
Polysorbate 80 in medical products and nonimmunologic anaphylactoid reactions

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Background: Polyoxyethylene-sorbitan-20-monooleate (also known as polysorbate 80 and Tween 80) is a solubilizing agent ubiquitously used in nutritives, creams, ointments, lotions, and multiple medical preparations (eg, vitamin oils, vaccines, and anticancer agents) and as an additive in tablets. Whereas its relevance as a contact allergen has declined during the past decades, it is of current relevance as a “hidden” inductor of anaphylactoid reactions.

Objective: To identify polysorbate 80 (generally believed to be an inert vehicle) as an inductor of a severe anaphylactoid reaction.

Methods: Skin prick testing, enzyme-linked immunosorbent assay, IgE immunoblotting, and flow cytometric detection of basophil activation were performed in controls and in a patient with a medical history of anaphylactic shock due to intravenous administration of a multivitamin product during pregnancy.

Results: Polysorbate 80 was identified as the causative agent for the anaphylactoid reaction of nonimmunologic origin in the patient. Polysorbate specific IgE antibodies were not identified in enzyme-linked immunosorbent assay and immunoblot examinations, confirming the nonimmunologic nature of the anaphylactoid reaction.

Conclusions: Polysorbate 80 is a ubiquitously used solubilizing agent that can cause severe nonimmunologic anaphylactoid reactions.

Ann Allergy Asthma Immunol. 2005;95:593–599.

INTRODUCTION

Polysorbates (also called Tweens) consist of a mixture of oleic esters of sorbitol and sorbitanes containing chains of polyethylene glycol (Fig 1). They are used as oil in water emulsifiers for ointments and lotions and as stabilizing and dissolving agents for flavors, essential oils, and medical infusions.¹ Table 1 summarizes the medical applications of polysorbate 80-containing products. The potency of polysorbate 80 as a type IV allergen is well known.^{2–4} In contrast, polysorbates are less well investigated as inductors of immediate-type reactions.^{5,6} We identified polysorbate 80, which is generally believed to be an inert vehicle, as an inductor of a severe nonimmunologic reaction.

METHODS

Characterization of Serum Samples From the Patient and Controls

Serum samples were taken from control individuals and from a 30-year-old patient referred to the Allergy Unit of the Department of Dermatology, University Hospital of Erlangen, to investigate an episode of anaphylactoid shock reaction on intravenous infusion of a multivitamin preparation (Multibionta N; Merck KgaA, Darmstadt, Germany) during her previous pregnancy. A few seconds after the start of the

infusion, the patient had experienced oral dryness, shortness of breath, bronchospasm, tachycardia, hypotension (blood pressure, 60/50 mm Hg), and, finally, unconsciousness. Skin symptoms were not reported. As a result of the immediate intervention of the treating gynecologist (intravenous prednisolone, clemastine, and volume substitution) and admission to the emergency department of the regional hospital, the pregnant patient recovered completely within 24 hours of supervision without harm to the unborn child. The allergologic workup was performed 4 months after delivery. The patient reported no previous contact with the culprit drug. The patient's medical history was unremarkable except for oral allergy syndrome (due to apple, pear, peach, cherry, hazelnut-containing cross-reactive allergens homologous to birch pollen allergens [eg, Bet v 1]) and seasonal and perennial allergic rhinoconjunctivitis (due to pollens from birch, hazel, alder, ragweed, grasses, rye, and house dust mites). In vitro (UniCap 100 system; Pharmacia, Freiburg, Germany), specific IgE antibodies were detected in the patient's serum for timothy grass (3.38 kU/L), birch (7.45 kU/L), mugwort (2.34 kU/L), *Dermatophagoides pteronyssinus* (13.8 kU/L), and *Dermatophagoides farinae* (12.8 kU/L). Results of CAP-FEIA (Pharmacia) for latex and Ficus species were negative. Total IgE levels were elevated to 452 kU/L (reference range, 0–100 kU/L). As controls, an otherwise healthy patient allergic to birch pollen and a nonallergic individual volunteered for all in vivo and in vitro examinations.

Skin Prick Test

Skin prick tests using latex fluids, extracts from pollen and molds (eg, *Alternaria*, *Cladosporium*, and *Aspergillus* species), preservatives, and Multibionta N were performed ac-

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Received for publication October 11, 2004.

Accepted for publication in revised form March 3, 2005.

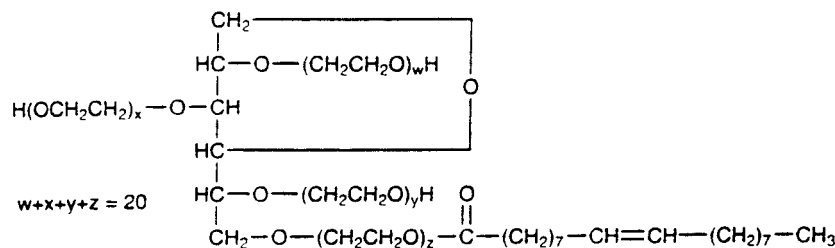


Figure 1. Chemical structure of polyoxyethylene-sorbitan-20-monooleate (polysorbate 80).

according to the methods of Dreborg.⁷ In a second session, the ingredients of the multivitamin preparation, kindly provided by the pharmaceutical company, were tested separately (Fig 2).

Basophil Activation Test

Flow cytometric detection of basophil activation by CD63 expression was performed as described previously.⁸ In brief, 100 μL of heparinized peripheral blood from all the participants was preincubated with 20 μL of stimulation buffer (containing interleukin 3 at a final concentration of 2 ng/mL) at 37°C for 10 minutes. A total of 100 μL of the cell suspension distributed in 5-mL polystyrene tubes was mixed in parallel with 100 μL of wash buffer (negative control), 100 μL of N-formyl-methionyl-leucyl-phenylalanine (fMLP) (1 $\mu\text{mol/L}$ as positive control), and 100 μL of Multibionta N in dilutions of 1:1,000, 1:100, 1:50, and 1:10 and then was incubated for 20 minutes at 37°C. Degranulation was stopped by chilling on ice for 5 minutes. Thereafter, phycoerythrin-conjugated anti-IgE (PE anti-IgE) and fluorescein isothiocyanate (FITC)-conjugated anti-CD63 monoclonal antibodies were added and incubated for 20 minutes on ice. Finally, the heparinized whole blood samples were lysed, fixed, and washed. Centrifuged tubes (250g for 5 minutes at 4°C) were analyzed within 2 hours using a flow cytometer (FACScan; BD Biosciences, San Jose, CA). Interleukin 3, fMLP, PE anti-IgE, and FITC-conjugated anti-CD63 monoclonal antibodies were purchased from Orpegen Pharma, Heidelberg, Germany. The basophil population was gated by the expression of PE anti-IgE. The expression of CD63 was analyzed on this gated cell population. At least 500 IgE-expressing basophils per probe were acquired, and the results are given as percentages of basophils expressing CD63 according to the following formula: number of PE anti-IgE⁺ and FITC-conjugated anti-CD63⁺ basophils/number of PE anti-IgE⁺ basophils (Fig 3).

Direct and Indirect Enzyme-Linked Immunosorbent Assay for the Detection of Specific IgE Antibodies

For direct enzyme-linked immunosorbent assay (ELISA), plates (Nunc-Maxisorb; Nunc, Roskilde, Denmark) were coated in duplicate with 100 μL /well of Multibionta N or its components (undiluted and diluted to a final concentration of 0.1%, 1%, and 10% in aqua dest or 12.5% human serum albumin [HSA] solution, respectively) overnight at 4°C. For

controls, 6 wells were coated with 100 μL /well of a 10- $\mu\text{g/mL}$ rBet v 1a diluted in 0.1M sodium bicarbonate (pH 9.6). Plates were washed twice (Tris-buffered saline Tween-20 [TBST]/0.5% wt/vol bovine serum albumin [BSA]) and blocked (TBST/1% wt/vol BSA) at 37°C for 2 hours. One hundred-microliter aliquots of each serum sample (diluted 1:5 in TBST containing 0.5% wt/vol BSA) were added and incubated overnight at 4°C. Plates were then washed 5 times (TBST/0.5% wt/vol BSA) and incubated with an alkaline phosphatase-coupled mouse anti-human IgE monoclonal antibody (PharMingen, San Diego, CA) diluted 1:1,000 in TBST/0.5% wt/vol BSA at 37°C for 1 hour, then at 4°C for 1 hour. After washing 5 times, bound anti-human IgE antibodies were visualized by color reaction using alkaline phosphatase substrate (Sigma-Aldrich Corp, St Louis, MO).

For indirect ELISA, plates were coated in duplicate with 100 μL /well of the serum samples diluted 1:5 in TBST/0.5% wt/vol BSA overnight at 4°C, then washed and blocked as described previously herein. Thereafter, 50 μL of Multibionta N or its components (undiluted and diluted to 0.01%, 1%, and 10% in 12.5% HSA solution) together with 50 μL of the serum samples diluted 1:5 in 0.5% HSA solution were added and incubated overnight at 4°C. For controls, 6 wells were incubated with 50 μL /well of 10- $\mu\text{g/mL}$ rBet v 1a (diluted in 0.1M sodium bicarbonate [pH 9.6]) together with 50 μL of the serum samples diluted 1:5 in 0.5% HSA solution. Plates were then washed 5 times (TBST/0.5% wt/vol BSA) and incubated with an alkaline phosphatase-coupled mouse anti-human IgE monoclonal antibody as described previously herein. The optical densities corresponding to the amounts of bound antibodies were determined at 405 nm in an ELISA reader (Dynatech MR 7000; Dynatech, Denkendorf, Germany) after the same incubation periods.

IgE Immunoblot

One hundred microliters of Multibionta N (diluted 1:1 with a 25% HSA solution, substituted with probe buffer, and boiled at 95°C for 5 minutes) per centimeter of 12.5% sodium dodecyl sulfate-polyacrylamide gel⁹ was separated and blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany).¹⁰ A benchmark prestained protein ladder (GibcoBRL, Karlsruhe, Germany) served as the molecular weight standard. IgE binding to the nitrocellulose-blotted

Table 1. Polysorbate 80-Containing Medical Products on the German Market*

Application	Intravenous/subcutaneous/ intramuscular administration	Oral/rectal administration	Topical administration
Analgetics/antirheumatics	—	X	X
Anthelmintics	—	X	—
Antiallergics	—	X	X
Antianemic preparations	X	X	—
Antiarrhythmics	X	—	—
Antiasthmatics	—	X	—
Antibiotics/anti-infectives	X	X	—
Antidiabetics	—	X	—
Antiemetics	—	X	—
Antiepileptics	—	X	—
Antihemorrhagic preparations	X	X	—
Antimycotics	—	—	X
Antiphlogistics	—	X	X
Antitussives/expectorants	X	X	—
Appetite depressants	—	X	—
Balneotherapeutics and preparations for thermotherapy	—	—	X
β -Blockers, calcium channel blockers, ACE inhibitors, antihypertensives	—	X	—
Cardioactive agents	—	X	—
Cholagogues and preparations for bile ducts	—	X	—
Coronary remedia	—	X	—
Corticosteroids	X	X	X
Cytostatics and other neoplastic preparations	X	X	X
Dermatics	—	—	X
Diuretics	—	X	—
Fibrinolytic agents	X	—	—
Hypnotics/sedatives	—	X	—
Immunomodulators	X	X	—
Inhibitors of platelet aggregation	X	X	—
Laxatives	—	X	—
Lipid reducer	—	X	—
Nootropics	—	X	—
Ophthalmics	—	—	X
Pituitary hormones and hypothalamic hormones	X	—	—
Preparations against gout	—	X	—
Preparations against hypotonia	—	X	—
Preparations for arteriosclerosis	—	X	—
Preparations for diagnostic investigation	—	X	—
Preparations for enzyme deficiency	X	—	—
Preparations for gastrointestinal tract	—	X	—
Preparations for hemorrhoids	—	X	X
Preparations for improvement of perfusion	X	X	—
Preparations for liver	—	X	—
Preparations for migraine	—	X	—
Preparations for mineral metabolism	—	X	—
Preparations for mouth and pharynx	—	—	X
Preparations for neuropathy	—	X	—
Preparations for osteoporosis	—	X	—
Preparations for veins	—	X	X
Preparations for withdrawal symptoms	—	—	X
Preparations for wound treatment	—	—	X
Preparations in gynecology	See pituitary hormones	X	X
Preparations in otology	—	—	X
Preparations in Parkinson disease	—	X	—
Preparations in rhinology	—	—	X
Psychotropic drugs	X	X	—
Roborantia	—	X	—
Sera, immunoglobulins, vaccines	X	—	—
Sexual hormones and their inhibitors	X	X	—
Spasmolytics	—	X	—
Urologic preparations	—	X	—
Vitamins	X	X	—

Abbreviation: ACE, angiotension-converting enzyme.

*According to Rote Liste.³⁹ Polysorbate 80 is used in products for topical administration (creams, ointments, solutions, jellies, lotions, fluids, emulsions, and plasters), oral administration (syrup, [fizzy] tablets, capsules, and aerosols), and rectal administration (suppositories) and for intravenous, subcutaneous, or intramuscular administration. The complete list of polysorbate 80-containing products used in all other modes of administration is available from the authors.

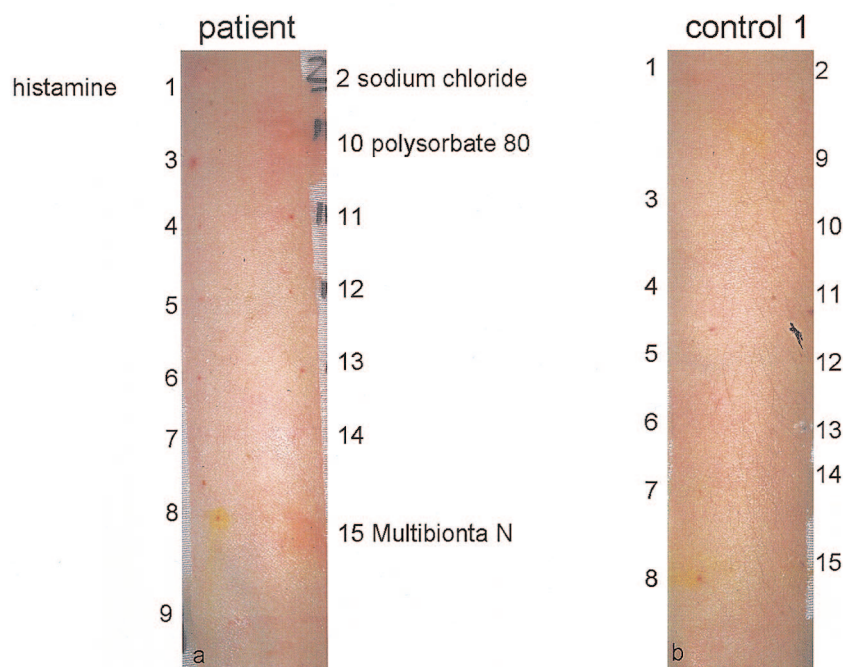


Figure 2. Skin prick test results to a polysorbate 80-containing multivitamin product (entire product at position 15 and its 12 components at positions 3 to 14) in a patient who had experienced an anaphylactoid shock reaction after intravenous administration (a) and in a control (b).

vitamin preparation was examined. In brief, after blocking 3 times for 15 to 30 minutes in buffer A (50-mmol/L sodium phosphate, pH 7.5, 0.5% wt/vol BSA, 0.5% vol/vol Tween, 0.05% wt/vol sodium azide), membranes were incubated overnight at 4°C with serum samples from the patient or controls diluted 1:5 in buffer A. After washing 3 times in buffer A, they were incubated with iodine 125-labeled anti-IgE antibodies from rabbit/mouse (Pharmacia CAP System Specific IgE RIA; Pharmacia & Upjohn, Freiburg, Germany) diluted 1:10 in buffer A. After washing in buffer A, bound antibodies were visualized using autoradiography (X-OMAT film; Kodak, Heidelberg, Germany).

RESULTS

In the skin prick tests, the 20-minute reading exhibited a 4+ reaction (>6-mm wheal reactions) to extracts from hazel and mugwort pollen, *D pteronyssinus*, and Multibionta N and its component polysorbate 80 (Fig 2A); 3+ reactions (4- to 6-mm wheals) to birch, timothy grass, and rye; and 2+ reactions (3-mm wheals) to *D farinae*, *Penicillium* species, own house dust, and Ficus leaves in the patient. Results were negative for latex fluid and preservatives. Skin prick test results were negative for Multibionta N and its components in a control (Fig 2B). For the patient, significant basophil activation was demonstrated on contact with the multivitamin product Multibionta N at all dilutions (Fig 3). No basophil activation was induced in the controls. Except for a significant absorbance indicating bound IgE to the coated control

antigen from birch in control serum 2 in the direct and indirect ELISAs, no IgE binding was observed to Multibionta N and its components in the patient and controls. Similarly, in the immunoblot experiments, no drug specific IgE antibody binding was detected.

DISCUSSION

Recent experimental data¹¹ have indicated that nonionic surfactants, such as polysorbate 80 and Cremophor EL (polyoxy-ethyleneglycerol triricinoleate 35), are not inert vehicles but biologically and pharmacologically active compounds. Except for eczematous reactions and a tuberculin-type hypersensitivity,¹² few studies exist on polysorbate-induced contact urticaria.^{5,6} We reveal a nonimmunologic method of inducing an anaphylactoid shock reaction by a polysorbate 80-containing multivitamin product. Besides a clearly positive skin prick test reaction to the entire multivitamin product and its component polysorbate 80, basophil activation testing confirmed mediator release on activation with the incriminated agent in dilution series in the patient. In contrast, in the controls, skin prick test results and basophil activation were negative. An immunologic IgE-mediated reaction and a nonimmunologic intolerance mechanism may share the same final pathways.¹³ Mast cells and basophils play dominant roles as effector cells in immediate-type hypersensitivity and intolerance reactions. Activation of basophils induces fusion of cytoplasmic granules with the plasma membrane, resulting in quantifiable changes to its

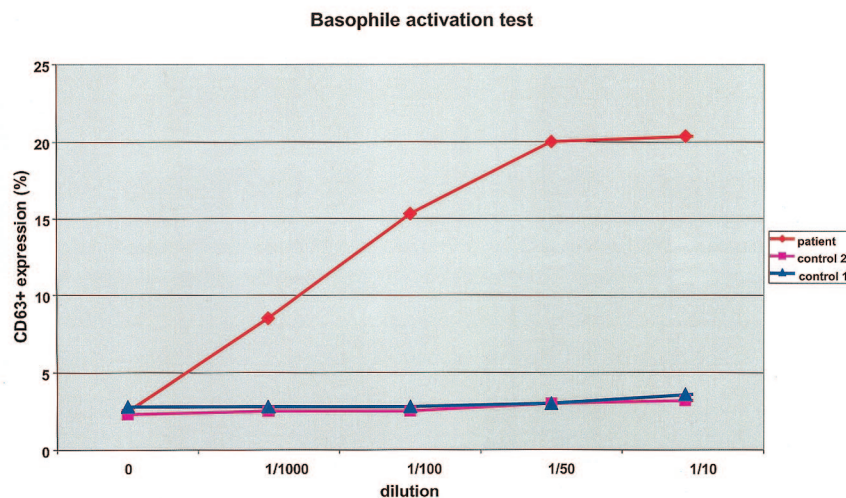


Figure 3. Basophil activation test results in a polysorbate 80–sensitive patient and 2 controls.

surface structure and successive release of stored or de novo synthesized inflammatory mediators in the extracellular medium.^{14,15} The surface marker CD63 is present on the membrane of cytoplasmic granules and is expressed only to an extent of less than 5% on the outside membrane of resting basophils but with high density on activated basophils after the fusion of these granules with the cytoplasmic membrane.^{16–22} Flow cytometric detection of basophil activation by CD63 expression (commonly referred to as the basophil activation test²³), or the BASIC test (basophil-altered surface immunofluorescence detected by cytometry²⁴), has previously been studied in diagnosing immediate-type allergy to various allergens (eg, natural rubber latex, foods, hymenoptera venoms, and aeroallergens) and drugs.^{8,23–36} Simultaneously, this new *in vitro* test also enables the detection of non-IgE-mediated basophil degranulation,^{36,37} as we showed for polysorbate 80, in which non-IgE mechanisms are responsible for the release of inflammatory mediators. Non-IgE-mediated hypersensitivity (previously called *pseudoallergy*) is characterized as a reaction with symptoms similar to those of an allergy but with different (nonimmunologic) causes. The pathogenetic mechanisms of these symptoms vary with the culprit drug or substance. Some drugs (eg, quaternary ammonium muscle relaxants and vancomycin) can cause non-IgE-mediated and immunologically mediated reactions. The distinction between IgE-mediated and non-IgE-mediated hypersensitivity can sometimes be made by the concentration of a reagent required to elicit a wheal-and-flare skin reaction.

It is well known that basophils can be activated by mechanisms other than allergen specific IgE antibodies. A variety of chemical compounds, such as 48/80, ionophore, and fMLP, have been shown to be obligate but nonspecific basophil activators. Some drugs (eg, muscle relaxants) in high

concentrations have also been shown to be nonspecific activators, at least in some susceptible individuals.³⁷ Products of complement activation, such as C3a and C5a, are also well-known basophil activators.³⁷ Polysorbate 80 acts as an enhancer of the systemic exposure to orally administered agents by increasing biomembrane permeability. The mechanistic basis for the acute nonimmunologic hypersensitivity reaction of polysorbate 80 in the presented case remains elusive. Although the pathogenetic mode of action in acute hypersensitivity reaction due to polysorbate 80 has not been fully elucidated, several seminal studies^{11,38} indicate that complement activation plays a significant role. Regarding the frequent use of polysorbate 80, severe reactions have rarely been reported in the literature, perhaps because of lack of information about the underlying cause of the reaction in similar cases.

For the culprit drug in our patient, several hypersensitivity reactions have been reported to the Drug Commission of the German Medical Association and have led to consideration of a modification of the drug formulation. Furthermore, the use of polysorbate 80 as a drug formulation vehicle has recently been implicated in clinically important adverse effects, including acute hypersensitivity and peripheral neuropathy after intravenous administration of the polysorbate 80–containing anticancer agent docetaxel.¹¹ Early clinical studies with this anticancer drug revealed an incidence of hypersensitivity ranging from 5% to 40%.¹¹ Patients allergic to intravenously administered etoposide tolerated very well the oral formulation, which is devoid of polysorbate 80.¹¹

The prescribing physician may be unaware of the ubiquitous presence of polysorbate 80 or of its potential biological and pharmacologic activity. Therefore, in cases of unclear hypersensitivity reactions, especially after medical infusions or injections, polysorbates may be taken into consideration as causative agents.

ACKNOWLEDGMENTS

We thank A. Wüst, Department of Dermatology, University of Erlangen-Nuremberg, and S. Moll, Department of Dermatology, University of Aachen, for their technical assistance.

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