

An immunoassay for the detection of IgE antibodies to trimethoprim in the sera of allergic patients

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Summary

An immunoassay was developed to detect IgE antibodies to the widely used antibacterial drug trimethoprim. Significant levels of trimethoprim-reactive IgE antibodies were found in the sera of two patients who had experienced life-threatening allergic reactions following administration of a combination of trimethoprim and sulphamethoxazole. No IgE antibodies reactive with sulphamethoxazole were found in the sera of either patient. Inhibition experiments revealed that a high degree of cross-reactivity occurs between the drug-reactive IgE antibodies and two structural analogues of trimethoprim, 6-hydroxy- and 6-chlorotrimethoprim. These experiments also indicated that the combining sites of the trimethoprim-reactive IgE antibodies in the two sera were probably complementary to different parts of the trimethoprim molecule. The assay should supplement skin testing in determining the offending drug in patients with suspected allergic sensitivity to trimethoprim-sulphamethoxazole complex.

Introduction

The combination of trimethoprim and sulphamethoxazole is a very widely used drug formulation for the treatment of bacterial infections. In common with almost all other drugs there is little clinical information available on allergic reactions to trimethoprim and sulphamethoxazole, although a recent report showed that in 165 patients treated with co-trimoxazole, 7% and 10% of patients had detectable levels of IgE antibodies to sulphamethoxazole and trimethoprim, respectively, in their serum [1, 2]. In the Boston Collaborative Drug Surveillance Program [3] involving 22 227 consecutively monitored medical inpatients, fifty-seven drugs were implicated in allergic skin reactions. Trimethoprim-sulphamethoxazole complex was found to be responsible for the

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highest individual number of reactions with an incidence of fifty-nine reactions per 1000 patients and was one of only five drug preparations causing an adverse reaction in greater than 2% of treated individuals [3, 4]. Currently there are four reports of anaphylaxis to the combination of trimethoprim and sulphamethoxazole. However, as far as we are aware, the offending drug(s) has not been identified in any of the studies [5-7].

In previous studies with sera from patients who experienced life-threatening anaphylactic reactions during anaesthesia, we developed radio-immunoassays (RIAs) for the detection of drug-reactive IgE antibodies to neuromuscular blocking (NMB) drugs [8-12] and the induction agent thiopentone [13]. Following a review of two individuals with life-threatening allergic reactions to trimethoprim-sulphamethoxazole and implication, by skin testing, of trimethoprim as the responsible agent, we attempted to develop a drug-specific immunoassay as a diagnostic test for allergy to this compound. Such assays, if successful, can also provide a suitable basis for studies aimed at investigating the molecular basis of an allergic drug reaction [11, 12, 14]. This report gives details of the assay developed and the specificities of the IgE-reactive antibodies detected.

Subjects and methods

Subjects and sera

Subject Cl was a 34-year-old woman, otherwise non-atopic, who, 6 years previously, had experienced generalized urticaria and angio-oedema, hypotension and upper airways obstruction within 10 min of ingesting two tablets of trimethoprim-sulphamethoxazole complex (80 mg trimethoprim and 400 mg of sulphamethoxazole per tablet). Although the subject required resuscitation, several prior courses of therapy with the drug combinations had been uneventful. In view of the subject's predisposition to recurrent urinary tract infection, identification of the drug responsible for the adverse reaction was seen as conferring a practical advantage in her management.

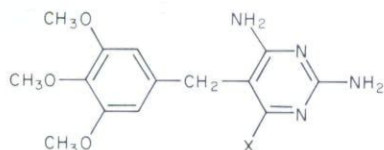
Subject Sa was a 42-year-old female with a documented past history of allergic rhinitis and asthma. In May 1981 she had suffered an anaphylactoid reaction during general anaesthesia. Intradermal testing at that time revealed positive reactions to alcuronium and d-tubocurarine. Courses of trimethoprim-sulphamethoxazole for recurrent bronchitis had been tolerated until March 1985 when she ingested a standard oral dose (*vide supra*) and within 7 min suffered an anaphylactoid reaction manifest as generalized pruritus and erythema, hypotension, urgent defaecation and oedema of the tongue and extremities. The subject required resuscitation.

Fifty millilitres of blood were obtained from each subject, the sample from Sa being taken 23 days after the anaphylactoid reaction. Sera were stored at -20°C .

Eight control subjects (five atopic, three non-atopic), none of whom were allergic to trimethoprim, were investigated for skin-test reactivities. The results for one atopic subject (Na) are representative of the other controls. Na had been exposed to trimethoprim-sulfamethoxazole but showed no adverse reactions to the drug complex.

Skin tests

Skin-prick tests were performed with undiluted Seprin Parenteral Infusion® (Wellcome Australia Pty, Ltd) using histamine 1 mg/ml and physiological saline as positive and negative controls, respectively.



Trimethoprim	X = H
6-Hydroxytrimethoprim	X = OH
6-Chloro-6-hydroxytrimethoprim	X = Cl

Fig. 1. Structures of trimethoprim and two structurally-related analogues.

For intradermal testing, trimethoprim and sulphamethoxazole were dissolved in the minimum quantity of 0.1 M sodium hydroxide and benzyl alcohol, respectively, before diluting with physiological saline to dilutions of 0.1 and 1 mg/ml. Saline solutions containing the same amounts of sodium hydroxide or benzyl alcohol were used as controls. Intradermal tests were performed by injecting 0.02 ml of test or control solutions.

Chemicals

Trimethoprim and sulphamethoxazole were donated by Roche Products Pty Ltd, Dee Why, Australia. 6-Chloro- and 6-hydroxytrimethoprim were synthesized as described by Smal *et al.* [15]. The structures of the three compounds are shown in Fig. 1.

Preparation of drug-solid phase covalent complexes

Sulphamethoxazole (11 mg) was suspended in water (10 ml) and the pH adjusted with 2.5 M NaOH to 10.8. The resulting clear solution was shaken with Epoxy-Activated Sepharose (Pharmacia) (200 mg) at 35°C for 16 hr. The gel was washed with water, 0.1 M borate buffer pH 8.0, 0.1 M acetate buffer pH 4.0 and water. Excess reactive sites were blocked by incubating the gel with 1 M ethanolamine (pH 9) for 5 hr. Finally the gel was washed as above and resuspended in water (20 ml).

Trimethoprim (100 mg) was dissolved in dioxane (9 ml), the solution diluted with water (28 ml) and the pH adjusted to 12.0 with 2.5 M NaOH. Epoxy-Activated Sepharose (1.0 g) was added and the mixture shaken at 30°C for 20 hr. The gel was washed successively with dioxane-water (1:2) (100 ml), water, 0.1 M borate buffer pH 8.0, 0.1 M acetate buffer pH 4.0 and water. Excess reactive sites were blocked by incubation with 1 M ethanolamine (pH 9) for 5 hr. The gel was washed as above and resuspended in water (100 ml).

Detection of drug-reactive IgE antibodies by RIA

Serum (50 µl) was added to 4 mg of the solid-phase complex (trimethoprim-, sulphamethoxazole- or ethanolamine-Sepharose or Sepharose alone) and left at room temperature for 3 hr. Tubes were then washed and centrifuged three times with phosphate-buffered saline pH 7.4 (PBS) containing Tween 20 (0.1%) before adding 20 000–30 000 c.p.m./tube of ¹²⁵I-anti-human IgE (Pharmacia). After standing overnight, tubes were washed three times and counted in a Packard Auto-Gamma Spectrometer. The presence of reactive IgE antibodies in a serum was determined by the per cent radioactive uptake of ¹²⁵I-anti-human IgE, i.e. the per cent of counts added.

Table 1. Skin test responses to trimethoprim and sulphamethoxazole by the patients and by an atopic subject exposed to the drugs

Tests	Weal size (mm) with subject		
	Cl	Sa	Na*
<i>Skin prick</i>			
Trimethoprim-Sulphamethoxazole (16:80 mg/ml) parenteral solution†	—‡	2 × 2	—
Histamine (1 mg/ml)	3 × 3	3 × 3	3 × 3
Physiological saline	—	—	—
<i>Intradermal</i>			
Trimethoprim (0.1 mg/ml)	—	6§	—
(1 mg/ml)	6.5§¶	n.d.**	—
Sulphamethoxazole (0.1 mg/ml)	—	—	—
(1 mg/ml)	—	—	—
Trimethoprim diluent††	—	—	—
Sulphamethoxazole diluent‡‡	—	—	—

* Atopic subject, showing no adverse reactions to trimethoprim-sulphamethoxazole therapy.

† Septrin Parenteral Infusion® (Wellcome Australia Pty, Ltd), used undiluted.

‡ —: no reaction.

§ Increase in weal diameter.

¶ Surrounding flare 14 mm.

** n.d.: not done.

†† Sodium hydroxide/physiological saline (see Subjects and methods).

‡‡ Benzyl alcohol/physiological saline (see Subjects and methods).

Inhibition assay

Serum (50 µl), appropriately diluted, was incubated for 1 hr with 50 µl of a solution of the compound being tested before addition of the solid phase (4 mg in 100 µl). After 3 hr, tubes were washed and centrifuged three times with PBS-Tween before the addition of ¹²⁵I-anti-human IgE (20 000–30 000 c.p.m./tube). After overnight incubation at room temperature, tubes were washed three times and counted.

Results

Skin tests

Results of prick and intradermal tests are shown in Table 1. When prick tested with undiluted commercial parenteral solution containing 16 mg/ml of trimethoprim and 80 mg/ml of sulphamethoxazole, subject Cl and the controls were unreactive while subject Sa responded with formation of a small weal. All subjects gave positive reactions to histamine. Following separate intradermal administration of the drugs, Cl and Sa, but not the controls, produced clear, positive reactions to trimethoprim but responses to sulphamethoxazole were again negative. The positive reaction obtained

Table 2. IgE antibodies to trimethoprim in patients' sera

Sera	Radioactive uptake (%) of ^{125}I -anti-human IgE with:		
	Trimethoprim-Sepharose	Ethanolamine-Sepharose	Sepharose alone
<i>Controls</i>			
Cord	0.8	0.5	0.4
Normals*	$1.1 \pm 0.2^\dagger$	0.6 ± 0.2	0.4 ± 0.1
<i>Patients</i>			
Cl	9.1	0.5	0.4
Sa	24.3	0.6	0.5

* Ten adult subjects (eight females and two males).

† Mean $\pm$ s.d.

with subject Sa occurred at one-tenth the concentration needed to produce a weal in subject Cl. Neither of the diluents produced weal or flare reactions in any of the subjects.

Examination for sulphamethoxazole-reactive IgE antibodies

Use of the sulphamethoxazole-Sepharose solid support in experiments with control sera and sera from patients Cl and Sa, who had each experienced an anaphylactoid reaction following ingestion of the trimethoprim-sulphamethoxazole drug combination, revealed no uptake of ^{125}I -anti-human IgE by the drug-solid phase. Radioactive uptakes of the labelled second antibody were almost the same (approximately 0.5–1.0%) with cord and normal sera and with sera Cl and Sa.

Detection of trimethoprim-reactive IgE antibodies

Table 2 summarizes results obtained from direct binding RIA studies in which trimethoprim-Sepharose was incubated with control sera and sera from patients Cl and Sa. Sera Cl and Sa gave uptakes of ^{125}I -anti-IgE of 9.1% and 24.3%, respectively. Based on previous experience by ourselves [9, 16], and others [17], where a RAST value of greater than 2.5 times the cord/normal value is considered positive, the uptakes by Cl and Sa clearly demonstrate the presence of trimethoprim-reactive IgE antibodies. In contrast, control solid supports of ethanolamine-Sepharose and Sepharose alone yielded low radioactive uptakes of second antibody with both the patients' sera and control sera.

Specificities of the trimethoprim-reactive antibodies

In order to examine the specificities of the IgE antibodies in the sera of patients Cl and Sa, inhibition studies with trimethoprim and two analogues were undertaken. Inhibitory dose responses obtained when the compounds were used with both sera are shown in Fig. 2 and quantities needed for 50% inhibition of the binding of IgE antibodies to trimethoprim-Sepharose are included. With serum Cl, trimethoprim and 6-chloro-trimethoprim proved to be of similar potency causing inhibition at concentrations of 16 and 19 nmol, respectively. In contrast, 220 nmol of 6-hydroxytrimethoprim was required to produce 50% inhibition. With serum Sa, all three compounds showed almost identical inhibitory potencies (Fig. 2), but these values were 160–3000

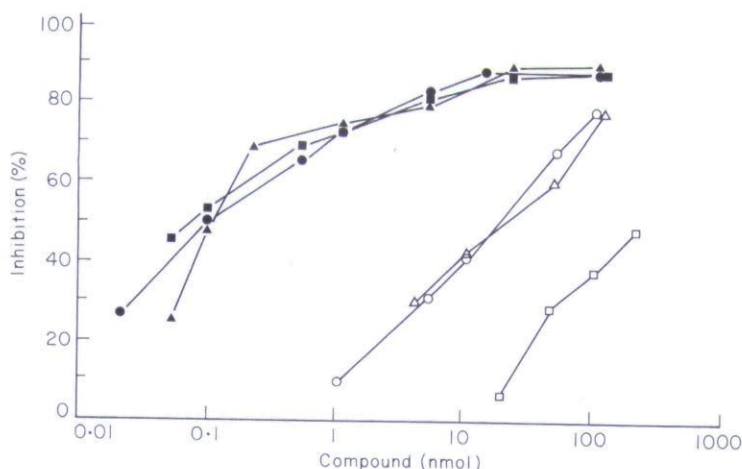


Fig. 2. Inhibition by trimethoprim, and two structurally-related analogues, of IgE binding in sera Cl (serum dilution 1:2, open symbols) and Sa (serum dilution 1:4, closed symbols) to trimethoprim–Sepharose complex. Amount (nmol) needed for 50% inhibition of the uptake of ^{125}I -anti-human IgE for Cl and Sa, respectively: (○ ●) trimethoprim (16 and 0.10) (□ ■) 6-hydroxytrimethoprim (220 and 0.07) (△ ▲) 6-chlorotrimethoprim (19 and 0.11).

times as potent as those obtained with serum Cl. Sulphamethoxazole did not inhibit the binding of either serum to trimethoprim–Sepharose.

Discussion

Although adverse reactions to the frequently used drug combination of trimethoprim and sulphamethoxazole are not uncommon [3, 4] the lack of clinical and laboratory information available has usually made it impossible to conclude whether patients are reacting to either or both drugs. In the present study, skin tests indicated that for two patients at least, trimethoprim was implicated in life-threatening anaphylactoid reactions. Taking into account the case histories of patients Cl and Sa, the positive and negative skin tests to trimethoprim and sulphamethoxazole, respectively, for both patients, and the presence in both sera of trimethoprim-reactive IgE antibodies, it seems reasonable to conclude that both patients experienced true anaphylactic, i.e. type 1, IgE-mediated allergic reactions to trimethoprim.

In the preparation of the trimethoprim solid phase support the mode of coupling of trimethoprim to Epoxy- (bis-oxirane-) Activated Sepharose is presumably through the nucleophilic N atom on the primary amino groups of the diaminopyrimidine ring (Fig. 1). The direct binding and inhibition studies confirmed that trimethoprim had bound to the activated support.

Results from direct binding immunoassay studies led to the conclusion that the IgE antibodies detected were probably specific for trimethoprim. Inhibition experiments with the drug and two structural analogues supported this conclusion and, in addition, revealed heterogeneity in the patients' responses to the drug. Apart from the large differences in inhibitory potencies exhibited by the compounds with the two sera, the inhibition patterns with each sera were also different. The latter findings suggest heterogeneity at the IgE antibody-combining site specificity level and demonstrate not

only that the trimethoprim molecule is antigenic but also that more than one antigenic determinant exists on the molecule. A more complete and accurate identification of these determinants will be possible by employing carefully selected structural analogues of trimethoprim in quantitative inhibition studies with IgE antibodies from sera Cl and Sa. No specificity studies on the combining sites of the trimethoprim-reactive IgE antibodies appear to have been carried out in the Wälti *et al.* study [2].

An alternative, but less likely, explanation for the specificities of the IgE antibodies and the patients' resultant reactions to trimethoprim is that IgE formation, particularly in the case of patient Cl, was not directed against trimethoprim but to an antigenically related or cross-reacting substance. It is recognized, however, that we are unlikely to find proof one way or the other for this speculation.

From the practical point of view, the assay for trimethoprim-reactive IgE antibodies should prove a valuable adjunct to the case history and skin testing in the diagnosis of allergic reactions to the drug. The laboratory procedures involved in detection of the antibodies are simple and easy to carry out. Preparation of the drug-solid phase complex is straightforward and the test procedure could probably be applied equally well as a radio- or enzyme-immunoassay.

Together with recent results obtained with other drug-induced allergic reactions [8-14], the findings presented here add to the list of chemical structures known to be responsible for the induction of allergic reactions, apparently via their capacity to induce the production of complementary IgE antibodies. From the diagnostic and mechanistic points of view, these findings are a considerable advance over our previous level of understanding of allergic reactions to drugs [18]. However, many clinical, chemical and immunological difficulties remain before we are likely to have at our disposal a large number of different but specific assays applicable to the diagnosis of the wide range of individual drug allergies.

Acknowledgment

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