

OCCUPATIONAL ASTHMA INDUCED BY INHALED CARMINE AMONG BUTCHERS

BELÉN AÑIBARRO¹, JAVIER SEOANE¹, CONCEPCIÓN VILA¹,
VICTORIA MÚGICA¹ and MANUEL LOMBARDEO²

¹The Allergy Unit
Severo Ochoa Hospital
Leganés, Spain

²Investigation Department
ALK-Abelló Laboratory
Madrid, Spain

Abstract

Background: Carmine is a natural coloring agent for food and cosmetics. There have been several reports of occupational asthma among employees at factories making natural dyes, however, there are no cases reported among butchers. **Objectives:** We report on two male patients who presented with a history of occupational asthma. Both patients are butchers and used a mixture of additives with carmine as dye in sausages. **Methods:** Skin prick tests were performed with common aeroallergens, commercial mixture additives and separated compound of the additive mixture. Total IgE, metacholine test, specific inhalatory challenge tests with carmine and mites, SDS-PAGE and IgE-immunoblotting were carried out. **Results:** Skin tests were positive to mites, additive mixture and carmine in both cases. Specific IgE to mites was positive only in patient one. Bronchial provocation tests were positive to metacholine and carmine and negative to *Dermatophagoides pteronyssinus* in both cases. IgE-immunoblot showed a specific IgE-binding band at 10 kDa and at a large number of bands along all molecular weights. No inhibition was achieved in carmine-IgE-specific bands with the *D. pteronyssinus* extract. **Conclusions:** We report on two butchers with occupational asthma due to an IgE-mediated sensitization to carmine. There are several proteins that may act as allergens, and these may be conditioned by the route of exposure and are not cross-reactive with mite proteins. Carmine may be considered to be an etiologic agent in butcher's asthma.

Key words:

Carmine, Allergy, Butchers, Occupational asthma, Cochineal, Dyes, E-120

INTRODUCTION

Carmine (E-120) is a natural red dye extracted from the dried female bodies of insect *Dactylopius coccus costa* (cochineal). It has been reported to cause hypersensitivity reactions in dye manufacture workers [1–6]. The coloring principle is a hydrated aluminum chelate of carminic acid. Today carmine is used in the food (butchery and confectionery), cosmetic and pharmaceutical industries, and also as a histologic dye.

We describe two cases of occupational asthma and rhinitis resulting from exposure to carmine which was used in the production of sausages.

CASE REPORTS

Case 1

The patient was a 47-year-old butcher. He was referred to our department because he exhibited a history of rhinitis and asthma. There was no known history of atopy. He was a nonsmoker and had worked in the same butcher's, a small family business, since he was 12 years old. Fifteen years ago he began to experience perennial rhinitis and conjunctivitis, associated in the last two years with cough, shortness of breath, dyspnea and chest tightness. He noticed that his symptomatology was related with the manufacture of burger, sausage, salami and "chorizo"

Received: March 25, 2003. Accepted: May 9, 2003.

Address reprint requests to B. Añibarro MD, C/Arzobispo Morcillo 42 9C, 28029 Madrid, Spain (e-mail: banibarro@hsvoinalud.es).

that he prepared with meats and a powdered mixture of food additives (*Colorsan-10*: sugar, starch, salt, Na-metabisulphite, ascorbic acid, Na-ascorbate and carmine). The patient did not report allergic reactions on eating delicatessen meats.

Case 2

A 24-year-old man reported recent episodes of rhinitis and asthma. He worked in a butcher's for 4 years. His job was to prepare "chorizo", sausages and burgers using a mixture of additives (sugar, salt, Na-metabisulphite, ascorbic acid, Na-ascorbate and carmine), garlic and paprika which are added to the meat. He had a history of rhinoconjunctivitis due to pollen allergy in the last 2 years.

Seven months before the first visit he began to experience escalated symptoms of rhinitis, cough, shortness of breath and wheezing. These symptoms recurred daily but improved on leaving work. They usually appeared 10 min after the beginning of work and then worsened.

MATERIALS AND METHODS

Extracts

Mixture of additives (*Colorsan-10*, Sta Catalina del Monte, Murcia, Spain) and their different components separately (Na-metabisulphite, ascorbic acid, Na-ascorbate and carmine) were provided as powder form. For skin-prick tests (SPTs) a 10% w/v (weight/volume) extract was prepared by dissolving these materials in phosphate-buffered saline (PBS) solution at room temperature.

Carmine powder was incubated at 10% in PBS for 2h at 4°C with gentle agitation. After it had been dialyzed against purified water, the solution was passed through a Millipore filter (0.22 µ). This extract was used for skin and inhalation tests and for the *in vitro* studies.

Skin tests

Skin prick tests (SPTs) were performed with the extracts described previously. A battery of common inhalant allergens, including mites, cockroach, pollens, moulds and dander (ALK-Abelló, Madrid, Spain) was tested. Garlic and meats were also tested in a prick by prick with the

fresh products. Histamine phosphate 10 mg/ml, and PBS were used as positive and negative controls. Responses were examined after 15 min. A wheal with a mean diameter greater than 3 mm of the negative control wheal was considered a positive response.

Five atopic and five non-atopic subjects were tested with carmine extract to determine non-irritating concentrations.

Total and specific IgE

Total IgE levels and specific IgE antibodies against *D. pteronyssinus* and *D. farinae* were measured by Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden).

Bronchial provocation tests

These tests were done with the informed consent, when the patients were free of symptoms and not receiving medication that could affect the results.

Methacholine inhalation test was performed according to the abbreviated method described by Chatham et al. [7] using an MEFAR dosimeter MB3 (MEFAR S.R.L, Bovezzo, Italia). The results of this test are expressed in terms of the dose necessary to decrease forced expiratory volume in 1 sec (FEV₁) at provocative dose causing a 20% fall in FEV₁ (PD₂₀), given in cumulative inhalation units (1 inhalation unit = 1 breath of solution with 1 mg of methacholine/ml). Specific bronchial provocation tests (BPTs) were performed according to the guidelines of the American Thoracic Society [8] with *D. pteronyssinus* (biological units – BU) and carmine (w/v) extracts described previously. Diluted extracts were generated by an MEFAR dosimeter. No response to the PBS solution challenge was required to start the allergen challenge. The initial extract solution was 10⁻⁶, and then the allergen was given in progressively increasing concentrations at intervals of 10 min. Patients took 5 consecutive aerosol-containing, slow, deep breaths. Baseline pulmonary function tests were performed and repeated 10 min after each allergen inhalation. A positive immediate response was defined as a 20% fall in FEV₁. Then hourly controls were performed for 6 h after challenge and the peak-flow measurements were recorded for 24 h.

Two asthmatic patients with allergy to mites were challenged as controls for carmine inhalation challenge to rule out a non-specific response.

IgE-Immunoblotting and immunoblot inhibition

SDS-PAGE was carried out according to Laemmli [9], with use of nonreducing conditions and 15% polyacrylamide running gel. The carmine extract proteins separated by SDS-PAGE were transferred onto nitrocellulose membranes as described by Towbin et al. [10].

Immunoblotting of IgE-binding proteins was achieved by enhanced chemiluminescence according to the manufacturer's instructions (ECL-Amersham). As negative controls, blots were also incubated with dilution buffer instead of patient's serum.

In the immunoblot inhibition experiment, carmine extracts were electrophoresed and immunoblotted as described before [10]. The sera of the patients, at a 1/10 dilution, were inhibited with carmine and *D. pteronyssinus* extracts respectively for 1 h and incubated overnight with the strips of carmine extract. The strips were washed and incubated with 125 I-labeled anti-human IgE for 6 h and then exposed to x-ray film at -70 C.

RESULTS

Skin prick tests

Patient 1 had SPTs positive to *D. pteronyssinus* and *D. farinae* as were to the additive mix (*Colorsan-10*) and carmine extracts. Results of SPTs with other common inhalant allergens were all negative as were the results of the tests with meats, garlic, paprika and the other additives tested. Total IgE was 516 kU/l. Specific IgE against *D. pteronyssinus* was 2.33 kU/l and 0.99 kU/l to *D. farinae*.

Patient 2 had SPTs positive to grass pollens and *D. pteronyssinus* as were to the additive mix and carmine extracts. No SPT response was registered to the other common inhalants, meats, garlic, paprika and additives tested. Total IgE was 149 kU/l. Specific IgE to *D. pteronyssinus* was < 0.35 kU/l.

No response was observed in SPTs of the control group.

Bronchial provocation tests

Methacholine inhalation test revealed the presence of bronchial hyperresponsiveness in both subjects, with a PD₂₀ value of 50 cumulative breath units in patient 1 and 175 in patient 2.

Both patients showed a negative result in the bronchial inhalation challenge with *D. pteronyssinus* extract (BU) up to a total dose of 93 cumulative inhalation units (1 inhalation unit = 1 breath of solution with 1 BU).

Specific BPTs with carmine extract elicited isolated immediate responses in both patients, with a PD₂₀ of 39 cumulative inhalation unit in the first patient and 70 in the second (1 inhalation unit = 1 breath of 1/5000 w/v dilution).

None of the control subjects with a similar grade of bronchial hyperresponsiveness showed reaction in the bronchial challenge with carmine extract.

IgE-Immunoblotting and immunoblot inhibition

Immunoblot with individual sera from the two patients (Fig. 1) showed a specific IgE-binding band at 10 kDa and at a great number of bands along all molecular weights mainly in patient 1.

To confirm the specificity of the detection and to rule out the presence of cross-reactivity with *D. pteronyssinus*, immunoblot-inhibition were carried out (Fig. 2). A complete inhibition was obtained with carmine extract in both sera, but no inhibition was achieved with the *D. pteronyssinus* extract.

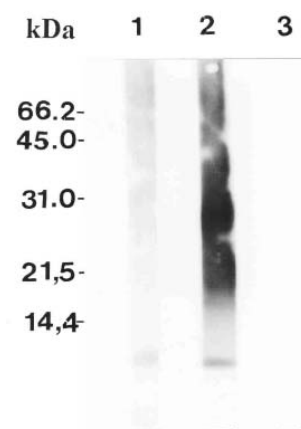


Fig. 1. Autoradiograph of IgE-immunoblotting from the SDS-PAGE gel of carmine extract and the patients' sera: serum from patient 1 (line 2), patient 2 (line 1) and negative control (line 3).

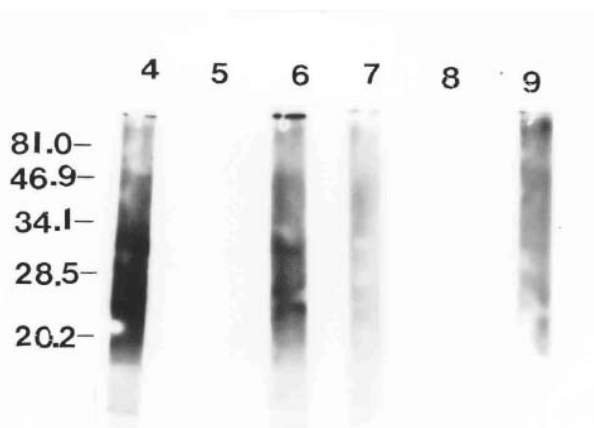


Fig. 2. IgE-immunoblotting inhibition of carmine allergen recognition by 2 patient sera: serum dilution of 1:10 from patient 1 (lines 4, 5, 6) and serum dilution of 1:5 from patient 2 (lines 7, 8, 9). Lines 4 and 7, noninhibited; lines 5 and 8, inhibited with carmine; lines 6 and 9, inhibited with *D. pteronyssinus* extract.

DISCUSSION

Carmine (E-120) is a natural red dye derived from the dried bodies of female cochineal insects (*Dactylopius coccus costa*), a parasite on some cacti, mainly *Nopalea cochenillifera*. Due to its strength and stability, carmine is widely used in the cosmetic (blushes, lipsticks), pharmaceutical (vitamins, syrup), food (juice, fruits, yoghurts, confectionery and butchery) and dyeing industries. It contains 50% of carminic acid, its coloring principle, and 15–25% of insect-derived proteins from the extraction process may be the implicated allergens in patients with carmine allergy.

There are several reports of carmine allergy resulting from inhalation [1–6] and ingestion [11–14] or contact exposure. In 1979, Burge et al. [1] first reported two patients with asthma due to carmine inhalation. In 1987, Tenabene et al. [2], first found positive skin tests to carmine in two of three patients with carmine-induced asthma, and in 1994, Quirce et al. [4] also demonstrated the presence of specific IgE antibodies against carmine and cochineal in one patient with carmine-induced asthma. These studies demonstrated that carmine may induce antibody responses, most likely IgE-mediated, in workers with occupational asthma, however, identification of responsible carmine allergens seem to be difficult. Previous data suggest that patient's reactivity to proteins from carmine may vary [4,6,14].

Carmine has been shown to have both high and low molecular weight components, either of which may theoretically cause sensitization. Quirce et al. [4] demonstrated with RAST inhibition studies that the main allergen had a molecular weight between 10 and 30 kDa. Recently, Chung et al. [14] showed that several bands between 23–88 kDa were variably recognized by all three reported patients. Lizaso et al. [6] by IgE-immunoblotting demonstrated specific binding bands at 17 kDa in cochineal raw extract, at 50 kDa in the boiled one, and at 28 kDa in carmine extract. In the present report, immunoblotting showed in both patients specific binding band at 10 kDa and several bands along a wide range of molecular weight. These findings and those cited above suggest that there are several proteins that may act as aeroallergens in exposed patients. It is possible that the sensitization to one or other protein may be conditioned to the type of exposure, but further studies investigating allergens in carmine allergic patients are required in order to get further insight into this aspect.

The coccoid-derived proteins are potential aeroallergens, similar to other arthropod-derived proteinaceous material. Our two patients have a sensitization to mites without clinical significance; however, in this study we could not demonstrate the existence of cross-reactivity between carmine proteins and mites.

Most of the reported carmine allergic patients are employed at factories that produce natural dye from cochineal, but other sources may also induce sensitization as demonstrated in the present report. We can conclude that carmine may be considered to be an etiologic agent in butchers with occupational asthma.

REFERENCES

1. Burge PS, O'Brien IM, Harries MG, Pepys J. *Occupational asthma due to inhaled carmine*. Clin Allergy 1979; 9: 185–8.
2. Tenabene A, Bessot JC, Lenz D, Kopferschmitt-Kubler MC, Pauli G. *Occupational asthma due to cochineal carmine*. Arch Mal Prof 1987; 48: 569–71.
3. Durham SR, Graneek BJ, Hawkins R, Taylor AJ. *The temporal relationship between increases in airway responsiveness to histamine*

- and late asthmatic responses induced by occupational agents. *J Allergy Clin Immunol* 1987; 79: 398–406.
4. Quirce S, Cuevas M, Olaguibel JM, Tabar AI. *Occupational asthma and immunologic responses induced by inhaled carmine among employees at a factory making natural dyes*. *J Allergy Clin Immunol* 1994; 93: 44–52.
 5. Acero S, Tabar AI, Alvarez MJ, García BE, Olaguibel JM, Moneo I. *Occupational asthma and food allergy due to carmine*. *Allergy* 1998; 53: 897–901.
 6. Lizaso MT, Moneo I, García BE, Acero S, Quirce S, Tabar I. *Identification of allergens involved in occupational asthma due to carmine dye*. *Ann Allergy Asthma Immunol* 2000; 84: 549–52.
 7. Chatham M, Bleecker ER, Norman P, Smith PL, Mason P. *A screening test for airways reactivity. An abbreviated methacholine inhalation challenge*. *Chest* 1982; 82(1):15–8.
 8. Cropp GJA, Bernstein IL, Boushey HA, Hyde RW, Rosenthal RR, Spector SL, et al. *Guidelines for bronchial inhalation challenges with pharmacologic and antigenic agents*. *Am Thorac Soc News* 1980; 6: 11–9.
 9. Laemli UK. *Cleavage of structural proteins during the assembly of the head of bacteriophage T4*. *Nature* 1970; 227: 680–5.
 10. Towbin H, Staehelin T, Gordon J. *Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications*. *Proc Nat Acad Sci USA* 1979; 76: 4350–4.
 11. Beaudouin E, Kanny G, Lambert H, Fremont S, Moneret-Vautrin DA. *Food anaphylaxis following ingestion of carmine*. *Ann Allergy Asthma Immunol* 1995; 74: 427–30.
 12. Baldwin JL, Chou AH, Solomon WR. *Popsicle-induced anaphylaxis due to carmine dye allergy*. *Ann Allergy Asthma Immunol* 1997; 79: 415–9.
 13. Wüthrich B, Kagi MK, Stucker W. *Anaphylaxis reactions to ingested carmine (E-120)*. *Allergy* 1997; 52: 1133–7.
 14. Chung K, Baker JR Jr, Baldwin JL, Chou A. *Identification of carmine allergens among three carmine allergy patients*. *Allergy* 2001; 56: 73–7.