

## Reaginic Antibodies to Drugs Used in Anesthesia

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Twenty-four patients survived life-threatening clinical anaphylaxis due to anesthesia. In each case the diagnosis of anaphylaxis was confirmed and the responsible drug was found by intradermal testing. To determine whether the reactions were anaphylactic or anaphylactoid, serum from each patient was tested for reaginic activity using Prausnitz-Kustner (PK) testing in human subjects and monkeys and passive cutaneous anaphylaxis (PCA) testing at four and 24 hours in monkeys. Positive results of PCA testing at four hours were repeated with serum that had been heated to 56 C for two hours. Drugs used in testing were Althesin®, thiopental, succinylcholine, gallamine, *d*-tubocurarine, and alcuronium. Vehicles and antioxidants were tested separately. Positive tests suggestive of the presence of reaginic antibodies occurred with sera from 15 patients who had had previous exposure to the drug. Nine patients had tests suggestive of IgE antibodies on first exposure, suggesting that cross-sensitivity may be a factor in such reactions to muscle relaxants. Two patients had positive tests for IgG antibodies. This is further evidence of the role of this mechanism in immediate allergy and demonstrates another mechanism by which anaphylaxis can occur without previous sensitization. Four patients had positive tests for IgE antibodies after previous exposure. It was concluded that it is not possible to determine the mechanism of anaphylaxis from a history of previous exposure. (Key words: Allergy. Anesthetics, intravenous: alphadione; thiopental. Complications: anaphylaxis. Neuromuscular relaxants: alcuronium; gallamine; succinylcholine; *d*-tubocurarine.)

THE INCIDENCE of severe hypersensitivity reactions to intravenous drugs used in anesthesia appears to be increasing.<sup>1</sup> The clinical syndrome of anaphylaxis can be produced by more than one mechanism. Where there is no evidence of an immune mechanism, such reactions are called anaphylactoid. Anaphylactoid reactions may be produced by direct histamine release<sup>2</sup> and complement activation.<sup>3</sup> Subsequent exposure to the drug may be safe after such reactions.<sup>4</sup> Anaphylaxis may also be produced by IgE-mediated reactions, which require previous sensitization, and IgG-mediated reactions,<sup>5,6</sup> which may not require previous sensitization.<sup>5</sup> Subsequent exposure to the drug is contraindicated.

In 35 patients who had experienced life-threatening clinical anaphylaxis, we found a positive intradermal test to the drug causing the reaction.<sup>7</sup> Positive intra-

dermal tests suggest true IgE-mediated anaphylaxis,<sup>8,9</sup> but they may be also produced by either direct histamine release,<sup>10</sup> or IgG antibodies.<sup>11</sup> Since the majority of reactions that we have seen occurred on the patients first exposure to a drug, and because anaphylaxis requires previous sensitization, this study was undertaken to elucidate further the mechanism of such reactions.

### Materials and Methods

Studies were made of 24 patients who had clinical anaphylaxis during anesthesia. The diagnosis was confirmed and the responsible drug was determined by intradermal testing using the method of Fisher.<sup>7</sup> Blood was taken from the patients, separated by centrifugation, and tested for hepatitis antigen. The serum was stored at -20 C and transported frozen. The serum was tested using Prausnitz-Kustner (PK) and passive cutaneous anaphylaxis (PCA) tests. Medical practitioners were used as experimental subjects for PK testing, since it was believed that the risks of serum hepatitis could not be adequately explained to lay volunteers for purposes of obtaining informed consent. For the PK tests, 0.1 ml of serum was injected into multiple sites on the forearm of physician volunteers and into the anterior abdominal wall of pharmacologically virgin *Macaca neminstrina* monkeys anesthetized with ketamine, 50 mg, intramuscularly. The serum sites were challenged by direct intradermal injections of the suspect drug and control drugs in dilution fractions of 1/100 in monkeys and 1/1000 in human subjects. Control injections were made directly intradermally. A positive result was recorded when a wheal-and-flare reaction occurred, with a wheal larger than 1 cm that persisted 30 min, with no reaction from control drugs in control sites.

For the PCA test, *Macaca neminstrina* monkeys were anesthetized with ketamine, 50 mg. Anesthesia was maintained with nitrous oxide, 3 l/min, oxygen, 1.5 l/min, and halothane, 0.5 per cent, using the Jackson Rees modification of an Ayres' T-piece circuit. Serum was injected intradermally into the anterior abdominal wall. At four and 24 hours, the monkeys were given Evans blue dye intravenously. If no alteration occurred in serum sites within 20 min, the monkeys were given an intravenous dose of the suspect drug mixed with Evans blue dye, and the serum sites were observed for 45 min. When positive results occurred in four-hour tests, the tests were repeated, using

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serum heated to 56 C for two hours. A result was recorded as positive when there was an easily detectable blue discoloration of the serum sites and an absence of discoloration of the control sites. If no reaction occurred within 45 min, another drug was tested. The drug dosages used (per kg) were Althesin®, 0.06 ml, gallamine, 1.5 mg, succinylcholine, 1 mg, *d*-tubocurarine, 0.5 mg and alcuronium, 0.25 mg.

The PCA test at 24 hours is a minor modification of the method of Layton, Lee, and De Elds.<sup>12</sup> The four-hour test is the method of Parish.<sup>13</sup> If vehicles and antioxidants were present in the ampules, these were tested independently.

In five patients, C<sub>3</sub> and C<sub>4</sub> complement levels were measured sequentially for 24 hours after the reactions by using radial immunodiffusion with Behring plates. When abnormalities occurred, total hemolytic complement (THC) was measured serially.

**Results**

In nine patients, all tests were negative (table 1). No patient showed a positive response to any vehicle or additive. Positive results were obtained in 15 patients (table 2). Four patients who had received the drug on a previous occasion had positive PK tests in volunteers. In two instances, these results were confirmed by both PCA and PK testing in monkeys.

Two patients who had reactions to muscle relaxants on first exposure had negative PK tests in volunteers and positive PK tests in monkeys. These results are surprising and may result from the greater concentrations of the drug used in the animals. Nine patients who had had reactions to muscle relaxants on first

TABLE 1. Negative Results

|                        | Patients Tested | Negative Results |
|------------------------|-----------------|------------------|
| Althesin               | 9               | 5                |
| Thiopental             | 1               | —                |
| Succinylcholine        | 4               | 1                |
| Gallamine              | 4               | 1                |
| <i>d</i> -Tubocurarine | 2               | 1                |
| Alcuronium             | 4               | 1                |

exposure had positive PK tests in volunteers, and of these nine patients, four had positive PK tests in monkeys. Two patients had positive PCA tests at four hours in monkeys, and these tests were also positive after the serum was heated. Both these patients showed decreased values for C<sub>4</sub> and THC and normal levels for C<sub>3</sub>.

**Discussion**

Determination of the mechanism of acute allergic reactions to intravenous drugs is difficult but worth while. If a reaction is caused by direct histamine release, the reaction is related to volume, concentration, and rate of injection.<sup>14</sup> Subsequent administration of the drug may be safe.<sup>4</sup> True anaphylaxis requires previous sensitization. A history of previous exposure is not, however, helpful in determining the responsible drug or mechanism. Fifty per cent of patients who reacted to induction agents and only three patients who had documented reactions to muscle relaxants had received the drug previously.<sup>15-17</sup>

Most reactions to muscle relaxants and induction agents give positive results when intradermal testing is performed under controlled conditions.<sup>7</sup> A number of authors have suggested that such tests indicate

TABLE 2. Positive Results of Antibody Testing in Patients Reacting to Anesthetic Drugs

|            | Sex | Drug                   | Previous Exposures to Drug | Results of Testing* |     |               |              |                      |
|------------|-----|------------------------|----------------------------|---------------------|-----|---------------|--------------|----------------------|
|            |     |                        |                            | Man                 |     | Monkey        |              |                      |
|            |     |                        |                            | PK†                 | PK† | PCA† 24 Hours | PCA† 4 Hours | PCA† 4 Hours Heated‡ |
| Patient 1  | M   | Thiopental             | 5                          | +                   | ND  | ND            | ND           | ND                   |
| Patient 2  | F   | Althesin               | 0                          | —                   | —   | —             | +            | +                    |
| Patient 3  | F   | Althesin               | 0                          | —                   | —   | —             | +            | +                    |
| Patient 4  | F   | Althesin               | 1                          | +                   | +   | +             | —            | ND                   |
| Patient 5  | F   | Althesin               | 1                          | +                   | —   | —             | —            | ND                   |
| Patient 6  | F   | Succinylcholine        | 0                          | —                   | +   | —             | —            | ND                   |
| Patient 7  | F   | Succinylcholine        | 0                          | +                   | —   | —             | —            | ND                   |
| Patient 8  | F   | Succinylcholine        | 0                          | +                   | +   | —             | —            | ND                   |
| Patient 9  | F   | Gallamine              | 0                          | +                   | —   | —             | —            | ND                   |
| Patient 10 | F   | Gallamine              | 0                          | +                   | —   | —             | —            | ND                   |
| Patient 11 | F   | Gallamine              | 0                          | —                   | +   | —             | —            | ND                   |
| Patient 12 | M   | Alcuronium             | 1                          | +                   | +   | +             | —            | ND                   |
| Patient 13 | F   | Alcuronium             | 0                          | +                   | +   | —             | —            | ND                   |
| Patient 14 | F   | Alcuronium             | 0                          | +                   | +   | —             | —            | ND                   |
| Patient 15 | F   | <i>d</i> -Tubocurarine | 0                          | +                   | +   | —             | —            | ND                   |

\* Results: + = positive; — = negative; ND = not done.

† PK = Prausnitz-Kustner test; PCA = passive cutaneous anaphylaxis test.

‡ Serum heated to 56 C for two hours.

§ Serum heated to 56 C for two hours.

true IgE-mediated antibodies.<sup>8,9</sup> However, direct histamine release,<sup>10</sup> and IgG antibodies, which may not require a latent period of sensitization, may also produce positive skin tests.<sup>11</sup>

Passive-transfer tests do not constitute absolute proof of the presence of antibodies, but they are highly reliable, and correlate well with specific tests such as radioallergic sorbent testing.<sup>18</sup> Prausnitz-Kustner testing is one such form of testing in man and, although such antibodies are said to be homocytotropic, it has been shown that modifications of PK testing are reliable in higher apes.<sup>11</sup> Passive cutaneous anaphylaxis tests in monkeys are less sensitive than PK tests.<sup>10</sup>

Parish<sup>13</sup> has described the differences between short-acting, heat-stable IgG antibodies and heat-labile IgE antibodies. Although Parish does not believe that such antibodies are involved in immediate allergy,<sup>20</sup> their role has been demonstrated by other workers.<sup>5,6</sup> In our study, four patients had positive PK tests to drugs they had been previously exposed to. This suggests true IgE-mediated anaphylaxis. Eleven patients had positive tests to muscle relaxants, and they had not received muscle relaxants previously. This suggests the presence of IgE antibodies. Since a latent period of sensitization is required for IgE-mediated anaphylaxis, the patients may have been sensitized when they receive the drug, *i.e.*, perhaps cross sensitivity is involved, at least in women in Australia. This finding has been previously documented in one patient in Australia.<sup>21</sup>

Two patients who had reacted to Althesin had positive tests for IgG antibodies. These reactions were associated with nonspecific changes in complement levels, and probably represent an acute phase response. This is another mechanism in which anaphylaxis may occur without previous exposure and a negative PK test. Mathieu, Goudsouzian, and Snider<sup>14</sup> suggested that in the event of a positive intradermal test and a negative PK test, subsequent administration of the drug is safe. Our study does not support that view. False negatives may occur, and reactions caused by other mechanisms, such as IgG antibodies, will not be detected. In severe allergic reactions to anesthetic agents, it is important to determine the causative agent and prevent its subsequent use.

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