necessary to do it.

Food labeling is very important, as it should reveal all substances able to cause, not only secondary effects or toxicity, but also allergic diseases. Exact labeling of food additives is also important because sensitized patients can react to minimal amounts of an allergen, as this patient developed symptoms from the carmine contained in a sweet (much less than the maximum permitted dose of 5 mg/kg body weight).

We conclude by saying that, to our knowledge, this is the first case of a patient occupationally sensitized to carmine in which

- 1) asthmatic symptoms were triggered either by ingestion of a food containing the dye or inhalative exposure
- 2) symptoms were reproducible by inhalative as well as oral challenge test

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.