

Case report

IgE-mediated anaphylaxis to Thiomucase[®], a mucopolysaccharidase: allergens and cross-reactivity

Background: Thiomucase[®] is a mucopolysaccharidase obtained from ovine tissues mainly used to facilitate the diffusion of local anaesthetics and in the treatment of cellulitis. A patient with an anaphylaxis in relation to the intramuscular administration of Thiomucase[®] is reported.

Objective: To investigate Thiomucase[®] allergens and their possible relationship with dander allergens and animal albumins.

Material and methods: Skin prick tests (SPT) and serum-specific IgE were performed with Thiomucase[®] and danders. Thiomucase[®] SDS-PAGE immunoblotting was performed in order to study allergens. RAST/CAP inhibition and SDS-PAGE immunoblotting inhibition were carried out to study the cross-reactivity.

Results: Skin prick tests (SPT) were positive to Thiomucase[®], animal dander (cat, dog, sheep, other), bovine serum albumin (BSA), and echinococcus. Specific IgE was also positive to Thiomucase[®], animal dander (cat, dog, sheep, other), BSA and echinococcus. In the RAST-CAP inhibition assays BSA was nearly completely inhibited by Thiomucase[®], Thiomucase[®] was partially inhibited by BSA and cat and *Echinococcus granulosus* was partially inhibited by sheep and Thiomucase[®]. In the Thiomucase[®] SDS-PAGE immunoblotting several proteins fixed IgE, ranging from 20 kDa to > 94 kDa, the strongest with 43 kDa. The IgE fixation to BSA, cat and sheep in the SDS-PAGE immunoblotting was completely inhibited by the preincubation of the serum with Thiomucase[®].

Conclusions: An IgE-mediated anaphylaxis to Thiomucase[®] is documented. Multiple allergens are recognized in Thiomucase[®] by the patient serum, the main with 43 kDa. Partial cross-reactivity with BSA, cat dander and sheep dander is documented.

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Key words: anaphylaxis; animals; BSA; cross-reactivity; *Echinococcus granulosus*; IgE; allergens; mucopolysaccharidase; Thiomucase[®].

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Thiomucase[®] (Almirall Prodesfarma, Barcelona, Spain) is a mucopolysaccharidase obtained from ovine tissues and with an activity similar to hyaluronidase and chondroitin sulfate depolymerase. It is usually used to facilitate the diffusion of local anaesthetics and in the treatment of cellulitis because of its diffusion and depolymeration capacity amongst hyaluronic and chondroitin sulfuric acids (1). Thiomucase[®] has also been used occasionally to improve the quality of motor blockade and to decrease the intraocular pressure in peribulbar anaesthesia, so it is a useful regional anaesthesia and akinesia technique for eye surgery (2). It has been also used in the treatment of cytotoxic agents extravasation, Peyronie's disease, extra-articular rheumatism and pre-menstrual syndrome. We report the case of a patient who suffered an anaphylaxis after the administration of Thiomucase[®] used as a treatment for cellulitis.

A 24-year-old woman, with rhinoconjunctivitis

owing to dander allergy for the last 8 years, received two intramuscular doses of Thiomucase[®] (mucopolysaccharidase, mannitol) as a mesotherapy treatment for cellulitis in an aesthetics centre in 1998. Five minutes later intense palm pruritus, generalized urticaria, facial angioedema, dyspnea, sick feeling and loss of consciousness developed. These symptoms disappeared within 20 min after treatment with 6-methylprednisolone, dextrochlorpheniramine and epinephrine. She had tolerated Thiomucase[®] (mucopolysaccharidase) suppositories when she was 12 years old.

Material and methods

Extracts

Commercial Thiomucase[®] was used. Its protein concentration was assessed according to the method of Lowry et al. (5) and came out as 1 mg per 100 TRU (1 mg/vial).

Cat dander and sheep dander extracts were kindly provided by ALK-Abelló (Madrid, Spain).

BSA with analytical grade was purchased from SERVA (Heilderberg, Germany).

Skin prick tests (SPTs)

SPTs were performed on the volar side of the forearm with a prick standardized lancet (DHS, Leverkusen, Germany) according to the Subcommittee on skin tests of the European Academy of Allergology and Clinical Immunology (3). Available commercial prick test extracts from mites, moulds, pollens, dander and foods (ALK-Abelló, Madrid, Spain; CBF-LETI, Barcelona, Spain; DHS-Bayer, Leverkusen) were tested. Commercial Thiomucase® was tested at different concentrations (1, 0.5, 0.05 mg protein/ml). *Echinococcus granulosus* prick (0.5 mg dry weight/ml) was kindly donated by IPI (Madrid, Spain).

Total serum IgE

Total serum IgE was measured by MEIA IMx system (Abbot Diagnostics, Chicago, IL, USA) following the instructions of the manufacturer.

Serum-specific IgE, RAST inhibition and CAP inhibition

Specific IgE to commercial extracts was measured by CAP FEIA (Pharmacia Diagnostics, Uppsala, Sweden) following the instructions of the manufacturer.

Specific IgE to Thiomucase® and BSA was measured by a radioimmunoassay technique (DDV Diagnostika, Hamburg, Germany) following the instructions of the manufacturer and using a RAST score. Thiomucase® and BSA were coupled to cyanogen-bromide activated paper disks according to the procedure of Ceska et al. (4).

A CAP level equal or greater than class 1 (0.35 KU/l) and a RAST level equal or greater than class 1 (0.35 KU/l) were both taken as positive.

RAST inhibition was performed according to Gleich et al. (6). The maximum inhibition is shown.

The CAP inhibition procedure was a modification of the RAST inhibition method of Gleich et al. (7).

Complementary tests

Blood and urine analysis, hydatid cyst serology, thorax X-ray, and abdominal ultrasound were performed as a part of the anaphylaxis study protocol.

One-dimensional electrophoresis (SDS-PAGE) and immunoblotting

Protein separation of Thiomucase®, BSA, sheep and cat extracts was accomplished by polyacrilamide slab-gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) according to a modification of Sutton et al. (8). The polyacrilamide gel concentration was 4% for the stacking gel and 12% for the running gel. Twenty µl of a 1-mg/ml solution of each allergenic extract were applied to each well (except Thiomucase® 0.333 mg/ml). Molecular weight markers were purchased from Pharmacia (Uppsala, Sweden) (94, 67, 43, 30, 20, 14 kDa).

Immunoblotting was performed according to Towbin et al. (9) with the patient serum, a negative control serum (SPTs negative with cat and Thiomucase®) and a positive control serum (SPTs positive with cat and other dander and negative with Thiomucase®).

Thiomucase®, cat dander, sheep dander, and BSA were subjected to SDS-PAGE and then electroblotted onto PDVF membranes (Millipore, Bedford, MA, USA). After removal from the transblot apparatus, the PDVF membrane was blocked with TRIS buffer

saline (TBS) Tween for 1 h at room temperature. The PDVF blot was then washed four times with TTBS (TBS with 0.1% Tween 20) and incubated with the sera (1 : 3 v/v dilution in TTBS) for 3 h at room temperature with rocking. The PDVF blot was again washed with TBS four times, and alkaline phosphatase-conjugated goat antihuman IgE (1 : 500 v/v of TBS, Caltag Laboratory, San Francisco, CA, USA) was added and incubated at room temperature with rocking for 3 h. After an additional wash with TBS three times, the blot was developed with an alkaline phosphatase conjugate substrate kit (Bio-Rad) of nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate at room temperature for 15 min. The reaction was stopped by incubating the PDVF blot for 10 min with distilled water.

Immunoblotting inhibition

Aliquots of the patient serum (1:3 v/v dilution in TTBS) were incubated overnight with equal volumes of Thiomucase® (1 mg/ml). Mixtures were then centrifuged (1300 g) and their supernatants incubated separately with Thiomucase®, cat dander, sheep dander and BSA blotted PDVF strips, followed by immunodetection as described above.

Results

SPTs were positive to Thiomucase® (1 mg protein/ml: wheal 4 × 4 mm plus erythema, 0.5 mg protein/ml: wheal 3 × 3 mm plus erythema, 0.05 mg protein/ml: wheal 3 × 3 mm plus erythema), animals (cat, dog, horse, hamster, sheep, guinea pig, rabbit, cow, mouse), echinococcus, BSA, another cow's milk proteins and some animal meats. SPTs with Thiomucase® were negative in 100 control patients (60 atopic and 40 nonatopic). Even 25 patients with dander allergy had negative skin tests to Thiomucase®.

The total IgE was 316 UI/l and specific IgE to Thiomucase® was positive (RIA: 1.09 PRU/ml). Specific IgE was also positive to animal dander (cat, dog, horse, hamster, sheep, mouse, guinea pig, goat, rabbit, cow), echinococcus, BSA and some animal meats.

Complementary tests were all normal (including echinococcus serology). An abdominal echography was performed in order to discard an hydatid cyst and was normal.

In the RAST inhibition assays BSA was inhibited up to 82% by Thiomucase® and Thiomucase® was inhibited up to 46% by BSA and 34% by cat (see Table 1).

Table 1. Maximum inhibition in CAP and RAST inhibition systems

Solid phase	Liquid phase	Technique	Inhibition (%)	Concentration
BSA	Thiomucase®	RAST	82%	500 µg/ml
	BSA	RAST	46%	4 mg/ml
Thiomucase®	Cat	RAST	34%	50 mg/ml
	Thiomucase®	CAP	54%	1 mg/ml
Echinococcus	Sheep	CAP	73%	1 mg/ml

In the CAP-inhibition procedures echinococcus was inhibited up to 73% by sheep and 54% by Thiomucase® (see Table 1 for the concentrations used).

A Thiomucase® SDS-PAGE showed multiples bands of different molecular weight.

Thiomucase® SDS-PAGE immunoblotting with patient serum showed IgE to several proteins with molecular weights ranging from 30 kDa to 94 kDa, the strongest with 43 kDa (Fig. 1A). The patient serum recognized several bands in sheep extract ranging from 43 to > 94 kDa and in cat extract ranging from 20 kDa to > 94 kDa and a big band with 67 kDa in BSA. The negative control serum showed no IgE to Thiomucase®, cat, sheep and BSA. The positive control serum with dander allergy showed weaker IgE fixation to cat and sheep extracts and a different IgE pattern to Thiomucase® with a weak fixation to the 43 kDa allergen and a stronger fixation to an allergen with molecular weight next to 67 kDa. The control positive serum showed no fixation to BSA, although recognized the other bands.

In the immunoblotting inhibition assay BSA, sheep, cat and Thiomucase® IgE were completely inhibited by preincubation of the patient serum with Thiomucase® (Fig. 1B).

Discussion

We report on a patient with IgE-mediated anaphylaxis to Thiomucase®, a mucopolysaccharidase, as demonstrated by means of *in vivo* and *in vitro* tests. These results are specific as other patients were tested with negative results.

No provocation test was performed with Thiomucase® because of the severity of the reaction, the clear-cut relationship between its administration and the reaction and the demonstration of specific IgE to the product by means of SPTs, serum specific IgE (RIA) and immunoblotting.

The ovine origin of mucopolysaccharidase together with the presence of multiple dander and BSA sensitizations in this patient suggested the possibility of cross-reactivity owing to a BSA-like panallergen. The study was directed to confirm this hypothesis and the BSA was chosen as a representative of the animal serum albumins.

In the RAST and CAP inhibition assays Thiomucase® was partially inhibited by BSA (46%) and cat (34%). On the contrary, BSA was nearly completely inhibited by Thiomucase® (82%). These data confirmed the existence of partial cross-reactivity between Thiomucase®, BSA and cat.

In the SDS-PAGE immunoblotting Thiomucase® had multiple allergenic proteins, one of them coincided with BSA known molecular weight (67 kDa), but the band that fixed most IgE was between 30 and 43 kDa.

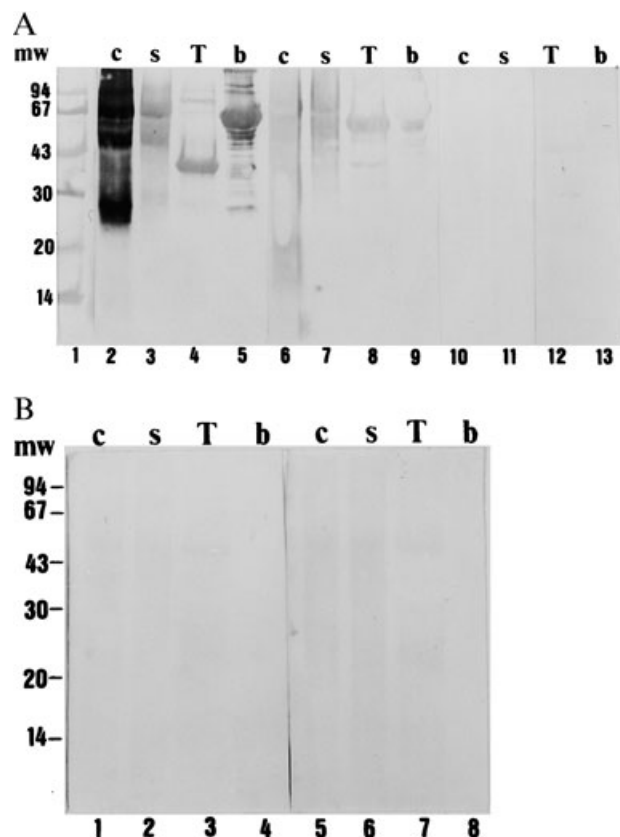


Figure 1. (A) SDS-PAGE immunoblotting (c, cat; s, sheep; T, Thiomucase®; b, BSA). Rows 1: Molecular weight standard. Rows 2–5: Patient serum. Rows 6–9: Control serum (patient with multiple dander allergy). Rows 10–13: Control serum (atopic patient without dander allergy). (B) SDS-PAGE immunoblotting inhibition with Thiomucase® (c, cat; s, sheep; T, Thiomucase®; b, BSA). Rows 1–4: Patient serum. Rows 5–8: Control serum (patient with multiple dander allergy).

BSA showed multiple bands in the SDS-PAGE that could be owing to impurities (BSA with analytical grade was used), to the degradation of BSA with formation of several peptides with lower molecular weights and/or subsequent aggregation with generation of peptides with higher molecular weight.

In the SDS-PAGE immunoblotting inhibition studies BSA, cat and sheep IgE were completely inhibited by Thiomucase®, that demonstrated the existence of cross-reactivity between Thiomucase®, BSA, sheep and cat. Thiomucase® would contain all the allergens of cat, sheep and BSA recognized by the patient, but not vice versa.

Taking together the clinical data, CAP/RAST inhibition results and SDS-PAGE immunoblotting inhibition results the hypothesis of a reaction owing to the BSA behaving as a panallergen in animal dander, meats and Thiomucase® could be discarded.

The positive IgE to echinococcus by CAP and prick test could be owing to the contamination of the echinococcus extracts with animal proteins, as they are usually extracted from sheep, goats or cows.

Echinococcosis is an endemic infectious disease in Spain that has been associated with anaphylaxis but which was discarded in this patient. Thus, positive CAP to echinococcus could be a false positive result in patients with dander sensitization, specially sheep and should be interpreted with caution in patients with dander sensitization. The high inhibition of echinococcus CAP with sheep (73%) supports this hypothesis. Other explanations could be that echinococcus had mucopolysaccharidases in its structure (inhibition by Thiomucase®) or both Thiomucase® and echinococcus extracts were contaminated by sheep tissues.

After an intense search into Medline (1966–2001) using different entries we found only one article about mesotherapy that mentioned an anaphylactic shock after a mesotherapy session with procaine plus Thiomucase® (10), with no allergological study. No other references to hypersensitivity or allergy to Thiomucase® or mucopolysaccharidase were found.

In conclusion, we report the first documented case of IgE-mediated anaphylaxis (11) owing to Thiomucase®, a mucopolysaccharidase with multiple allergens. Some of these allergens are shared with BSA, cat, sheep and echinococcus. Fortunately, other patients with dander allergy did not recognize Thiomucase®.

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