controls, respectively. Immunoblot under reducing conditions was performed as well. Electrophoresis of extracts (15 μg/lane) was carried out in a 10% polyacrylamide precast Nupage Bis-Tris gel with 2-(*N*-morpholino) ethane sulfonic acid buffer at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane that was saturated with 0.1 M tris buffered saline containing 5% of BSA and incubated for 16 h at 4°C with serum. Bound specific IgE were detected by peroxidase-conjugated anti-human IgE antibodies from goat (1 : 1000) using an enhanced chemiluminescence western blotting kit as substrate.

Patient's serum showed a strong IgE reactivity to sheep milk, a slighter reactivity to goat milk, but no reactivity to cow milk (Table 1), whereas the positive control serum strongly reacted to milk proteins from all three animals. On immunoblot, an intense IgE reactivity at about 55 kDa, and a less evident reactivity at about 28 kDa with sheep milk, but no reactivity to cow milk were observed (Fig. 1). Preadsorption of serum with 130 μg of sheep milk extract totally inhibited IgE reactivity to sheep milk, whereas 130 μg of house dust mite or cow milk did not exert any effect. The negative control serum did not react to any milk.

Although it is generally believed that the significant homology between milk from cow, sheep, and goat results in clinical cross-reactivity, cases of ovine milk allergy in the absence of cow milk allergy are being increasingly reported (2, 3). Several patients were sensitized to sheep and goat casein, and reactivity to a 14 kDa allergen in goat milk was found in a patient who tolerated cow and sheep cheese (4). Other patients reacted to multiple ovine milk allergens

Table 1 IgE reactivity to sheep, goat, and cow milk proteins

Serum	Sheep	Goat	Cow
Patient	1578	568	216
Positive control	3515	3571	3443
Negative control	272	387	212

Figures represent absorbance (OD) at 450 nm.

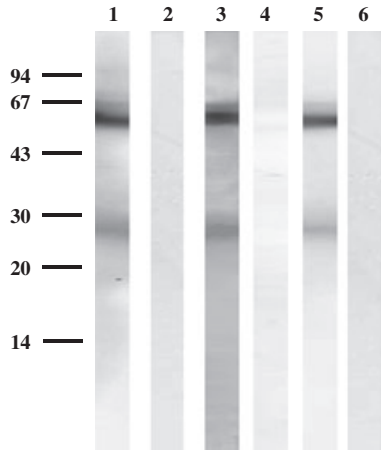

Figure 1 Analysis of patient's IgE reactivity to ovine and cow milk by immunoblot. Lane 1: sheep cheese (uninhibited); lane 2: cow milk; lane 3: sheep cheese (serum preadsorbed with cow milk); lane 4: sheep cheese (serum preadsorbed with sheep cheese); lane 5: sheep cheese (serum preadsorbed with house dust mite); lane 6: sheep cheese (normal serum).

(5), but following the report by Alvarez (6), this is the first report of selective IgE reactivity to a 55 kDa protein in ovine milk. This molecular weight corresponds to serum albumin. In a case of allergy to pork serum albumin (7), we observed that only raw or semi-raw meats, but not cooked meats induced symptoms, suggesting the heat sensitivity of the allergen. In conclusion, serum albumin can be associated with allergy to dairy products, and this kind of allergy can be species specific.

*Ambulatorio di Allergologia
Clinica San Carlo
Via Ospedale 21
20037 Paderno Dugnano (MI)
Italy
Tel.: +39 0299038470
Fax: +39 0299038223
E-mail: r.asero@libero.it

Accepted for publication 14 November 2009
Allergy 2010: 65:1067–1068

DOI: 10.1111/j.1398-9995.2009.02298.x

References

1. Asero R, Mistrello G, Roncarolo D, Casarini M, Falagiani P. Allergy to nonspecific lipid transfer proteins in Rosaceae: a comparative study of different in vivo diagnostic methods. *Ann Allergy Asthma Immunol* 2001;**87**:68–71.
2. Wüthrich B, Johansson SG. Allergy to cheese produced from sheep's and goat's milk but not to cheese produced from cow's milk. *J Allergy Clin Immunol* 1995;**96**:270–273.
3. Calvani M Jr, Alessandri C. Anaphylaxis to sheep's milk cheese in a child unaffected by cow's milk protein allergy. *Eur J Pediatr* 1998;**157**:17–19.
4. Tavares B, Pereira C, Rodrigues F, Loureiro G, Chieira C. Goat's milk allergy. *Allergol Immunopathol (Madr)* 2007;**35**:113–116.
5. Martins P, Borrego LM, Pires G, Pinto PL, Alfonso AR, Rosado-Pinto J. Sheep and goat's milk allergy: a case study. *Allergy* 2005;**60**:129–130.
6. Alvarez MJ, Lombardero M. IgE-mediated anaphylaxis to sheep's and goat's milk. *Allergy* 2002;**57**:1091–1092.
7. Asero R, Mistrello G, Falagiani P. Oral allergy syndrome from pork. *Allergy* 1997;**52**:684–686.

Severe anaphylaxis to the antiseptic polyhexanide

O. Kautz, H. Schumann, F. Degerbeck, L. Venemalm, T. Jakob*

Keywords: chlorhexidine; IgE; polyaminopropylbiguanide; polyhexamethylenebiguanide; polyhexanide.

An 81-year-old female patient experienced symptoms of a grade III anaphylaxis with palmar pruritus, flush, swelling of the lips, swallowing difficulties, hypotension and loss of consciousness while using a new brand of wet toilet paper containing polyhexanide as disinfectant. The detailed allergy history revealed that the patient had experienced three similar anaphylactic episodes (grade II) during wound care of an existing leg ulcer, once when using a new wound

First report of anaphylaxis to polyhexanide, a widely used antiseptic, with confirmed IgE-mediated mechanism.

dressing that contained polyhexanide (Suprasorb PHMB) and twice after wound cleansing with two different polyhexanide disinfectants (Lavanid 1 and Prontosan). The patient had no known allergies or atopic diseases. Total IgE was elevated (411 kU/l), there were no specific IgE detected for common seasonal and perennial inhalation allergens, base line mast cell tryptase was normal (3.07 μg/l). Skin prick tests (SPT) were positive for Lavanid 1 in a 1 : 10 dilution corresponding to 20 μg/ml polyhexanide. Except for polyethylene glycol (PEG) 4000, Lavanid 1 contains no other additives. SPT performed with PEG 4000 was negative. SPT with chlorhexidine in different commercial preparations was positive. None of five healthy volunteers tested positive using the same test preparations for polyhexanide and chlorhexidine. Analysis of specific IgE (ImmunoCAP; Phadia, Uppsala, Sweden) revealed specific IgE to chlorhexidine (0.88 kU_A/l) and polyhexanide (16.5 kU_A/l) (a result ≥0.35 kU_A/l was considered positive). The polyhexanide ImmunoCAP was an experimental prototype, and the chlorhexidine ImmunoCAP was the regular product c8 (ImmunoCAP; Phadia).

Polyhexanide is a poly-biguanide antiseptic, which is widely used, for example, in contact lens solutions, wound dressings, pool cleaners and in cosmetics. It is reported to have an excellent tissue compatibility, to be non-cytotoxic, nonirritating and nonsensitizing (1). Only two reports about allergic reactions to polyhexanide exist, in each case anaphylactic reactions during surgery are described (2, 3). Allergic reactions to another biguanide antiseptic, chlorhexidine, are well known (4), and an IgE-mediated mechanism has been established (5, 6). The diagnosis of polyhexanide allergy in previous reports was based on history and SPT (2) or history alone (3). To our knowledge, the patient presented here is the first case of anaphylaxis to polyhexanide with a confirmed IgE-mediated mechanism.

The way of sensitization in the presented case is unclear. The use of the polyhexanide containing wound dressing, which led to anaphylaxis, was the first documented contact to polyhexanide. It may be speculated that the former use of chlorhexidine sensitized the patient. No studies on the allergenic determinants of polyhexanide have been performed. Polyhexanide is a hexamethylene biguanide polymer. With chlorhexidine, it shares the hexamethylene biguanide, but lacks the 4-chlorophenyl aromatic ring present in chlorhexidine (Fig. 1). Therefore, a partial cross-reactivity with chlorhexidine could be expected. To address this question, we performed inhibition studies: varying concentrations of polyhexanide and chlorhexidine digluconate were added to the patient serum, and a 50% inhibition of the assay response was achieved at a polyhexanide concentration of 0.34 μg/ml and a chlorhexidine concentration of 145 μg/ml (Fig. 1). The results from this inhibition study indicate a limited *in vitro* cross-reactivity between polyhexanide and chlorhexidine, with a higher relative avidity of the patient's IgE antibodies to polyhexanide compared with chlorhexidine. As a clinical consequence, patients with known chlorhexidine allergy could be at risk for anaphylactic reactions to polyhexanide. However, sera from three patients with a history of chlorhexidine allergy and sIgE to chlorhexidine ranging from 1.0 to 28.1 kU_A/l tested negative for sIgE to polyhexanide, suggesting that the earlier reported patient displayed a genuine polyhexanide sensitization. Patients and health care workers should be aware of polyhexanide as a potential allergen and need to be informed that polyhexanide is often also declared as polyhexamethylene-biguanide or as polyaminopropyl-biguanide.

This work was in part funded by a research grant from Phadia to TJ.

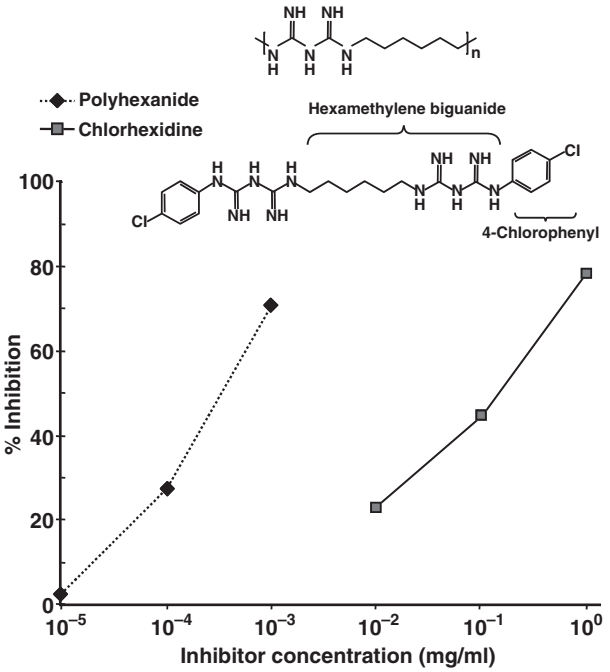

Figure 1 Inhibition of sIgE binding to polyhexanide ImmunoCAP prototype by preincubation of patient's serum with increasing concentrations of polyhexanide (diamonds) or chlorhexidine (squares).

*Allergy Research Group
Department of Dermatology
University Medical Center Freiburg
Hauptstrasse 7
79104 Freiburg
Germany
Tel.: +49 761 270 6781
Fax: +49 761 270 6655
E-mail: thilo.jakob@uniklinik-freiburg.de

Accepted for publication 19 November 2009
Allergy 2010: 65:1068–1070

DOI: 10.1111/j.1398-9995.2009.02299.x

References

1. Rietkötter J, Körber A, Grabbe S, Dissemond J. Eradication of methicillin-resistant *Staphylococcus aureus* in a chronic wound by a new polyhexanide hydrogel. *J Eur Acad Dermatol Venereol* 2007;**21**: 1416–1417.
2. Olivieri J, Eigenmann PA, Hauser C. Severe anaphylaxis to a new disinfectant: polyhexanide, a chlorhexidine polymer. *Schweiz Med Wochenschr* 1998;**128**: 1508–1511.
3. Ferrarini A, Baggi M, Flückiger R, Bianchetti MG. Intraoperative anaphylaxis to a chlorhexidine polymer in childhood. *Paediatr Anaesth* 2006;**16**:705. (abstract).
4. Krautheim AB, Jermann TH, Bircher AJ. Chlorhexidine anaphylaxis: case report and review of the literature. *Contact Dermatitis* 2004;**50**:113–116.
5. Pham NH, Weiner JM, Reisner GS, Baldo BA. Anaphylaxis to chlorhexidine. Case report. Implication of immunoglobulin E antibodies and identification of an allergenic determinant. *Clin Exp Allergy* 2000;**30**:1001–1007.
6. Garvey LH, Krøigaard M, Poulsen LK, Skov PS, Mosbech H, Venemalm L et al. IgE-mediated allergy to chlorhexidine. *J Allergy Clin Immunol* 2007;**120**:409–415.

Immediate rhinoconjunctivitis induced by metamizole: an allergic reaction?

E. Di Leo, E. Nettis*, G. F. Calogiuri, A. Ferrannini, A. Vacca

Keywords: aspirin; hypersensitivity reactions; metamizole; oral challenge test; rhinomanometry test.

Hypersensitivity reactions to nonsteroidal anti-inflammatory drugs (NSAIDs) have been frequently described (1). Metamizole, a pyrazolone derivative with analgesic properties, frequently causes hypersensitivity reactions (2).

A 49-year-old male patient suffering from rhinitis and conjunctivitis after metamizole intake came to our observation because of need for aspirin for hypersensitivity. His clinical history gave nasal and ocular symptoms 30–60 min after the metamizole intake. As he had not taken other NSAIDs, his medical practitioner suggested the allergological evaluation before starting aspirin.

We investigated a rhinopathy provoked by metamizole in one patient performing skin and oral provocation tests by monitoring the nasal respiratory function by anterior rhinomanometry.

To confirm the causative role of metamizole, skin prick and intradermal tests with the drug (Novalgina® 1000 mg/2 ml, Sanofi-Aventis S.p.A., Milan, Italy) were carried out with negative results. Because of the described poorly dangerous adverse reactions, an oral challenge test (OCT) was performed using metamizole (Novalgina® 500 mg/ml drops). An oral tolerance test with acetylsalicylic acid (Aspro® 500 mg tablets, Bayer S.p.A., Milan, Italy) was carried out too. During the challenge tests, the respiratory functions were monitored with anterior rhinomanometry (ARM) and spirometry test upon the written informed consent. Before the OCT, the measured forced expiratory volume in 1 s (FEV1) must be at least 70% of the predicted value, and the subject must have nasal ways totally opened as assessed by ARM [total nasal resistances (R) of adult patients are considered as normal when lower than 0.50 Pa/cc^3/s on a resistance of 150 Pa) (3)]. In addition, at the baseline, the patient must have (i) absence of nasal (rhinorrhea, sneezing) or ocular (lacrimation, eye pruritus or redness) symptoms and (ii) normal R after challenge with saline.

We made the OCTs with metamizole and aspirin using the provocation protocol recommended by the EAACI/GA2LEN guideline for aspirin hypersensitivity (4). We thus performed two single-blind, placebo-controlled challenge tests on two different days with a two-week interval between the two tests (for metamizole and for aspirin). For each drug on day 1, three placebo doses were administered at 1.5–2-h intervals: for metamizole, the placebo was saccharose diluted in water, for aspirin was talc inserted into inert capsules. One week later, on day 2, the drug was administered. Each successive dose was administered after 2 h if no symptoms had developed. During the test, spirometry, rhinomanometry, and arterial pressure evaluation were monitored both before and 30 min after the drug or placebo or as soon as any symptom arose. OCT was considered as positive in case of: (i) R increase $\geq 100\%$ than baseline on ARM and/or (ii) symptoms like rhinitis or conjunctivitis (also in absence of rising R), and/or (iii) FEV1 decrease $\geq 20\%$ than the baseline. If positive reaction had occurred, the drug administration would be stopped.

The test was considered as negative in the absence of any reaction (symptomatic and/or functional) in the two protocol days after the intake of the cumulative dose of metamizole (500 mg) and aspirin (500 mg).

The metamizole OCT gave negative response on the spirometry test, and no cutaneous symptoms developed. However, patients showed rhinitis (rhinorrhea, nasal congestion) and conjunctivitis in conjunction with significant increase in nasal resistance ($R = +120$) with a sudden latency time of symptoms' development (30 min) after the first dose (Table 1). He did not show any reaction after the aspirin challenge.

Rhinoconjunctivitis reactions induced by metamizole are circumstantial (5): more frequently asthma, cutaneous, or anaphylactic reactions occur (6). This report first describes naso-ocular reactions to metamizole by oral provocation test and measured by ARM, which were not associated with other respiratory (e.g., asthma) or skin symptoms.

In the case of hypersensitivity reactions to NSAIDs, especially pyrazolones, *in vitro* diagnostics is not reliable, and the involved pathogenetic