

Acetophenone azine: a new allergen responsible for severe contact dermatitis from shin pads

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Summary

Background. Contact dermatitis resulting from the use of shin pads is usually caused by rubber components, dyes, benzoyl peroxide, or formaldehyde resins.

Objectives. To investigate and identify a new allergen in shin pads that was responsible for severe contact dermatitis in a young football player.

Methods. High-performance liquid chromatography (HPLC) of samples of shin pads was performed. The boy was patch tested with pieces of shin pads and with acetophenone azine, a chemical substance identified by HPLC in the foam of the shin pads.

Results. HPLC identified acetophenone azine at concentrations of approximately 20 µg/g of shin pad samples. Patch tests gave strongly positive reactions to pieces of shin pads and to acetophenone azine down to 0.001% in acetone, whereas acetophenone and hydrazine sulfate were both negative. Twenty controls were negative for acetophenone azine 0.01% in acetone.

Conclusions. Acetophenone azine is a new, strong allergen of shin pads, and more generally of other sport equipment based on ethylene vinyl acetate. It may be used as a biocide, but this has to be confirmed. Further investigations are needed to understand factors such as exposure, cross-reaction patterns, metabolism, and the optimal patch test preparation.

Key words: acetophenone azine; allergic contact dermatitis; chemical analysis; EVA copolymers; patch testing; shin pads; sport equipment; CAS no. 729-43-1.

Allergic contact dermatitis among people playing sport is well described, with their equipment often being the cause of such allergic reactions. We report a case of severe allergic contact dermatitis resulting from the use of shin pads in a young boy who played football. A new, strong allergen, acetophenone azine, present in the shin pad foam made of the copolymer of ethylene and vinyl acetate (EVA), has been identified.

Materials and Methods

Case history

A 13-year-old boy with no history of atopy or contact dermatitis presented with acute, vesicular dermatitis on his shins 1 month after wearing shin pads for playing football as a goalkeeper (Fig. 1).

This eruption became generalized 1 week later, and resulted in hospitalization. Hypereosinophilia was noted ($1120/\text{mm}^3$; normal, $<700/\text{mm}^3$). A skin biopsy confirmed the diagnosis of eczema. Three months after complete resolution of the eruption, the patient was referred to us for investigation.

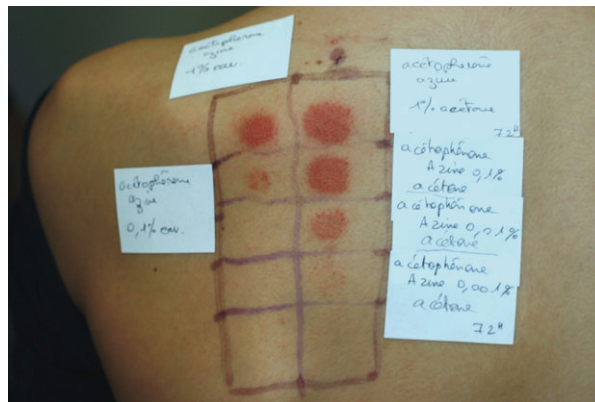
Because of the high number of patch tests, the patient was patch tested over several sessions: first, with the

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European baseline series (Trolab, Stallergènes, Antony, France) and plastics/glues and rubber series (Chemotechnique, Vellinge, Sweden), and at a second time with dyes and preservative series (Chemotechnique), with dimethylfumarate 0.1% and 0.01% wt/wt in petrolatum and with all of the topical medicaments used. Large pieces of the black shin pad foam in close contact with the skin were also tested 'as is', simply moisturized with acetone, water, and ethanol.

Acetophenone (Sigma-Aldrich) was also tested at dilutions of 0.1%, 0.03%, 0.01%, 0.003%, 0.001%, 0.0003%, 0.0001% and 0.00003% wt/wt in pet. Hydrazine was not available, so patch testing was performed with hydrazine sulfate 2% in pet. (Chemotechnique).



Tests were applied on the upper back, in IQ Chambers® (Chemotechnique). Readings were performed on day (D) 2 and D3, according to ICDRG guidelines.

We observed strong reactions to pieces of the black foam moisturized with ethanol, acetone, and water (2+ on D2; 3+ on D3).

Twenty control subjects did not react to acetophenone azine at 0.01% wt/vol in acetone (1 of 1 versus 0 of 20; $p < 0.05$, Fisher's exact test, two-sided).

Chromatographic analyses were performed with a high-performance liquid chromatography (HPLC) method suitable for identifying allergens in rubber items (1).

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Table 1. Results of patch tests with acetophenone, hydrazine sulfate, and acetophenone azine

Concentration (%)	Acetophenone (pet.) D2/D3	Hydrazine sulfate (pet.) D2/D3	Acetophenone azine (aq.) D2/D3	Acetophenone azine (acetone) D2/D3
2	NT	–/–	NT	NT
1	NT	NT	++/++	++/++
0.1	–/–	NT	+/+	++/++
0.03	–/–	NT	NT	NT
0.01	–/–	NT	–	+?/+
0.003	–/–	NT	NT	NT
0.001	–/–	NT	–/–	–/+?
0.0003	–/–	NT	NT	NT
0.0001	–/–	NT	–/–	–/–
0.00003	–/–	NT	NT	NT

NT, not tested.

then dissolved in 1 ml of acetonitrile, which was filtered before injection onto the HPLC column.

A reversed-phase column [Alltima C18, 4 mm, 150 × 4.6 mm, polyether ether ketone (PEEK)-lined; Alltech Associates, Deerfield, IL, USA] was eluted with acetonitrile and aqueous zinc sulfate (10^{-5} mol/l) at a ratio of 50:50 for 5 min, and then a linear gradient to 100% acetonitrile for 35 min. The eluent was pumped with an Agilent 1260 (Agilent Technologies, Santa Clara, CA, USA) at a flow rate of 1 ml/min, and monitored at 280 nm with an Agilent 1100 Series diode-array detector (Agilent Technologies). All devices in contact with the mobile phase after the injector were made of PEEK.

Acetophenone azine was identified after comparison of retention times and ultraviolet (UV) spectra recorded by the diode-array detector, and the concentration in the shin pads was determined after comparison of areas of sample peaks with the area of a sample of acetophenone azine with a known concentration. Duplicate analyses were performed for each sample.

Results

Acetophenone azine was identified in the shin pad by an external chemical laboratory (Service commun des laboratoires, Massy, France). Further analyses were performed by our own laboratory with HPLC to determine the amount of acetophenone azine in the product and to search for thiourea derivatives and other rubber allergens. The chromatograms showed a few dominant peaks with absorption at 280 nm (Fig. 3). Comparison of retention time and the UV spectrum with a reference sample of acetophenone azine established the identification of the peak eluting at ~20 min. The spectrum had a characteristic shape that was very different from those of other known rubber additives normally screened with this

HPLC method (1). No other peaks in the chromatograms could be identified. The concentrations were found to be 19, 24 and 23 µg/g, respectively, in the three samples from the shin pad. These concentrations correspond to 1.2, 1.0 and 1.7 µg/cm², respectively.

Discussion

Most cases of contact dermatitis caused by shin or knee pads seem to be irritant reactions resulting from friction and sweating (2), but allergic contact dermatitis may be under-reported. The main allergens are rubber additives that are included in the baseline series (mercapto and thiuram derivatives, and *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine) and in rubber series (thioureas and carbamates) (3, 4) or dyes (3), benzoyl peroxide (3), and urea formaldehyde or phenol formaldehyde resins (5, 6). When allergic contact dermatitis caused by protective equipment is suspected, it is always important to test a large piece of the equipment, as we did with the shin pads. Occasionally, patch testing with ultrasonic bath extracts of the shin pads may help to detect a contact allergy (7). Topical ointments and lotions used in the same skin area should also be tested.

Our patient suffered from allergic contact dermatitis caused by his shin pads, but he did not react to any of the allergens mentioned above. However, acetophenone azine was identified in the shin pads. At patch testing, he reacted positively to acetophenone azine in acetone down to 0.001%, whereas 20 controls did not react to a 10-fold higher concentration (0.01%).

Acetophenone azine was found as a component of EVA of the black foam of the patient's shin pads, but we do not know why and how it is incorporated in EVA. It is perhaps used as a catalyst during the process of polymerization of EVA. It is also known from older publications to have a

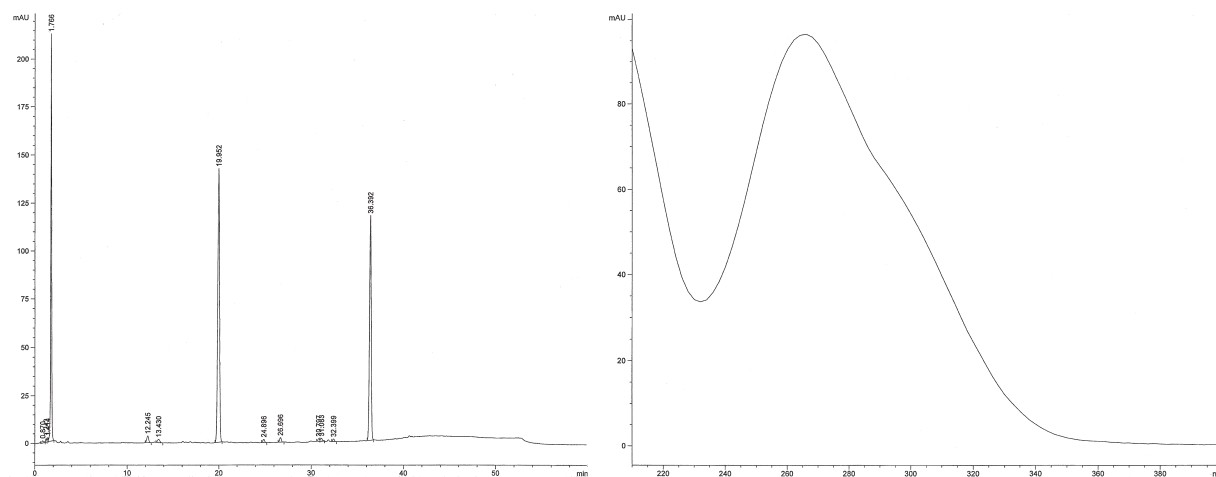


Fig. 3. Left: high-performance liquid chromatography chromatogram of an extract of the shin pad monitored at 280 nm. Right: ultraviolet spectrum obtained from the peak eluting at 19.952 min in the chromatogram. Analysis of a reference sample of acetophenone azine showed an identical retention time as the reference sample. The peak at 36.392 min could not be identified.

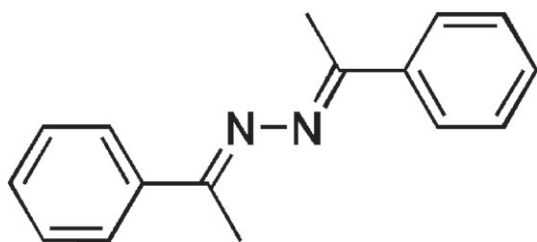


Fig. 4. Chemical structure of acetophenone azine.

broad spectrum of antimicrobial activity, and in particular antihelminthic activity (8). More generally, ketazines are used as industrial microbiocides with bactericidal and fungicidal properties (9).

Acetophenone azine, also called bis(1-phenylethylidene)hydrazine or methylphenylketazine (CAS no. 729-43-1), has the molecular formula $C_{16}H_{16}N_2$ and a molecular weight of 236.31 (Fig. 4). It is marketed as a white powder.

Acetophenone azine has been produced by reacting hydrazine hydrate with acetophenone.

It is mentioned in the Safety Data Sheet that acetophenone azine may cause allergic reactions in certain sensitive individuals after prolonged or repeated exposure (10).

Acetophenone azine itself could be the sole allergen responsible for this severe contact dermatitis. Under conditions with increased sweating, such as playing football, acetophenone azine could be hydrolysed to acetophenone and hydrazine. Hydrazine and some of its derivatives are known irritants and contact sensitizers (11–13). Severe contact dermatitis has

been reported in chemistry students after handling of hydrazine derivatives (13). Patch test reactions were strongly positive with the suspected product called *N*-(α -chlorobenzylidene) phenylhydrazine at 0.01% in acetone, and cross-sensitization was observed between various hydrazine derivatives.

Five of 6 students who were exposed to these chemicals felt ill, and 3 further students became ill a few months later. A biphasic course, as in our case, was described: circumscribed lesions developed quickly in directly contaminated areas, and this was followed by widespread erythema and oedema with papular, vesicular and urticaria-like lesions after 4–12 days.

Recently, when the local lymph node assay was used in combination with examination of the structural and physicochemical properties to identify dermal sensitizers, haloalkanes and hydrazines were among the most potent ones (14).

However, benzophenone azine, an azine derived from another cyclic ketone, did not show thermal decomposition even at temperatures as high as 230°C when analysed by gas chromatography, and homogeneous hydrolysis of benzophenone azine was reported only in the presence of various strong acids in a previous article (15). We therefore think that hydrazine is not the allergen concerned in our case. Furthermore, our patient did not react to the two possible hydrolysis substances, hydrazine (tested as hydrazine sulfate) and acetophenone.

In conclusion, acetophenone azine is a strong sensitizer that is possibly used as a biocide in the plastic industry. Further investigations are needed to investigate

factors such as exposure, cross-reaction patterns, metabolism, and the optimal patch test preparation. It is important to know why this product is used by the manufacturers of EVA copolymers; these thermoplastic copolymers are widely used all over the world by industries and consumers.

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