

Detection of thiopentone-reactive IgE antibodies following anaphylactoid reactions during anaesthesia

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Summary

A radioimmunoassay was developed to detect IgE antibodies to the anaesthetic induction agent thiopentone. Significant levels of thiopentone-reactive IgE antibodies were found in the sera of two patients who had experienced life-threatening anaphylactoid reactions following administration of the drug. Inhibition experiments revealed that cross-reactivity occurs between the drug-reactive IgE antibodies and four barbiturate analogues pentobarbitone, phenobarbitone, barbitone and methohexital. The assay should supplement skin testing for the detection of patients with potentially lethal sensitivity to thiopentone.

Introduction

There have been several reports of anaphylactoid, life-threatening reactions to thiopentone during anaesthesia [1–5]. The majority of reactions occur after multiple exposures to thiopentone; however, cases have been described on first and second exposure [2]. Although the underlying mechanism(s) of these reactions is unclear, the clinical features indicate that at least some may be true type I hypersensitivity responses. In view of the extensive use of thiopentone for inducing anaesthesia, there is a need for a diagnostic test to distinguish patients who are allergic to the drug and ideally, to provide information on the mechanism of the reaction. Challenge with the drug is potentially too dangerous to be of practical use [6]. Although intradermal testing has been claimed to be the method of choice for investigating drug allergy [6,7], controversy surrounds its use [8–10] and the predictive value of the test in patients without a history of a previous reaction is limited [11].

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As very little is known about the mechanisms underlying adverse drug reactions [12], assays that detect drug-specific IgE antibodies may greatly assist attempts to distinguish those reactions that are truly allergic. We have previously reported the development of radioimmunoassays (RIAs) that detect muscle relaxant drug-reactive IgE antibodies in the sera of patients who experienced a life-threatening anaphylactoid reaction during anaesthesia [13–16]. Based on the successful methodology developed for the muscle relaxant drug studies and following life-threatening reactions to thiopentone in two patients, we attempted to develop a drug-specific RIA by covalently coupling thiopentone to a solid support and using the drug-solid phase complex to detect drug-specific IgE antibodies in patients' sera. This report provides details of the assay and results of investigations of the specificity of the drug-reactive IgE antibodies detected.

Materials and methods

Chemicals

Epoxy-activated Sepharose 6B was obtained from Pharmacia, Uppsala, Sweden; thiopentone from Abbott Laboratories, Sydney, Australia; phenobarbitone from Sigma Chemical Co, St Louis, Mo, U.S.A.; barbitone from BDH, Poole, England and methohexital from Eli Lilly, Sydney, Australia. Structures of the barbiturates examined are shown in Fig. 1.

Subjects and sera

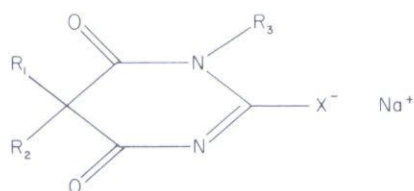
Patient Ol was a 58-year-old woman with a history of allergy to antihistamines, tetracyclines and penicillin. She had, over a 2-year period, ten anaesthetics for back manipulations and after the last anaesthetic was told she could not have any more manipulations. In 1981 she was anaesthetized for a haemorrhoidectomy and after a thiopentone induction developed cardiovascular collapse and a diffuse erythematous rash. She was referred to the Anaesthetic Allergy Clinic at Royal North Shore Hospital where intradermal testing showed a weal of 15 mm and flare of 50 mm to a 0.02 ml injection of 1:100 dilution of 2.5% thiopentone in normal saline. There was no response to intradermal testing with neuromuscular blocking drugs or saline.

Patient Be was a 52-year-old woman with a history of penicillin allergy who had undergone multiple anaesthetics for urological procedures. In 1984 and 1985 she received thiopentone on five separate occasions and developed cardiovascular collapse on each occasion and bronchospasm on one. One month after the last anaesthetic she was intradermally tested with 1:100 dilution of 2.5% thiopentone, and dilutions of suxamethonium, atracurium, pancuronium, gallamine, d-tubocurarine, lignocaine, bupivacaine, procaine, diazepam, propanidid, methohexitone and fentanyl. She developed a 12 mm weal with pseudopod formation at the thiopentone site, and no reaction at any of the other sites.

Both patients were bled 1 month after their last reaction to thiopentone. Although both patients had received the drug on previous occasions, patient Be had probably reacted to the drug on four occasions. Control sera were obtained from fourteen members of the hospital staff with no history of adverse reactions to anaesthetic agents or any other drugs. Cord sera were obtained from the Department of Obstetrics, Royal North Shore Hospital.

Preparation of thiopentone-Sepharose complex

Thiopentone (80 mg) was dissolved in water (12 ml) and covalently coupled to 500 mg



Thiopentone :	$R_1 = \text{CH}_3\text{CH}_2-$	;	$R_2 = \text{CH}_3\text{CH}_2\text{CH}_2\underset{\text{CH}_3}{\text{CH}}-$
	$R_3 = \text{H}$	;	$X = \text{S}$
Pentobarbitone :	$R_1 = \text{CH}_3\text{CH}_2-$	;	$R_2 = \text{CH}_3\text{CH}_2\text{CH}_2\underset{\text{CH}_3}{\text{CH}}-$
	$R_3 = \text{H}$	;	$X = \text{O}$
Phenobarbitone :	$R_1 = \text{CH}_3\text{CH}_2-$	;	$R_2 = \text{C}_6\text{H}_5$
	$R_3 = \text{H}$	;	$X = \text{O}$
Barbitone :	$R_1 = \text{CH}_3\text{CH}_2-$	;	$R_2 = \text{CH}_3\text{CH}_2-$
	$R_3 = \text{H}$	;	$X = \text{O}$
Methohexital :	$R_1 = \text{CH}_2=\text{CHCH}_2-$	;	$R_2 = \text{CH}_3\text{CH}_2\text{C}\equiv\underset{\text{CH}_3}{\text{CCH}}-$
	$R_3 = \text{CH}_3$	;	$X = \text{O}$

Fig. 1. Structure of thiopentone and four structurally-related barbiturate drugs.

of Epoxy-activated Sepharose 6B by adjusting the pH to 12.1 with 2.5 M sodium hydroxide and gently shaking at 25°C for 18 hr. After washing with water, 0.1 M borate buffer, pH 8 and 0.1 M acetate buffer, pH 4, remaining free activated groups were blocked by incubation with 1 M ethanolamine, pH 9 at room temperature for 4 hr. The gel was then washed as described above and resuspended in water (65 ml). Ethanolamine-Sepharose complex was prepared by using 300 mg of ethanolamine and 500 mg of activated Sepharose.

Table 1. IgE antibodies to thiopentone in sera from patients who experienced anaphylactoid reactions during anaesthesia

Sera	Radioactive uptake (%) of ^{125}I -anti-human IgE with	
	Thiopentone-Sepharose	Ethanolamine-Sepharose
Controls		
Cord	1.5	0.5
Normals*	1.9 ± 0.3	0.6 ± 0.2
Patients		
Be	16.2	2.3
Ol	6.2	0.7

* Fourteen adult subjects (nine females/five males). Mean and SD.

Table 2. Cross-reactivity between thiopentone and four barbiturate analogues; inhibition studies with sera containing IgE antibodies reactive with thiopentone

Serum	Amount (μ mol) of drug needed for 30% inhibition of the uptake of ^{125} I-anti-human IgE				
	Thiopentone	Pentobarbitone	Phenobarbitone	Barbitone	Methohexital
Be*	3.0	> 5	> 5	> 5	5.0
Ol*	< 0.5†	< 0.5†	> 5	> 5	2.9

* Serum Be was used at a dilution of 1:3. Serum Ol was used undiluted.

† Amounts for 50% inhibition were 1.5 μ mol for thiopentone and 1.4 μ mol for pentobarbitone.

Detection of IgE antibodies by radioimmunoassay

Serum (50 μ l) was added to 4 mg of the solid phase complex (thiopentone-, or ethanolamine-Sepharose) and left at room temperature for 3 h. Tubes were then washed and centrifuged three times with phosphate-buffered saline (PBS) containing Tween 20 before adding 20 000–30 000 cpm/tube of ^{125} I-anti-human IgE (Pharmacia). After standing overnight, tubes were washed three times and counted in a Packard Auto-Gamma Spectrometer. The presence of thiopentone-specific IgE antibodies was determined by the per cent radioactive uptake of ^{125} I-anti-human IgE, that is percent of counts added.

Inhibition assay

Serum (50 μ l) appropriately diluted was incubated for 1 h with 50 μ l of a solution of the compound being tested before addition of the thiopentone-Sepharose complex (4 mg in 100 μ l). After 3 hr, tubes were washed and centrifuged three times with PBS-Tween before the addition of ^{125} I-anti-human IgE (20 000–30 000 cpm/tube). After overnight incubation at room temperature, tubes were washed three times and counted.

Results

Table 1 summarizes results obtained from direct binding RIA studies with sera from the two patients who had experienced life-threatening anaphylactoid reactions after receiving thiopentone. Based on previous experience with other RAST assays [13–16], a positive value in the RAST assay was taken as greater than 2.5 times the cord/normal value [14].

Sera Be and Ol produced uptakes of ^{125} I-anti-human IgE of 16.2 and 6.2% respectively, the former being regarded as a strong positive. By contrast, a control solid support of ethanolamine-Sepharose gave low uptakes with both sera and, together with the low values obtained for cord and fourteen normal sera, indicated that the assay was detecting thiopentone-reactive IgE antibodies. To check the specificities of the IgE antibodies in both patients, inhibition studies were performed in which patients' sera were pre-incubated with thiopentone and four other structurally-related barbiturate drugs. The quantities (μ mol) of these drugs required to produce inhibition of the reaction of the IgE antibodies with thiopentone-Sepharose are given in Table 2. Figure 2 shows detailed inhibition results obtained with sera from both patients.

With serum Be, thiopentone was the best inhibitor of IgE binding requiring 3.0 μmol of drug to produce 30% inhibition whereas 5.0 μmol of methohexital was needed for the same degree of inhibition. Pentobarbitone and phenobarbitone were the next best inhibitors each requiring more than 5.0 μmol of drug while barbitone was a still weaker inhibitor.

With serum OI, pentobarbitone and thiopentone were the most potent inhibitors producing 50% inhibition with 1.4 and 1.5 μmol of drug respectively. Methohexital was the next best inhibitor requiring 2.9 μmol of drug to give only 30% inhibition. Barbitone and phenobarbitone proved to be the weakest inhibitors of IgE binding.

Discussion

The RIA described here will detect IgE antibodies in the sera of patients that bind to the frequently used induction agent thiopentone. The assay was also used in inhibition experiments to provide a quantitative assessment of cross-reactivity between thiopentone and other structurally-related barbiturate drugs. Thiopentone is used in the assay in a non-antigenic form (i.e., not coupled to a protein carrier) by covalently coupling the drug directly to activated Sepharose containing a spacer 'arm'. The mode of coupling of thiopentone to the solid phase would presumably be through the nucleophilic sulphur atom (Fig. 1) although a possible breakdown product resulting from the high pH used in the coupling procedure may be the form of the drug presented to the activated Sepharose. Detailed chemical studies are needed to establish the chemical linkages involved in the drug-Sepharose covalent complex.

Inhibition experiments designed to investigate the specificity of the assay showed that IgE binding was inhibited by thiopentone and four structurally-related barbiturates pentobarbitone, phenobarbitone, barbitone and methohexital. With patient OI, thiopentone and pentobarbitone were the most potent inhibitors while barbitone was clearly the weakest inhibitor of IgE antibody binding (Fig. 2). With patient Be,

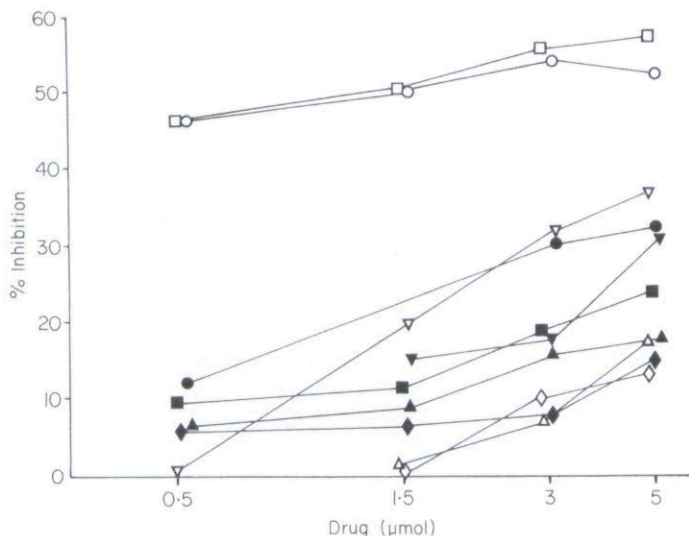


Fig. 2. Inhibition by thiopentone and four structurally-related barbiturate drugs of IgE binding in sera Be (closed symbols) and OI (open symbols) to thiopentone-Sepharose complex. Sera Be and OI were used at a dilution of 1:3 and undiluted respectively. Key to symbols: (○) thiopentone, (Δ) phenobarbitone, (◊) barbitone, (□) pentobarbitone, (▽) methohexital.

thiopentone was the strongest inhibitor followed by methohexital. Barbitone was again the least potent inhibitor. On a structure-activity basis (Figs 1 and 2, Table 2), it appears that the thiopentone-reactive IgE antibodies in both patients are recognizing the barbiturate ring and the size and composition of the side chain alkyl groups R_1 and R_2 are also important for recognition. Barbitone which has ethyl side chains at R_1 and R_2 proved to be the weakest inhibitor. In patient Be, the sulphur atom of thiopentone seems to be important for recognition by the IgE antibody molecule since the other four barbiturate drugs (which contain an oxygen atom instead) were considerably weaker inhibitors. Additional inhibition experiments with other structurally-related, and some unrelated, compounds should allow us to fully identify the thiopentone allergenic determinant(s).

In the meantime, the assay described here may prove valuable to anaesthetists as a diagnostic procedure and supplement skin testing for the detection of some patients with potentially lethal sensitivity to the commonly used induction agent thiopentone.

Acknowledgments

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