

Drugs as allergens

The molecular basis of IgE binding to trimethoprim

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The combining site specificities of IgE antibodies that react with the oral antibacterial agent trimethoprim and found in the sera of two subjects who experienced anaphylaxis after taking the drug, were investigated. Hapten inhibition studies with some close analogues of trimethoprim and a range of other structurally related compounds showed that the allergenic determinant complementary to the IgE antibodies in the serum of one of the subjects was the 3,4-dimethoxybenzyl group. The complementary allergenic structure recognized by the IgE antibodies in the serum from the second subject comprised both the trimethoxybenzyl and diaminopyrimidine rings of trimethoprim. Thus, as with thiopentone, but unlike the neuromuscular blocking drugs, the trimethoprim molecule has more than one determinant each with the capacity to provoke IgE formation, interact with the antibody combining site and provoke drug-induced allergic reactions. The general approach set out here employing carefully selected structural analogues in hapten inhibition studies should be invaluable for confirming specificity and identifying allergenic determinants in IgE antibody-mediated allergic drug reactions.

Key words: drug allergy; trimethoprim allergy; IgE to trimethoprim; trimethoprim allergenic determinants; trimethoprim immunoassay.

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CLINICAL ASPECTS

The combination of the antibacterial drugs trimethoprim and sulphamethoxazole is very widely used for the treatment of bacterial infections. Adverse reactions to this formulation are not uncommon and anaphylaxis in patients taking the drugs has been reported. Using a radioimmunoassay, IgE antibodies to trimethoprim have been detected in sera of patients apparently allergic to the drug(s). This assay should aid the diagnosis of immediate, IgE-mediated reactions to trimethoprim. By using carefully selected structural analogues of trimethoprim in hapten inhibition studies, the parts of the trimethoprim molecule involved in provoking IgE formation and allergic sensitivity may be identified. Knowledge of drug allergenic determinants will further our understanding of the sorts of structures likely to produce allergic reactions and the basis of allergic cross-reactions to drugs.

Apart from the progress made over the past 25 years in identifying penicillin allergenic determinants (1, 5, 9), and the more recently acquired insight into the nature of IgE-binding determinants on neuromuscular blocking drugs (2, 3, 6) and the anaesthetic induction agent thiopentone (7), our knowledge of drug allergy and allergens is

meagre. It is not clear, for example, which of the many drugs known to cause adverse reactions actually cause true allergic reactions by specific interaction with cell-bound IgE molecules in allergic subjects. It follows therefore that until more of these drugs are identified, our knowledge of drug allergenic determinants will not be increased.

We recently developed an immunoassay for the detection of IgE antibodies to trimethoprim (TMP) in the sera of subjects allergic to this widely used oral antibacterial agent (8). Specificity of this assay was established in three ways. Firstly, IgE antibodies in the sera of subjects who experienced an anaphylactic reaction following administration of the drug, bound strongly to TMP covalently coupled to a solid phase. Binding did not occur when control solid phases were used. Secondly, cord sera and sera from normal, non-allergic subjects and from allergic subjects (but not allergic to trimethoprim) did not bind to the TMP solid phase. Finally, pre-incubation of the subjects' sera with TMP strongly inhibited the binding of the IgE antibodies. Following development of the assay, we attempted to identify the complementary IgE-binding structures on the drug. This paper sets out a general procedure using hapten inhibition studies to probe the TMP-reactive IgE antibody combining sites and presents conclusions on the nature of the TMP allergenic determinants.

MATERIAL AND METHODS

Subjects and sera

Subject CI experienced generalized urticaria, angioedema, hypotension and upper airways obstruction within 10 min of ingesting two tablets containing 80 mg of trimethoprim and 400 of sulphamethoxazole per tablet. Subject Sa suffered an anaphylactoid reaction involving generalized pruritus and erythema, hypotension and oedema of the tongue within minutes of ingesting what was described by her physician as a "standard oral dose" of trimethoprim-sulphamethoxazole complex. Both subjects required resuscitation. Positive reactions to trimethoprim, but not to sulphamethoxazole, occurred in both subjects when challenged intradermally with each of the drugs (8). Blood was taken from CI and Sa 6 years and 23 days respectively after the reactions. Sera were stored at -20°C .

Sera from subjects allergic to common environmental allergens (mite and pollens) and normal and cord serum controls were obtained as before (7).

Chemicals

Trimethoprim [2,4-diamino-5-(3',4',5'-trimethoxybenzyl) pyrimidine] was donated by Roche Products Pty Ltd., Dee Why, Australia. 6-Chlorotrimethoprim (2,4-diamino-6-chloro-5-(3',4',5'-trimethoxybenzyl) pyrimidine and 6-hydroxytrimethoprim (2,4-diamino-6-hydroxy-5-(3',4',5'-trimethoxybenzyl) pyrimidine) were synthesized as described by Smal et al. (10). The following compounds were obtained from Sigma Chemical Co., St. Louis, Missouri: 4-amino-5-aminomethyl-2-methylpyrimidine, 2-amino-4-hydroxy-6-methylpyrimidine, 2-amino-6-chloro-4-pyrimidinol, 2-amino-4-chloro-6-methylpyrimidine, cytosine, 2,6-diaminopurine, diaveridine, 3,4-dimethoxybenzoic acid, 3,4-dimethoxyphenylethylamine, isocytosine, 4-methoxyphenylacetic acid, 4-methoxyphenylethylamine, 5-methylcytosine, pyrimethine, 2,4,6-triaminopyrimidine, 3,4,5-trimethoxybenzoic acid and 3,4,5-trimethoxycinnamic acid. 3-(3',4',5'-trimethoxyphenyl)propionic acid was from Ega-Chemie, Steinheim, W. Germany.

Detection of IgE antibodies

IgE antibodies that react with trimethoprim were detected as previously described (8) in a solid phase immunoassay by reaction with the drug covalently coupled to bis-oxirane-activated Sepharose. Bound IgE antibodies were detected by reaction with ^{125}I -horse-anti-human IgE (Kallestad Laboratories, Austin, Texas).

Inhibition assay

Serum (50 μl), appropriately diluted, was incubated for 1 h at room temperature with 50 μl of a solution of the compound being tested before addition of the trimethoprim-Sepharose complex (4 mg in 100 μl). After 3 h, tubes were washed and centrifuged three times with phosphate buffered saline pH 7.4 containing 0.1% Tween 20 before the addition of ^{125}I -anti-human IgE ($\sim 30,000$ cpm/tube). Tubes were incubated overnight at room temperature, washed three times as before and counted.

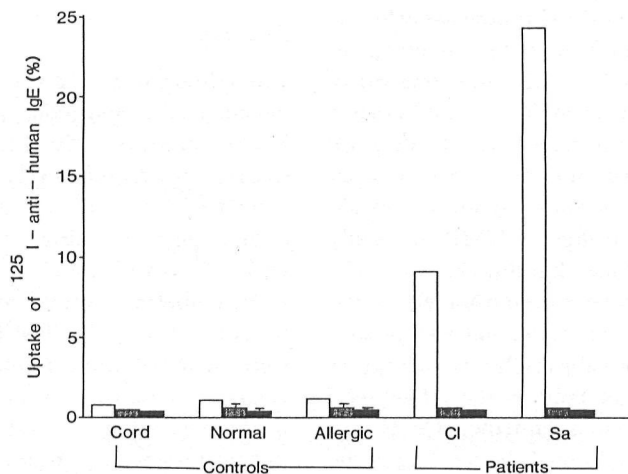


Fig. 1. Histogram showing uptakes of ¹²⁵I-anti-human IgE, and reflecting uptakes of human IgE antibodies, by trimethoprim- and control-solid phases used with human cord and "normal" (non-allergic) sera and sera from allergic subjects and two patients, CI and Sa, both of whom experienced an anaphylactic reaction after ingestion of trimethoprim. Fourteen sera from non-allergic healthy adults were used as the "normal" sera. The 9 sera from allergic subjects were from adults allergic to house dust mites and/or grass pollens but not trimethoprim. Open bars represent reactions with trimethoprim-Sepharose, hatched bars reactions with ethanolamine-Sepharose, and black bars reactions with Sepharose alone.

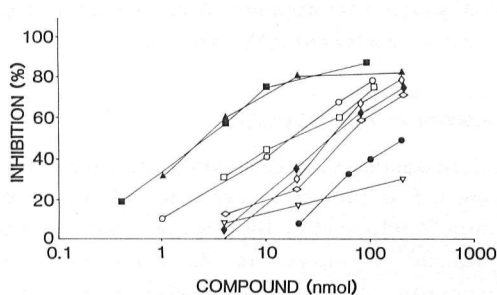


Fig. 2. Inhibition by trimethoprim and some structurally related compounds of IgE binding in serum CI to trimethoprim-Sepharose complex. Serum CI was used at a dilution of 1:2. See Table 1 for key to symbols.

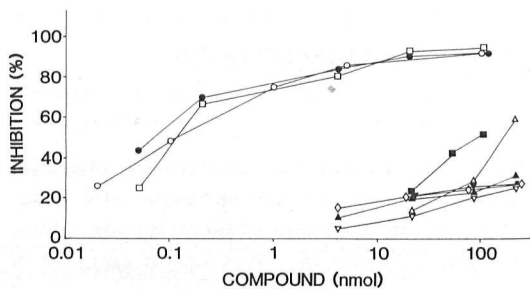


Fig. 3. Inhibition by trimethoprim and some structurally related compounds of IgE binding in serum Sa to trimethoprim-Sepharose complex. Serum Sa was used at a dilution of 1:4. See Table 1 for key to symbols.

RESULTS

Detection of IgE antibodies that react with trimethoprim

Since the anaphylactoid reactions occurred in subjects after they had ingested tablets containing trimethoprim and sulphamethoxazole, both drugs had to be considered as possible instigators of the reactions. Both subjects responded with positive,

immediate skin reactions to trimethoprim but not to sulphamethoxazole after intradermal injections of the drugs. Likewise, IgE-antibodies that reacted with trimethoprim but not sulphamethoxazole were detected in the sera of both subjects when immunoassays employing drug-solid phase complexes were used (8).

Fig. 1 summarizes results obtained from direct binding radioimmunoassay studies in which trimethoprim-Sepharose and control

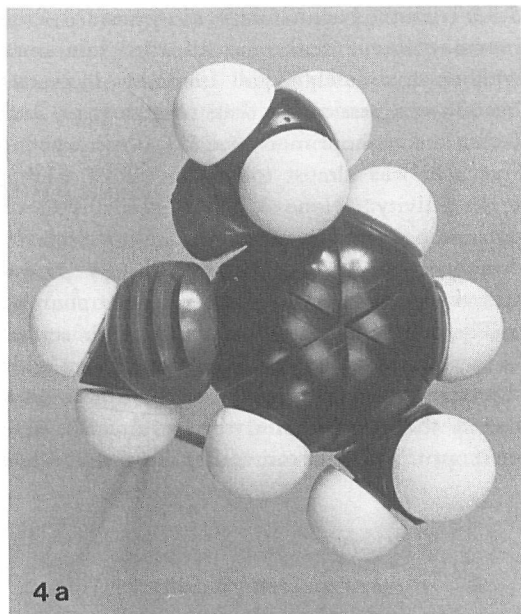
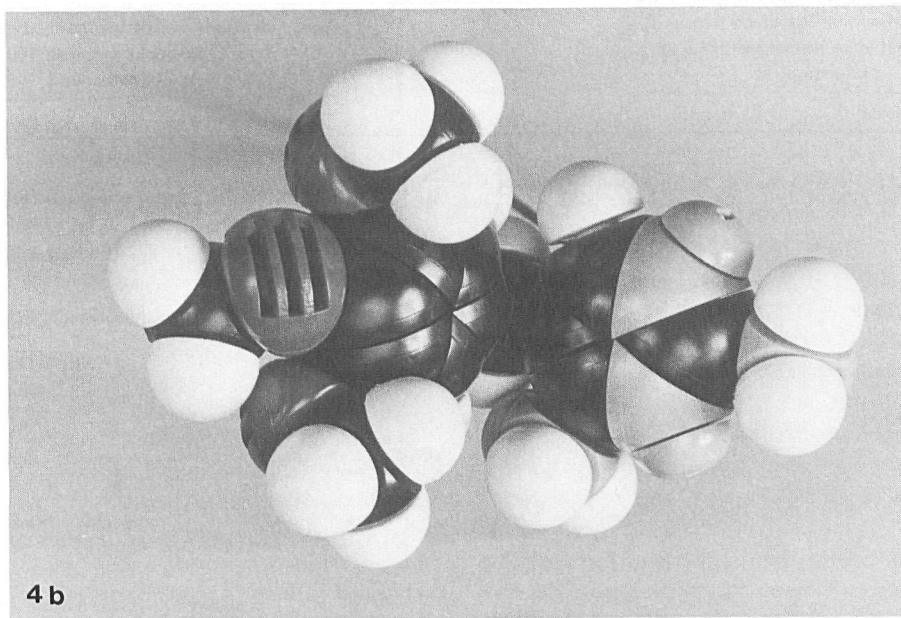


Fig. 4. a) CPK molecular model of the 3,4-dimethoxybenzyl group, the determinant recognized by the IgE antibodies in serum Cl. This group almost entirely constitutes the left-hand half of the trimethoprim molecule shown in b.

b) CPK molecular model of trimethoprim showing the trimethoxybenzyl group on the left (compare structure shown in (a) which has one less methoxy group) and the diaminopyrimidine ring which constitutes the right half of the molecule. Both groups together constitute the determinant that is complementary to the IgE antibody combining sites in serum Sa.



solid supports were incubated with sera from both allergic subjects and with control sera. For sera Cl and Sa, uptakes of ^{125}I -anti-human IgE were from 18 to 48 times higher than the radioactive uptakes these sera produced with the control supports, ethanolamine-Sepharose and Sepharose alone. Uptake ratios of labelled sec-

ond antibody by trimethoprim ranged from 11 to 30 when sera Cl and Sa were compared with cord serum, "normal" sera and sera from subjects highly allergic to house dust mites and grass pollens, the most important sources of inhalant allergy in our region.

Hapten inhibition studies

Results of inhibition studies with trimethoprim and some structurally related compounds are summarized in Fig. 2 for serum Cl and Fig. 3 for serum Sa. A side-by-side comparison of the per cent inhibition produced with 100 nmol of the compounds is listed in Table 1. Results with serum Cl showed that the best inhibitors were diaveridine and 3,4-dimethoxyphenylethylamine followed by trimethoprim and 6-chlorotrimethoprim. Surprisingly, 6-hydroxytrimethoprim was a very poor inhibitor. 3-(3',4',5'-Trimethoxyphenyl)-propionic acid, 3,4,5-trimethoxycinnamic acid and 3,4-di-

methoxybenzoic acid were all good inhibitors with approximately equal inhibitory potency, but all were less active than trimethoprim and 6-chlorotrimethoprim. 3,4,5-Trimethoxybenzoic acid was almost totally devoid of inhibitory activity. None of the derivatives of pyrimidine tested, except 2-amino-4-chloro-6-methylpyrimidine, showed inhibitory potencies that compared to the above compounds.

The inhibition profile obtained with serum Sa was far simpler, and strong inhibitors with this serum were from 50 to 100 times more potent than they were with serum Cl. Trimethoprim, 6-chlorotrimethoprim and 6-hy-

Table 1

Inhibition of trimethoprim-IgE antibody interaction by trimethoprim and some structurally related compounds

Compound	Structure	Symbol	% inhibition of IgE antibody binding to TMP-Sepharose with 100 nmol of compound	
			Serum Cl	Serum Sa
Trimethoprim (TMP)		○	77	92
6-Hydroxy TMP		●	40	91
6-Chloro TMP		□	78	94
Diaveridine		■	87	51
Pyrimethine			16	12
3,4,5-Trimethoxybenzoic acid		▼	12	23
3,4,5-Trimethoxycinnamic acid		◇	64	8

(cont.)

droxytrimethoprim were all very strong inhibitors and of approximately equal potency. Diaveridine, almost the only other compound that approached 50% inhibition, was over 100 times less potent on a molar basis.

DISCUSSION

Trimethoprim was covalently coupled to 1,4-bis-(2,3-epoxypropoxy)butane-activated Sepharose via the primary amino groups present to form stable alkylamine linkages. There appears to be no information available on the binding, if any, of trimethoprim to carriers *in vivo*. However, if such *in vivo* binding occurs it seems likely that it too would proceed via the reactive

amino groups on the drug molecule, particularly since electron resonance effects of the ring nitrogens make the amino groups even more reactive. It should also be remembered that the methoxy groups are electron donating and this makes the two unsubstituted carbons on the trimethoxyphenyl ring potentially more reactive to oxidative mechanisms. It should be emphasized, however, that no reactions of any sort have been shown to occur *in vivo* between trimethoprim and possible macromolecular carriers, such as proteins, carbohydrates or lipids nor, to our knowledge, have such possible reactions even been studied.

Results obtained in direct binding studies led

Table 1 (cont.)

Inhibition of trimethoprim-IgE antibody interaction by trimethoprim and some structurally-related compounds

Compound	Structure	Symbol	% inhibition of IgE antibody binding to TMP-Sepharose with 100 nmol of compound	
			Serum Cl	Serum Sa
3-(3',4',5'-Trimethoxyphenyl)-propionic acid		◇	69	23
3,4-Dimethoxyphenylethylamine		▲	81	24
3,4-Dimethoxybenzoic acid		◆	66	6
4-Methoxyphenylacetic acid			13	4
4-Methoxyphenylethylamine		▼	26	22
2-Amino-4-chloro-6-methylpyrimidine			48	4
2-Amino-4-hydroxy-6-methylpyrimidine			18	4
4-Amino-5-aminomethyl-2-methylpyrimidine		Δ	15	35

us to conclude that the IgE antibodies detected were reacting specifically with trimethoprim. Inhibition studies with trimethoprim and some structurally related compounds supported this conclusion and revealed differences in the combining site specificities of the antibodies in the two sera tested.

Although the interaction between the IgE antibodies in serum Cl and the trimethoprim-solid support was inhibited by trimethoprim and 6-chlorotrimethoprim, the most potent inhibitors of the binding were diaveridine and 3,4-dimethoxyphenylethylamine. These results immediately suggested that the antibodies were recognizing the portion of the trimethoprim molecule that contains the methoxy-substituted benzene ring and, in addition, that the complementary antigenic structure on the molecule contains two, and not three, methoxy groups attached to the ring. The latter conclusion is supported by the greater inhibitory potency obtained with 3,4-dimethoxybenzoic acid compared to 3,4,5-trimethoxybenzoic acid. Involvement of a methylene group in the allergenic determinant recognized by the IgE antibodies in serum Cl was suggested by comparing results obtained with 3-(3,4,5-trimethoxyphenyl) propionic acid and 3,4,5-trimethoxycinnamic acid on the one hand and 3,4,5-trimethoxybenzoic acid on the other. Taken together, all of these findings suggest that the trimethoprim allergenic determinant recognized by the IgE antibodies in serum Cl is the 3,4-dimethoxybenzyl group, which is almost identical to one half of the trimethoprim molecule (Fig. 4a). The low recognition of 6-hydroxytrimethoprim by serum Cl compared with the high recognition of TMP and 6-chlorotrimethoprim is puzzling. In view of the conclusions drawn above concerning the antigenic determinant, a possible explanation is intramolecular hydrogen bonding between the hydrogen of the hydroxyl group on the pyrimidine moiety and the oxygen of one of the methoxy groups on the benzene ring. This interaction may be sufficient to disturb the size and shape of the antigenic determinant.

With serum Sa, the almost equal activity found with trimethoprim and its 6-hydroxy and 6-chloro derivatives, and their greater inhibi-

tory potencies than diaveridine, methoxyphenyl derivatives and derivatives of 2,4-diaminopyrimidine, showed that the trimethoxybenzyl and diaminopyrimidine rings together constitute the complete complementary allergenic determinant (Fig. 4b).

Thus, as with the combining sites of IgE antibodies that recognize different allergenic determinants on thiopentone (7) but unlike those that react with neuromuscular blocking drugs where a common determinant is recognized (3,4,6) trimethoprim-reactive IgE antibodies from the sera of different subjects may be complementary to different structural determinants on the trimethoprim molecule.

As more sera from subjects allergic to trimethoprim are examined for IgE antibodies that react with the drug, it will be interesting to gauge the extent of the structural variation seen in the trimethoprim determinants that react with the antibodies.

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