

## Anaphylaxis during Anesthesia: Use of Radioimmunoassays to Determine Etiology and Drugs Responsible in Fatal Cases

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Skin (intradermal or prick) testing is usually used to investigate anaphylactoid reactions that occur during anesthesia.<sup>1-4</sup> Testing is usually carried out at least 1 month after the reaction with the intention of confirming the diagnosis, implicating the responsible drug and, ultimately, making subsequent anesthesia safe.<sup>1</sup>

In the deceased patient confirmation of an anaphylactic etiology may be relevant in establishing the cause of death and may have medicolegal significance. Establishing that the cause of a reaction was indeed anaphylactic is therefore of value, particularly when the *post mortem* findings following anaphylaxis are relatively nonspecific<sup>5</sup> and since an anesthetic related problem is a more common cause of mortality.<sup>6</sup> Since skin testing cannot be used in the case of the deceased subject, the examination of sera, taken before or after death, for the presence of specific immunoglobulin E (IgE) antibodies may be a valuable diagnostic aid.

Here, we present results of studies on two patients who experienced fatal drug-induced anaphylaxis during anesthesia and in whom confirmation of diagnosis by radioimmunoassay for drug-specific IgE antibodies, performed with sera taken prior to anesthesia, led to a change in the provisional *post mortem* diagnosis. This study was also noteworthy in that one of the subjects had serum antibodies to both the muscle relaxant drug and the induction agent used.

### CASE REPORTS

*Patient 1.* A 41-yr-old otherwise healthy man with no previous history of general anesthesia and no history of allergy, atopy, or asthma was admitted for bronchoscopy to investigate persistent lung collapse/consolidation. He was not taking any medications. Bronchoscopy under local anesthesia was unsuccessful.

On the subsequent day he was administered general anesthesia so that bronchoscopy could be performed. Preanesthetic medication was with papaveretum 20 mg/hyosine 0.4 mg (Omnopon-Scopolamine®, Roche). On arrival in the operating theater an ECG and automatic blood pressure cuff were applied. The initial blood pressure was 100/80 mmHg, and initial pulse rate was 60 beats per min. While breathing oxygen, 3 mg *d*-tubocurarine was administered, followed by thiopental 400 mg and succinylcholine 100 mg. The patient's lungs were ventilated with 100% oxygen and the trachea intubated with a 9.5-mm cuffed endotracheal tube. During administration of the thiopental he coughed profusely, and during intubation the arterial blood pressure was measured by oscillotonometry at 40/20 mmHg, and the carotid pulse was barely palpable.

While metaraminol was being drawn up, asystole occurred. The metaraminol was injected and a subclavian venous catheter rapidly inserted; cardiac massage was commenced and epinephrine given. Over the next 2 h epinephrine, calcium chloride, isoproterenol, norepinephrine, sodium bicarbonate, hydrocortisone, and methyl prednisolone were given. Four liters of colloid and 11 l lactated Ringer's solution were also administered. Arterial blood gases at 30 min after cardiac arrest showed a pH of 7.4, oxygen tension of 219 mmHg, and carbon dioxide tension of 20 mmHg, and 45 min later showed a pH of 6.9, oxygen tension of 117 mmHg, and carbon dioxide tension of 61 mmHg. Throughout resuscitation there was no cardiac electrical activity or spontaneous cardiac output. DC cardioversion and external and internal pacing was unsuccessful, and resuscitation was ceased after 2.5 h.

*Post mortem* examination showed hemorrhagic pericarditis and pulmonary interstitial edema, and the primary cause of death was described as hemorrhagic pericarditis.

*Patient 2.* A 28-yr-old woman was admitted to the hospital after 9 days of vaginal bleeding and abdominal pain. She had a history of asthma, for which she used a salbutamol inhaler as necessary; had never been hospitalized for asthma; and had no known allergies. She had had five previous uneventful anesthetics of which records of two were available; she had received thiopental on both occasions and alcuronium and pancuronium on one occasion. On admission she looked pale but was normotensive (blood pressure 115/70 mmHg and pulse rate 96 beats per min). Her hemoglobin concentration was 8.4 g/l, and prior to surgery she was given three units of packed cells. She was premedicated with 10 mg papaveretum (Omnopon®, Roche), 10 mg metoclopramide, and 1 ml salbutamol *via* nebulizer. Anesthesia was induced with thiopental 375 mg and succinylcholine 75 mg, and following tracheal intubation the lungs could not be ventilated. Surgery was commenced and the endotracheal tube was changed. There was no improvement in ventilation, and surgery was abandoned and the wound closed rapidly.

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There was no urticaria or angioedema. The patient's lungs remained difficult to ventilate, and cardiac arrest occurred. She was given 1 ml 1:1000 epinephrine *via* the endotracheal tube and 1 ml 1:10,000 epinephrine and 250 mg aminophylline intravenously and defibrillated twelve times over the next hour. An epinephrine infusion was commenced, and 2 l modified gelatin infusion was given. Her blood pressure stabilized but difficulty in ventilation persisted despite of repeated salbutamol and hydrocortisone. She developed bleeding from puncture sites and the wound and was given six units of blood, ten units of platelets, and four units of fresh frozen plasma. Five hours after induction she developed bradycardia, which rapidly progressed to asystole, and could not be resuscitated.

*Post mortem* examination showed pulmonary edema, hyperinflated lungs, and terminal narrowing of the bronchi. There was 1,500 ml blood in the peritoneal cavity. The *post mortem* report attributed death to intraperitoneal hemorrhage with bronchospasm in a patient with asthma as a contributing cause.

## Materials and Methods

### SERA

Blood taken from patient 1 preoperatively for laboratory tests and from patient 2 for cross-matching was supplied for radioimmunoassay studies. A sample of blood was also taken from patient 1 following administration of the anesthetic agents and during the reaction. Sera from adults allergic to house dust, mites, and/or grass pollens but with no history of adverse reactions to anesthetics were obtained after clinical diagnosis had been confirmed by skin prick testing with the appropriate allergen extracts and the detection of allergen-specific IgE antibodies in the radioallergosorbent test (RAST).<sup>7</sup> These sera showed radioactive uptakes of <sup>125</sup>I-antihuman IgE of approximately 10–50% with the allergen discs. Sera from 10 nonallergic, healthy adults with no history of adverse reactions to anesthesia were collected from hospital staff members. Cord serum was obtained from the Department of Obstetrics and Gynaecology, Royal North Shore Hospital. Control sera generally gave uptakes of 0.5–1% in the RASTs.

### RADIOIMMUNOASSAYS FOR THE DETECTION OF IgE ANTIBODIES TO THIOPENTAL AND NEUROMUSCULAR BLOCKING DRUGS

For the detection of IgE antibodies that react with succinylcholine, solid phases using choline, a compound whose structure exactly mimics the terminal groupings on the neuromuscular blocking drug (NMBD) relaxant, is used.<sup>8</sup> Choline-Sepharose, thiopental-Sepharose and ethanolamine-Sepharose for use as the solid phase in the immunoassays were prepared as previously described.<sup>8,9</sup> IgE antibodies were detected as previously reported using 6 mg solid phase complex per tube and <sup>125</sup>I-antihuman IgE (Pharmacia, Uppsala).<sup>8,9</sup> The presence of specific IgE antibodies was determined by the percent of counts of <sup>125</sup>I-anti-IgE remaining after the washing procedure; results are presented accordingly in tables 1 and 2. Uptakes of <sup>125</sup>I-anti-IgE that were at least three times the uptakes recorded with control sera were considered positive.<sup>7–9</sup>

## INHIBITION ASSAY

Quantitative hapten inhibition assays were performed as described.<sup>10</sup> Inhibitory amounts of drug are expressed as nanomoles per tube. Drugs and chemicals used have been described.<sup>9–11</sup>

### Results

#### DIRECT BINDING STUDIES FOR THE DETECTION OF IgE ANTIBODIES

Serum from subject 1 produced positive reactions when tested with choline and thiopental solid phases. Serum from subject 2 gave a positive reaction only with the thiopental complex. Both sera showed background binding with the ethanolamine-Sepharose control solid phase complex (table 1). None of the control sera showed any positive uptakes with any of the three solid phase complexes (table 1).

#### HAPTEN INHIBITION STUDIES WITH THE ANESTHETIC DRUGS USED CLINICALLY

To test the specificities of the drug-IgE positive reactions detected in the direct binding studies, sera 1 and 2 each were preincubated with succinylcholine and thiopental before addition of the drug-solid phases. Reactions of serum 1 with each solid phase were inhibited by the complementary free drug only. With serum 2, which gave a positive reaction with thiopental-Sepharose but not with choline-Sepharose, thiopental, but not succinylcholine, inhibited IgE-binding to the thiopental solid phase (table 1).

#### HAPTEN INHIBITION STUDIES TO INVESTIGATE CROSS-REACTIVITY WITH OTHER NEUROMUSCULAR BLOCKING DRUGS

Using the choline solid phase, all six NMBDs examined were found to inhibit strongly the IgE binding from the serum of patient 1. *d*-Tubocurarine and decamethonium were marginally more potent inhibitors than pancuronium, succinylcholine, and gallamine; however, even with alcuronium, the least potent, only 22 nmol compound was needed for 50% inhibition. Thiopental did not inhibit, in concentrations as great as 3,000 nmol per tube (table 2).

#### HAPTEN INHIBITION STUDIES TO INVESTIGATE THE IgE-THIOPENTAL INTERACTIONS

Thiopental inhibited binding to the thiopental solid phase of IgE antibodies in the sera of both patients. No inhibition was observed with up to 5  $\mu$ mol each of barbital, barbituric acid, hexobarbital, mephobarbital, methohexital, and phenobarbital and with the NMBDs succinylcholine, alcuronium, *d*-tubocurarine, decamethonium, gallamine, and pancuronium.

### Discussion

The RAST has been used previously to diagnose a fatal anaphylactic reaction to penicillin<sup>†</sup> and radioimmunoas-

§ Harle DG, Baldo BA, Wajon P, Fisher MM: Detection of thiopental-reactive IgE antibodies following anaphylactoid reactions during anaesthesia. *Clinical Allergy* 16:493–498, 1986.

† Jarisch R, Roth A, Boltz A, Sandor I: Diagnosis of penicillin allergy by means of Phadebas RAST penicilloyl G and V skin tests. *Clinical Allergy* 11:155–160, 1981.

TABLE 1. Direct Binding and Inhibition Results with NMBD- and Thiopental-reactive IgE Antibodies in the Sera of Two Patients Who Experienced Life-threatening Anaphylactoid Reactions during Anesthesia

Sera	Direct Binding : Radioactive Uptake (%) of <sup>125</sup> I-anti-human IgE with Sepharose Linked to:			Inhibition: With Drug Solid Support of:			
	Choline	Thiopental	Ethanolamine	Choline-Sepharose		Thiopental-Sepharose	
				Succinylcholine*	Thiopental*	Succinylcholine*	Thiopental*
Controls							
Cord	0.8	1.5	0.5				
Normals†	0.8 ± 0.3	1.9 ± 0.3	0.6 ± 0.2				
Allergic‡	0.7 ± 0.3	2.0 ± 0.3	0.8 ± 0.3				
Patients							
1§	17.7	14.5	0.9	84**	0††	0**	39††
1¶	18.2	11.9	0.5				
2	1.8	7.0	0.5	0	0	0	32

\* Used as the free drug in hapten inhibition studies.

† Sera from ten "normal" (nonallergic) adult subjects (eight women and two men).

‡ Sera from ten adults allergic to house dust mite and/or grass pollens (six women and four men).

§ Blood taken preoperatively.

¶ Blood taken postoperatively.

\*\* Percent inhibition produced with 100 nmol drug.

†† Percent inhibition produced with 3000 nmol drug.

says using drug-solid phase complexes have been developed and applied for the detection of IgE antibodies that react with muscle relaxants<sup>8,10,11</sup> or thiopental.<sup>12</sup> These immunoassays correlate well with case histories, skin tests, and basophil release findings for subjects who experienced a drug-induced anaphylactic reaction during anesthesia.<sup>4,9-11</sup>

Direct binding and hapten inhibition studies detailed here clearly showed that the serum of patient 1 contained IgE antibodies that reacted with the NMBD succinylcholine and the induction agent thiopental, both of which the patient received. Inhibition of IgE antibody binding by succinylcholine<sup>8,9</sup> and thiopental<sup>11</sup> to the complementary drug-solid phase complex and the failure of these two compounds to cross-inhibit (at concentrations as great as 100 nmol and 3000 nmol per tube)<sup>12</sup> demonstrated that the serum of patient 1 contained separate populations of IgE antibodies that reacted with succinylcholine and thiopental respectively.

Harle *et al.* have previously demonstrated, in the sera of some subjects allergic to muscle relaxants but not thiopental, IgE antibodies that react with both the muscle relaxants and the induction agent. This cross-sensitivity detected *in vitro* appears to be due to IgE antibodies' rec-

ognizing both substituted ammonium groups on the NMBDs and a determinant represented by, or derived from, the ring nitrogen atom of thiopental.<sup>12</sup> This *in vitro* cross-reactivity may be separated from *in vivo* sensitivity to thiopental by the failure of thiopental to inhibit IgE binding to the thiopental-solid phase.<sup>12</sup> Since inhibition with thiopental was observed with serum from patient 1, we concluded that this subject had experienced a true type I allergic reaction to the induction agent. The degree of inhibition obtained with thiopental in hapten inhibition studies using sera from thiopental-allergic subjects tends to be lower than we find in other drug inhibition studies.<sup>9-12</sup> Values of 20% or more inhibition with 3-5 μmol thiopental correlate well with clinical signs of anaphylaxis to the drug.<sup>11-13</sup> Both direct binding and hapten inhibition studies with the serum of patient 2 showed the presence of thiopental-reactive IgE antibodies with no indication of antibodies to succinylcholine. The antibodies to thiopental were readily inhibited (32%) by free thiopental. Inhibition of the choline-reactive IgE antibodies in the serum of patient 1 by succinylcholine and by comparable amounts of five different NMBDs suggests that this subject would have experienced a reaction if one of the alternative relaxants tested had been used.<sup>8,10</sup>

TABLE 2. Results of Inhibition Studies with Serum from Patient 1\* Containing Choline- (and Succinylcholine-) Reactive IgE Antibodies.† Demonstration of Cross-reactivity with Other NMBDs but Not Thiopental

Amount (nmol) of Drug Needed for 50% Inhibition of the Uptake of <sup>125</sup> I-anti-human IgE						
Succinylcholine	Alcuronium	d-Tubocurarine	Decamethonium	Gallamine	Pancuronium	Thiopental
10.5	22	6	6.5	13	8	>3000

\* Serum taken preoperatively and used at a dilution of 1-5.

† Hapten inhibition studies carried out with a choline-Sepharose solid phase.

Hence, on the basis of the immunoassay findings, we concluded that the sera of both patients contained IgE antibodies to a drug, or drugs, given during anesthesia. This conclusion is relevant to interpreting the *post mortem* findings and to establishing the cause of death, since in both of the patients a diagnosis of anaphylaxis was not obvious. In the first patient, cardiac arrest occurred with no other manifestations of anaphylaxis. Single organ system involvement in anaphylactic reactions occurs in fewer than 10% of patients.<sup>14</sup> In patient 2 the predominant features were bronchospasm, which is more commonly related to intubation,<sup>15</sup> and the late occurrence of disseminated intravascular coagulation, which is a rare complication of anaphylaxis.<sup>16</sup> The finding of free peritoneal blood *post mortem* was a contributing factor to the late hypotension. Furthermore, the anesthesia was induced with thiopental and succinylcholine through a butterfly needle in the dorsum of the hand. It has been suggested that under these circumstances, fatal reactions resembling anaphylaxis can be produced by the formation of aggregates in small intravenous cannulas<sup>16,17</sup> and that such reactions are due to insufficient care by the anesthesiologist.<sup>17</sup> There is, however, no convincing evidence that such reactions occur.

*Post mortem* findings in neither case were specific. In patient 2 the detection of IgE antibodies led to a revision of the initial diagnosis of internal hemorrhage with bronchospasm in an asthmatic as a secondary event. The final diagnosis was anaphylaxis to thiopental with internal hemorrhage as an associated event. In patient 1 the initial *post mortem* diagnosis of cardiac arrest, hemorrhagic pericarditis, and possible pneumonitis was changed at the inquest to anaphylactic shock. Assem and Ling<sup>18</sup> have previously used the drug immunoassays we developed<sup>8</sup> to determine the drug responsible for a fatal anaphylactic reaction in a case in which the diagnosis was not in dispute. In the cases described here, the use of the test has been taken a step further, not only in making the diagnosis, but in leading to a change of the preliminary *post mortem* diagnosis.

The detection of antibodies to both succinylcholine and thiopental in patient 1 was a surprising finding. Although cross-sensitivity between NMBDs is a common finding in patients who react to a NMBD,<sup>2,4,10,19</sup> we had not previously encountered allergy to both thiopental and a muscle relaxant in more than 300 patients studied. Subsequent to our investigations, two patients with proven allergy to both thiopental and a muscle relaxant have been documented.<sup>20</sup>

Application of the tests described here for the detection of drug-reactive IgE antibodies in sera taken preoperatively (for example, for cross-matching or other laboratory investigations) may provide valuable information in fatal cases where an anaphylactic etiology is suspected.

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