



Brief Communication

Postmortem diagnosis of fatal anaphylaxis during intravenous administration of therapeutic and diagnostic agents: Evaluation of clinical laboratory parameters and immunohistochemistry in three cases

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Abstract

This report documented three autopsy cases involving fatal shock during intravenous injection of therapeutic and diagnostic agents in a hospital setting. For postmortem diagnosis, clinical laboratory parameters for anaphylaxis, specificity of antibodies for allergens and mast cell numbers in tissue sections were examined. Elevated plasma tryptase levels were evident in the three adult males; two of the three victims displayed elevated IgE levels. However, immunoassay failed to detect antibodies specific to the relevant agent. Double immunostaining was performed employing anti-tryptase and anti-chymase monoclonal antibodies in order to count mast cells in lung sections. Increased numbers of mast cells were observed in anaphylactic tissues, which was particularly true for chymase-positive cells, in comparison with tissues associated with acute traumatic deaths. In addition to findings at autopsy, positive data obtained by laboratory examinations and immunohistochemical analyses indicated that fatal systemic anaphylaxis occurred during intravenous injection of clinical agents.

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1. Introduction

In conjunction with adverse medical events including negligence, acute death during intravenous administration of therapeutic and diagnostic agents is occasionally encountered in forensic autopsy. In most instances of fatality, anaphylaxis due to allergic reactions to agents is suspected rather than non-immune mechanisms including

direct toxicity, e.g., over-dose usage of anti-cancer drug. Systemic anaphylaxis occurs as a result of generalized mast cell degranulation caused by immunological reaction. Gross findings include laryngeal and epiglottic edema as well as common acute death symptoms; however, no specific findings of anaphylactic shock are apparent at autopsy [1,2]. Elevated levels of histamine and immunoglobulin E (IgE) are classical indicators in terms of confirmation of anaphylactic reaction [3]. In early 1991, Yunginger et al. [4] introduced the assay of mast cell tryptase, a major component of secretory granules, to the postmortem examination in forensic pathology. Several reports have demonstrated that tryptase level in the plasma of cadavers is an effective indicator of an ante-mortem anaphylactic event, consequent to a longer half-life in the circulation [5–7]. His-

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tamine, however, lacks stability due to degenerative release from basophils to plasma during the postmortem period [8]. Therefore, plasma tryptase level and specific-IgE to agent are valuable indicators with respect to the diagnostic profile.

Nishio et al. [9] demonstrated marked elevation of plasma chymase, another major component of mast cell granules, in victims of anaphylactic death, to date. An additional finding involved potential infiltration of mast cells in various edematous tissues, which supports the occurrence of an allergic reaction; however, the effectiveness of the patho-histological examination has not been fully established [10,11].

Recently, we encountered three cases involving adult males who suddenly died following intravenous administration of therapeutic and diagnostic agents. In the present study, clinical laboratory examination of plasma and immunoassay regarding specificity of antibodies to these agents were conducted. Moreover, immunohistochemistry to mast cells was evaluated in terms of the postmortem diagnosis of anaphylactic death.

2. Materials and methods

Plasma derived from intracardiac blood at autopsy was stored at -30°C within 2 h after separation by centrifugation. All measurements of biochemical laboratory parameters, including tryptase, IgE, histamine, albumin, aspartate 2-oxoglutarate aminotransferase (AST), creatine phosphokinase, etc., were conducted by Mitsubishi BCL (Tokyo, Japan); IgE values were determined with the radio-immunosorbent test. Four other cases, in which the cause of acute death was extrinsic trauma, served as controls. In order to detect allergen-specific IgE antibodies, a solid-phase immunoassay was developed by cross-linking to a cellulose sponge (ImmunoCAP, Pharmacia, Uppsala, Sweden) [12]. The purified agents were commercially available, except for the analgesic, Nolport. Victim plasma samples were analyzed with this assay, accompanied by measurement on the UNICAP system (Pharmacia).

The double immuno-staining procedure involving anti-tryptase and anti-chymase antibodies was utilized for detection of mast cell population [13]. Briefly, paraffin-embedded tissue blocks were cut into sections 4 mm thick. After deparaffinization and dehydration, sections were treated with 0.3% hydrogen peroxide to block endogenous peroxidase activity. Subsequently, sections were rinsed in phosphate-buffered saline. In the first staining, a mouse monoclonal antibody to chymase, CC1 (NOVO Castra,

Newcastle, UK), was incubated at a dilution of 1:100 for 1 h at room temperature, followed by introduction of the biotinylated second antibody and streptavidin-conjugated peroxidase (LSAB System, Dako Cytomation, Carpinteria, CA). After washing, enzyme activity was visualized with 0.02% 3,3'-diaminobenzidine containing 0.002% hydrogen peroxide [14]. In the second staining, sections were incubated continuously with a mouse monoclonal antibody to tryptase, AA-1 (Lab Vision, Fremont, CA), at a dilution of 1:200 for 1 h, followed by the addition of biotinylated second antibody and streptavidin-conjugated alkaline phosphatase. Staining was performed according to the Fast-blue RR salt procedure (Sigma–Aldrich, St. Louis, MO). Specimens were mounted with no counterstaining.

Results were assessed quantitatively based on the number of mast cells observed in 10 randomly selected fields of one section at 40-fold magnification under a light microscope, in which two specimens prepared from inferior lobes of the lungs were examined. In each instance, a total evaluated area was equivalent to 4.9 mm^2 . The number of mast cells represented a total of positive cells determined by quantitative morphometry. Six cases of traumatic acute death, in addition to the aforementioned four cases, served as controls. These control cases consisted of major injuries to the brain and the cervical spine characterized by a short postmortem interval within 38 h.

Values of the indicators are expressed as mean and standard deviation. A comparison of values in the two groups was conducted statistically employing the Mann–Whitney U-test due to the small sample size.

3. Results

3.1. Clinical course and autopsy findings

Full autopsy was performed on three victims who suffered from fatal shock during injection of solvent agents in a hospital setting. Table 1 summarizes clinical courses of the three cases (#1–#3) in which anaphylaxis was suspected consequent to the appearance of critical impairment during the intravenous administration. The causal agents included iodine-containing contrast agent, analgesics and antibiotics. All three subjects sustained extensive resuscitation after shock. None of the victims displayed history of episodes of allergy or anaphylaxis. Potential previous sensitization by administration of the relevant agent was confirmed in subject #1; however, the remaining subjects exhibited no apparent past history of agent usage.

Table 1
Summary of suspected anaphylaxis due to intravenous administration

Case	Age/sex	Agents	Time from shock to death (h)	Postmortem interval (h)
#1	56/M	Iodine-containing contrast agent (Ioxaglic acid)	4	18
#2	74/M	Analgesics (Nolport; extract of vaccinated rabbit skin)	3	26
#3	46/M	Antibiotics (Cefotiam hydrochloride)	1	22

Table 2

Clinical laboratory data for postmortem diagnosis

Parameters	Cut off value	Anaphylaxis (<i>n</i> = 3)	Control (<i>n</i> = 4)
Tryptase (μg/L)	<10	182, 395 and 433* (336.7 ± 135.3)	8, 12, 16 and 8 (11.0 ± 3.8)
IgE (IU/mL)	<170	59, 1100 and 430* (529.7 ± 527.6)	82, 17, 143 and 126 (92.0 ± 56.2)
Histamine (ng/mL)	<0.2	145, 23 and 146 (104.7 ± 70.7)	18, 57, 161 and 127 (90.8 ± 65.0)
Weight of the lungs (g)	–	Left: 549, 407 and 474 (477 ± 71) Right: 790, 538 and 569 (632 ± 137)	–

Real values are indicated with mean and SD in parenthesis. For anaphylaxis, the values are arranged in the order of case #1–#3.

* $P < 0.05$.

At postmortem autopsy, mild laryngeal edema and increased lung weight attributable to congestion were observed as were common findings of acute death. Weights of the lungs are presented in Table 2. Otherwise, remarkable changes in terms of gross findings were lacking in all subjects. Histological examination of major organs revealed common changes corresponding to edema and congestion, except for changes associated with chronic hepatitis were evident in subject #2.

3.2. Clinical laboratory parameters

A total of 20 clinical laboratory parameters, including tryptase, IgE and histamine, were measured in plasma obtained from the three victims and the four control subjects. Results are summarized in Table 2. Values of the three anaphylaxis-related parameters were elevated, with the exception of IgE in one case. However, histamine demonstrated a non-specific increase as did leaked enzymes such as AST.

3.3. Specific antibodies

The fixation of two ligands including cefotiam hydrochloride and ioxaglic acid was attempted. Effective fixation was achieved solely in the case of the former antibiotic. Insufficient signal, which was characterized by a value of less than 0.35 U/ml, was detected following application of the plasma of subject #3 to the immunoassay. This finding suggested the presence of small amounts of specific antibodies.

3.4. Mast cell determination

As shown in Fig. 1, double immuno-staining involving anti-tryptase and anti-chymase antibodies was conducted in lung tissues of the three victims and 10 control cases of traumatic death. Mast cells were concentrated in the vicinity of alveoli and capillaries. In a comparison of the total number of mast cells determined by quantitative morphometry, the anaphylactic cases exhibited a mean greater than that of the trauma death control group ($n = 10$). Differences were not statistically significant in terms of the tryptase-positive cells; in contrast, differences were meaningful with respect to the chymase-positive and doubly positive cells (Table 3). Additionally, no significant correla-

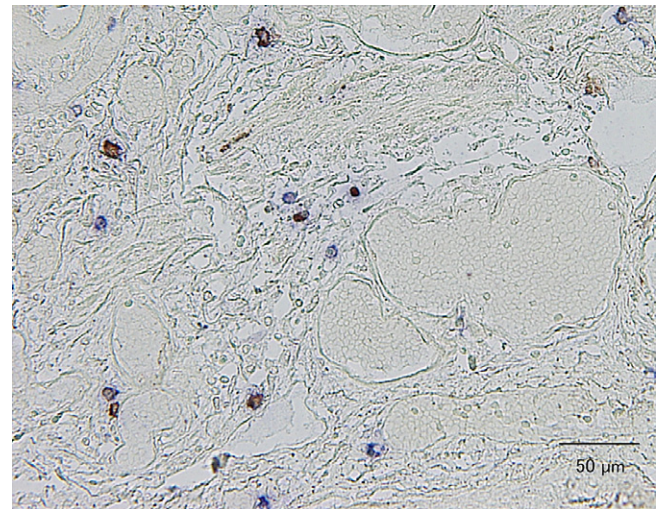


Fig. 1. Immunohistochemistry of a lung section of case #1 in microscopy (magnification; 40×). Mast cells are double-immuno-stained by anti-tryptase antibody (brown) and anti-chymase antibody (blue). A scale bar is indicated below.

tion was observed between mast cell number and plasma tryptase levels.

3.5. Other

In addition, following the accidental event experienced by subject #3, a portion of his plasma was transferred to the pharmaceutical company. Subsequently, measurement of cefotiam hydrochloride blood level by gas chromatography/mass spectrometry revealed a value of 13.0 mg/L, which was within the therapeutic range.

4. Discussion

The current study examined available laboratory parameters in order to facilitate postmortem diagnosis of suspected anaphylaxis. In comparison with other parameters such as electrolytes and leaked enzymes, including AST, tryptase and IgE appeared to be stable up to 18–26 h after death, the interval during which autopsies were conducted in the present cases. Horn et al. [7] documented a gradual albeit not significant increase in plasma tryptase in the time course following death. However, autopsy is expected within two days after a fatal adverse event, which

Table 3
Number of mast cells in the lung sections

Number of mast cells	Anaphylaxis (<i>n</i> = 3)	Trauma (<i>n</i> = 10)
Only tryptase-positive	127, 19 and 48 (64.7 ± 55.9)	15–115 (54.1 ± 28.7)
Only chymase-positive	27, 11 and 25* (21.0 ± 8.7)	0–8 (2.9 ± 2.8)
Both positive	29, 19 and 68* (38.7 ± 25.9)	8–36 (14.8 ± 8.6)

Cell numbers are indicated with mean and SD in parenthesis. A total area of 4.9 mm² was counted.

* *P* < 0.05.

indicates that plasma specimens should be viable for laboratory examination in the postmortem period. Another aspect of note is that other physical conditions of non-allergic reactions somehow affect tryptase value, e.g., amniotic fluid embolism [15], myocardial infarction [16] and hyperthermia [17]. Elevated tryptase does not merely indicate an allergic reaction, but Yunginger et al. [4] demonstrated that intravenous administration of a drug agent is associated with a high degree of tryptase increase in comparison with other types of anaphylaxis attributable to be stings and food ingestion.

Nishio et al. [9] demonstrated elevated plasma levels of chymase in anaphylactic deaths, a study in which two (#2 and #3) of the current plasma specimens were involved. A significant positive correlation was evident between tryptase and chymase. Moreover, plasma chymase is more stable than tryptase during the postmortem period; thus, chymase may prove to be another valuable indicator for the postmortem diagnosis of anaphylaxis.

Specific IgE induces anaphylaxis in the form of type 1 allergic reactions, in which binding of specific IgE to the allergen leads to explosive release of mediators by mast cells [3]. It is not clear as to whether total plasma IgE levels reflect the acute phase of allergic reactions; however, elevation of IgE is indicative of the occurrence of anaphylaxis to some degree. Assessment of IgE specificity for a suspected allergen via the allegro-sorbent test appears beneficial. Fixation was successful solely in the case of cefotiam hydrochloride; however, insufficient signal was obtained. The reason for this absence remains unclear; we thought that the specific antibodies were consumed during the anaphylactic reaction [2].

Fineschi et al. [10] observed mast cell levels 5-fold greater than normal in the lungs of an anaphylaxis victim employing fluorescein angiography. Perskvist and Edston [18] also noted significantly elevated numbers of infiltrated mast cells in the lungs and heart. The current findings were consistent with these observations; however, numbers identified using anti-tryptase were not statistically meaningful in the present study. We supposed that this situation might occur owing to the limited number of subjects. In contrast, the difference was greater and more striking in the chymase-positive cells, indicating that immuno-staining involving anti-chymase antibody may be indispensable for detection of mast cells.

In an extensive investigation employing a double immuno-staining procedure, Perskvist and Edston [18]

demonstrated that the appearance of tryptase-negative and chymase-positive mast cells was specific to those sections associated with fatal anaphylaxis. The current data supported their proposal in principle; this distinguished subset was observed with a mean (SD) of 21.0 (±8.7) in the sections. However, as shown in Table 3, despite limited numbers, this cell subtype was present in five traumatic death control cases. The three subtypes, tryptase-positive, chymase-positive and doubly positive, exhibited distinct distribution among organs; chymase displays lower expression in mast cells of the lung in comparison to those of the skin [19]. It is likely that the anaphylactic lung is characterized by a difference in terms of population subtypes, in which enhanced production of chymase should occur.

In conclusion, we examined clinical laboratory indicators and immunohistochemical results as possible markers of postmortem diagnosis of anaphylaxis. The victims exhibited a unique clinical course; furthermore, no evidence of an anatomical cause of death with nonspecific findings, e.g., pulmonary edema, existed. Relevant specific antibodies were not detected; however, due to the exclusion circumstances and the positive data, we concluded that systemic anaphylactic shock occurred in the three victims during intravenous injection of diagnostic and therapeutic agents.

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