

# Biochemical Markers of Anaphylactoid Reactions to Drugs

## Comparison of Plasma Histamine and Tryptase

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Adverse reactions to drugs require that their mechanisms be elucidated, particularly when anaphylaxis is suspected. Early diagnosis can be achieved by plasma histamine measurements. Unfortunately, the short plasma half-life of histamine and the difficulties in handling the sample usually preclude this measurement, although a sensitive radioimmunologic kit is routinely available. It has been recently suggested that mast cell tryptase, a component of the mast cell granules, could provide an alternative to histamine determination. We have measured plasma histamine and tryptase in 19 patients who developed possible anaphylactoid reactions to anesthetic or other drugs. Eight patients had increased values for both histamine and tryptase. In 4 a muscle relaxant drug was proved responsible for the reaction. Six patients had normal levels for both substances. In each case, the clinical signs of anaphylaxis were moderate. Two patients had normal histamine and high tryptase concentrations, due to late sampling (> 5 h). In 2 other patients, histamine was high, with normal tryptase: in 1, muscle relaxant allergy was further demonstrated. Tryptase half-life was equal to 90 min in 3 patients. At least 15 min was necessary to reach the peak level when the responsible drug was administered intravenously. The best time for measuring tryptase was 1-2 h after the reaction (not > 6 h), whereas for histamine it was 10 min to 1 h. We conclude that measurement of plasma tryptase along with measurement of plasma histamine may aid in diagnosis of anaphylaxis. (Key words: Immune response, anaphylactoid; histamine; tryptase.)

BIOCHEMICAL PROOF of anaphylaxis is often difficult to obtain.<sup>1</sup> Histamine, a major mediator involved in anaphylactic shock, has a plasma half-life of only a few minutes. Thus, it is necessary to collect blood samples within the first 30 min after onset of the reaction, often when other events requiring urgent action are occurring. Furthermore, because histamine is present in circulating basophils, which might be altered by the drawing of blood, hemolyzed samples should not be used for histamine determinations. Thus, the diagnosis of anaphylaxis is usually based on clinical signs.

Tryptase, a neutral protease stored in mast cell granules,<sup>2</sup> is liberated into blood if mast cell degranulation occurs. Tryptase is virtually absent from normal serum, as well as serum obtained during septic shock or myocar-

dial infarction.<sup>3</sup> An increased concentration of tryptase measured after an adverse reaction to drugs may signal mast cell-related events such as anaphylaxis.<sup>3</sup> Because tryptase is present in low concentration in white blood cells,<sup>4</sup> hemolysis and blood clotting have little influence on the measured concentration. Furthermore, the half-life of tryptase in plasma is approximately 2 h *in vivo*, and immunoreactive tryptase is stable in plasma or serum *in vitro*.<sup>5</sup> This allows for delayed sampling and measurement.

To determine if tryptase is a consistent and reliable marker of anaphylaxis, we compared plasma histamine and tryptase concentrations in patients having anaphylactoid reactions.

## Materials and Methods

### SUBJECTS

#### *Nonanesthetized Controls*

Thirteen healthy volunteers aged 22-44 yr (mean 32 yr, standard deviation [SD] 6.2) had plasma histamine and tryptase measurements 3 h after breakfast.

#### *Anesthetized Controls*

Plasma histamine was measured 15 min after induction in 35 control subjects aged 24-81 yr (mean 55.1 yr, SD 14.6) anesthetized without any adverse reaction. Tryptase was measured after surgery in 19 control subjects aged 18-79 yr (mean 51 yr, SD 17) anesthetized without any reaction suggesting anaphylaxis. The anesthetic protocols included various muscle relaxants.

#### *Patients*

Nineteen patients were referred to us for measurements of plasma histamine and tryptase during the hours following an adverse reaction to drugs. Sixteen patients had been anesthetized. All of them had previously received hydroxyzine as a premedication. In 12 cases, the reaction had immediately followed induction of general anesthesia. Eleven patients had received a muscle relaxant. In 4 cases the reaction had appeared unrelated to anesthetic drug injection. In 2 it appeared after gelatin infusion, and in 1 after dressing with Peruvian balsam. The 3 nonanesthetized patients had reacted immediately after a single drug injection (penicillin, tetanus vaccine, and contrast medium). The clinical signs ranged from those

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of a typical anaphylactoid reaction (hypotension, bronchospasm, and erythema) to cutaneous signs or bronchospasm alone. The severity of the reaction was classified according to Ring and Messmer severity scale<sup>6</sup> (table 1). Eight patients were further investigated by skin testing at 6 weeks or later; when appropriate, muscle relaxant-directed immunoglobulin E (IgE) and *in vitro* histamine release were performed. Three other patients had only muscle relaxant-directed IgE measured, 2 because they died soon after the reaction and 1 because he could not return (table 1). The remaining patients were not yet investigated or refused to return.

## METHODS

### Plasma Histamine Determination

Blood was withdrawn from a peripheral vein in EDTA-containing tubes that were immediately transported while in chilled water to the laboratory. After centrifugation (4° C, 1,500 rpm) plasma was gently aspirated and frozen at -20° C. Histamine was measured in duplicate by a competitive radioimmunoassay after acylation of histamine<sup>7</sup> (histamine radioimmunoassay kit, Immuno-tech, Luminy, France). The lower limit of detection of the assay is 0.2 nM (1 nM = 0.11 ng/ml).

### Tryptase Measurement

Mast cell tryptase concentration was measured in plasma or serum by an immunoradiometric assay<sup>8</sup> (Tryptase RIACT kit, Pharmacia, Uppsala, Sweden). The lower limit of detection of the assay is 0.5 U/l (1 U = 1 µg of purified human lung tryptase). The experiments were performed in duplicate.

### Immunologic Tests

Skin tests were performed according to Fisher<sup>9</sup> at 6 weeks of the reaction or later. The sensitivity of skin tests is 97%.<sup>9</sup> Muscle relaxant specific IgE antibodies were measured by a radioimmunoassay.<sup>10</sup> The sensitivity is 87.9%. *In vitro* histamine release was obtained by incubation of whole blood with increasing dilutions of drug. After a 30-min challenge at 37° C and centrifugation at 4° C, histamine was measured in the supernatant. In our laboratory, the sensitivity of this test is 37%.

## Results

### CONTROLS

#### Plasma Histamine

The normal mean histamine concentration measured in the 13 healthy volunteers was 0.8 nM (SD 0.4 nM). In

the 35 anesthetized control subjects, the mean concentration was 1.63 nM (SD 0.61 nM). The upper concentration for normal subjects, calculated from mean  $\pm$  3 SD (99% confidence interval), was 3.5 nM. The normal values usually reported for plasma histamine are < 9 nM<sup>11</sup> when measured by the fluorometric method. We considered the pathologic range as > 9 nM and the intermediate range as 3.5–9 nM.

### Tryptase

The concentrations measured in the 13 healthy volunteers were all less than 2 U/l. Eighteen of the 19 anesthetized control subjects had tryptase values < 2 U/l. In one the tryptase concentration was 2.2 U/l.

### PATIENTS

Table 1 shows the values of plasma histamine and tryptase concentrations in the first sample of all the patients obtained after the anaphylactoid reaction, as well as a second value of the concentration of tryptase, in 11 patients, observed 90 min to 24 h later.

Tryptase concentration was increased in the first sample for 11 subjects (cases 1, 2, 3, 5, 6, 7, 8, 13, 17, 18, and 19). Three of these samples had been kept frozen at -20° C before tryptase measurement, for 15 (case 6), 24 (case 7), and 42 (case 8) months. In eight patients (cases 1, 2, 5, 6, 7, 13, 18, and 19), plasma histamine also was increased. In one case (case 3), the sample was hemolyzed; thus, although plasma histamine was increased, it could not be accepted as a positive result. Histamine was normal in two cases (cases 8 and 17): the intervals in time between the reaction and the sampling were 24 and 5 h, respectively. Six of these 11 patients were further investigated. All had at least one positive test, suggesting anaphylaxis: four had positive skin tests and positive IgE directed against muscle relaxants. Two also had positive *in vitro* histamine release. Two patients, who died after sampling (cases 6 and 8), had positive muscle relaxant-directed IgE.

In eight patients, the tryptase concentration was normal in the first sample (cases 4, 9, 10, 11, 12, 14, 15, and 16). In six of these patients, plasma histamine concentration was normal as well (cases 9, 10, 11, 12, 14, and 15). The clinical signs were often minor. Three of these patients had further testing: two had negative tests (cases 9 and 10), but one (case 11) had slightly positive skin tests to atracurium, at the highest recommended concentration (100-fold dilution of the commercially available preparation). In contrast, increased histamine concentrations were found in two patients (cases 4 and 16) in whom tryptase concentration was normal. Muscle relaxant allergy was demonstrated in case 4. The circumstances of the reaction were unusual for case 16: tachycardia, hypotension, and erythema appeared after the end of anesthesia,

TABLE 1. Severity Grade of Anaphylactoid Reaction and Plasma Histamine and Tryptase Concentrations in 19 Patients

| Case | Circumstances                    | Severity Grade* | Time of Sampling | Plasma Histamine (nM) | Tryptase (U/l) | Responsible Drug         |
|------|----------------------------------|-----------------|------------------|-----------------------|----------------|--------------------------|
| 1    | Anesthesia (MR)                  | 3               | 20 min           | 110                   | 140            | Not tested               |
|      | Induction                        |                 | 5 h              | —                     | 13             |                          |
| 2    | Anesthesia (MR)                  | 3               | 10 min           | 5250                  | 39             | Pancuronium              |
|      | Induction                        |                 | 3 h, 30 min      | 27                    | 27             | Positive ST and IgE      |
| 3    | Anesthesia (MR)                  | 3               | 15 min           | Hemolyzed             | 110            | Succinylcholine          |
|      | Induction                        |                 | 2 h              | —                     | 49             | Positive ST, IgE, and HR |
|      |                                  |                 | 24 h             | —                     | <2             |                          |
| 4    | Anesthesia (MR)                  | 2               | 20 min           | 17                    | <2             | Vecuronium               |
|      | Induction                        |                 | 1 h, 30 min      | —                     | <2             | Positive ST and IgE      |
| 5    | Anesthesia (MR)                  | 3               | 1 h              | 38                    | 11             | Succinylcholine          |
|      | Induction                        |                 |                  |                       |                | Positive ST and IgE      |
| 6    | Anesthesia (MR)                  | 4               | 1 h              | 1120                  | 240            | Pancuronium              |
|      | Induction                        | Death           |                  |                       |                | Positive IgE             |
| 7    | Anesthesia (MR)                  | 3               | 30 min           | >150                  | 38             | Gallamine                |
|      | Induction                        |                 |                  |                       |                | Positive ST, IgE, and HR |
| 8    | Anesthesia (MR)                  | 4               | 24 h             | 6                     | 6              | Alcuronium               |
|      | Induction                        | Death           |                  |                       |                | Positive IgE             |
| 9    | Anesthesia (MR)                  | 2               | 20 min           | 2                     | <2             | Negative IgE             |
|      | Induction                        |                 | 1 h, 30 min      | —                     | <2             |                          |
| 10   | Anesthesia (MR)                  | 1               | 15 min           | 1                     | <2             | Negative ST, IgE, and HR |
|      | Induction                        |                 | 2 h              | —                     | <2             |                          |
|      |                                  |                 | 24 h             | —                     | <2             |                          |
| 11   | Anesthesia (MR)                  | 2               | 30 min           | 5                     | <2             | Atracurium               |
|      | Induction                        |                 |                  |                       |                | Slightly positive ST     |
| 12   | Anesthesia (without MR)          | 1               | 10 min           | 4                     | <2             | Negative IgE and HR      |
|      | Induction                        |                 | 1 h, 30 min      | —                     | <2             | Not tested               |
| 13   | Anesthesia                       | 3               | 40 min           | 92                    | 47             | Not tested               |
|      | 1-h, 30-min delay                |                 | 2 h              | —                     | 27             |                          |
|      | Gelatin infusion                 |                 |                  |                       |                |                          |
| 14   | Anesthesia                       | 2               | 30 min           | 5                     | <2             | Not tested               |
|      | 1-h delay                        |                 | 1 h, 30 min      | 3                     | <2             |                          |
| 15   | Anesthesia                       | 2               | 5 min            | 5                     | 2              | Not tested               |
|      | Gelatin infusion                 |                 |                  |                       |                |                          |
| 16   | Anesthesia (recovery)            | 2               | 15 min           | 300                   | <2             | Peruvian balsam          |
|      | Dressing                         |                 | 24 h             | 2                     | <2             | Negative ST and HR       |
| 17   | Penicillin injection (immediate) | 3               | 5 h              | 3                     | 18             | Not tested               |
| 18   | Tetanus vaccine (30 min after)   | 3               | 50 min           | 95                    | 16             | Not tested               |
|      |                                  |                 | 2 h              | —                     | 23             |                          |
| 19   | Venography (iodide)              | 4               | 2 h, 15 min      | 19                    | 31             | Not tested               |

Severity grade is according to the Ring and Messmer scale.<sup>6</sup>

MR = muscle relaxant administration; ST = skin tests; IgE = muscle

relaxant-directed IgE; HR = *in vitro* histamine release.

a few minutes after dressing a badly wounded hand with tulle gras Lumière®. Skin tests to Peruvian balsam elicited no reaction, and *in vitro* histamine-release was negative.

The *in vivo* plasma half-life of tryptase has been calculated in five patients (fig. 1). In three cases, the half-life was similar and was 90 min (cases 1, 3, and 13). For case 2, the apparent half-life was 5 h. For case 18, tryptase was higher 2 h after the reaction than 1 h before. The reaction was subsequent to a subcutaneous injection of tetanus vaccine.

### Discussion

This study describes 11 cases for which anaphylaxis, or at least degranulation of mast cells or basophils, was

suggested either by the increase of plasma histamine and tryptase or by the increase of one or the other associated with positive skin tests and/or positive IgE directed against muscle relaxant. Only four of these subjects (cases 1, 2, 5, and 6) presented the classical clinical triad—hypotension, bronchospasm, and flushing. In two patients, bronchospasm alone led to cardiac arrest and death or prolonged coma (cases 8 and 19). In such dramatic cases, the necessity of a reliable and early diagnosis is obvious.

The new radioimmunoassay for plasma histamine measurement is specific for histamine: histidine, N<sup>1</sup>-methyl-histamine, cimetidine, and related compounds do not interfere in the assay.<sup>7</sup> The normal values are lower and less scattered than with the fluorometric assay<sup>11</sup>

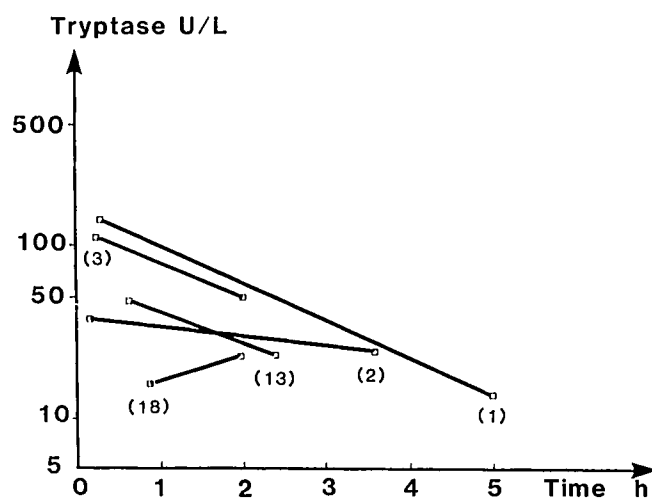


FIG. 1. Change with time of plasma tryptase after anaphylactoid reactions in five patients. The number in brackets refers to the case number in table 1.

(< 3.5 versus < 9 nM). This allows the determination of an intermediate range between normal values and pathologic values. In this range can be found cases with slight damage of cells due to improper handling of the sample and cases with minimal *in vivo* histamine release. This increases the specificity of the assay. In our study, the sample collection and the measurement of plasma histamine was much easier than expected. The blood sample could be centrifuged, and the plasma frozen and then sent for analysis. Of ten reactions with increased histamine concentrations, five occurred outside the reference hospital. When immunologic tests were possible, we found a good correlation between results of these tests and the concentration of plasma histamine, provided that the sampling was done no more than 1 h after onset of the reaction. However, two patients had persistently increased concentrations of histamine more than 2 h after the anaphylactic reaction.

Tryptase is an interesting alternative to histamine when sampling is delayed: the half-life of 90 min found here in three subjects is in good agreement with the 90–120 min previously reported.<sup>5</sup> In our study, the peak concentration was obtained sooner: 15 and 20 min, respectively, for cases 3 and 1, versus 1 h after venom challenge.<sup>5</sup> In case 2, the peak concentration was probably not obtained at the sampling time of 10 min, which explains the apparently prolonged half-life. The site of drug administration is important, as shown by the progressive increase in tryptase concentrations observed in case 18 after subcutaneous injection of tetanus vaccine. Thus, the sampling time for tryptase should be at least 15–20 min after signs of the reaction if the drug has been administered intravenously, and probably 1 h otherwise. With the tryptase plasma

half-life of 90 min, it can be calculated from cases 1, 3, and 13 that tryptase will reach a value of < 5 U/l 6–7 h after the onset of the reaction. Although in case 8 tryptase concentration was increased 24 h after the reaction, this is a very unusual feature (see case 3). Schwartz *et al.*<sup>3</sup> report that tryptase was < 5 U/l in four patients 24 h after the anaphylactic event. Thus, it is recommended that the blood sample be obtained within the first 6 h, with 1–2 h being optimal. Furthermore, plasma tryptase is very stable: high concentrations of tryptase could be found after storage at –20° C for more than 1 yr. Blood clotting, hemolysis, freeze–thaw cycles, and incubation at room temperature during 48 h have little influence on the measured values.<sup>5</sup> These reasons, and the large time interval for sampling, make tryptase a very easy-to-use biologic parameter for the diagnosis of anaphylaxis.

The presence of histamine in plasma at high concentrations does not indicate whether mast cells or basophils are involved,<sup>3</sup> and tryptase may help differentiate between basophil reaction and mast cell activation. The reaction observed after dressing (case 16) could be related to a toxic effect of Peruvian balsam on circulating basophils, which could explain the normal tryptase concentration and the negative skin tests. However, case 4 had normal tryptase concentration but IgE-induced reaction. This indicates either that the reaction was basophil-dependent or that mast cells were absent or contained no tryptase. However, the two cases did not have severe reactions, suggesting that mast cells did not react. The differentiation between immunologic and nonimmunologic reactions is more difficult: tryptase is localized in the secretory granules of all types of mast cells, together with histamine.<sup>3</sup> Thus, histamine and tryptase are released together when mast cells are activated. This is demonstrated *in vitro* during IgE-mediated reactions, or complement-dependent activation or other nonimmunologic reactions,<sup>12</sup> and *in vivo* during anaphylaxis.<sup>5</sup>

In conclusion, when anaphylaxis is suspected, it is recommended that plasma histamine be measured soon after the reaction and that a second sample, collected 1–2 h later (not more than 6 h), be assayed for tryptase. Furthermore, the patient should be evaluated 6 weeks later to elucidate the causative agent of the reaction.<sup>1</sup>

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