



Expression of FcεRIα and tryptase in human lung tissue during drug-induced anaphylactic death

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Abstract

The diagnosis of drug-induced anaphylactic death is an important component of forensic medicine. The purpose of this study was to explore the expression of FcεRIα and tryptase in human lung tissue during drug-induced anaphylactic death and its value for forensic medicine. The expression of FcεRIα and tryptase in lung tissues of the drug-induced anaphylactic death group (n = 30) and control group (who died due to sudden cardiac death, falling from a height, or traffic accidents, n = 30) was detected by immunohistochemistry and immunofluorescence staining. The difference in FcεRIα and tryptase expression in the lung tissue between the drug-induced anaphylactic death group and the control group was statistically significant (p < 0.05). The levels of FcεRIα and tryptase expression greatly increased in the lung tissue of the drug-induced anaphylactic death group, which may provide morphological evidence and a reference for the diagnosis of drug-induced anaphylactic death in forensic medicine.

Keywords Forensic pathology · Drug-induced anaphylactic death · Lung · FcεRIα · Tryptase

Introduction

Drug-induced anaphylactic death is a typical immediate hypersensitivity reaction. Death caused by anaphylactic shock can easily lead to a series of legal issues, such as medical disputes. The diagnosis of drug-induced anaphylactic

death is also an important component of forensic medicine [1]. IgE antibodies play a vital role in type I allergic reactions. The binding of IgE with the high-affinity IgE receptor (FcεRI) on the surface of target cells can trigger a series of signal transductions in cells and then induce mast cell degranulation and the release of inflammatory mediators, which are important to the pathogenesis of the type I allergic reactions [2, 3]. Increased total serum IgE levels in the body suggest that the individual has a hereditary allergic constitution or type I hypersensitivity reactions [4]. The expression of FcεRI on the surface of mast cells and basophils also increased in individuals with elevated circulating IgE in the serum, and the binding of IgE to the receptor resulted in the accumulation of FcεRI on the surface of these cells [5, 6].

FcεRI is a heterotetrameric structure composed of three subunits, one α subunit, one β subunit and two identical γ subunits (αβγ₂). The extracellular domain of the α chain contains a complete IgE-binding domain; the β chain is a transmembrane molecule that crosses the membrane four times and participates in the initiation of signal transduction; and the disulfide bond connects two identical γ chains, which are subunits that transmit signals, and one of its identified functions is to promote the expression of FcεRIα on the cell surface [7, 8]. Compared with the β and γ subunits, the α subunit is an indispensable component of the FcεRI

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tetramer and is a key site of IgE binding to FcεRI, and its important role has been demonstrated in animal experiments [9]. Changes in the FcεRI α subunit in tissues of individuals with drug-induced anaphylactic death are not clear and have not been reported. Therefore, the study of the expression of the FcεRI α subunit in tissues during drug-induced anaphylactic death will provide important information for both the clinical treatment and postmortem diagnosis of drug-induced anaphylactic death in forensic medicine.

Tryptase is a serine protease synthesized and released mainly by mast cells. It can be used in biopsy as a marker for detecting the number of mast cells (α-tryptase) and the degree of mast cell activation (β-tryptase). The sign of mast cell activation is the release of β-tryptase [10]. Due to the high stability of tryptase, the degradation of tryptase in the early stage of anaphylactic death is not significant. Therefore, the detection of tryptase for the diagnosis of anaphylactic death has been recognized by experts and scholars [11]. In this study, tryptase was used as a marker protein of mast cells and as a reference index for detecting FcεRIα.

In this study, immunohistochemistry and immunofluorescence were used to analyze the expression of FcεRIα and tryptase in lung tissues of the drug-induced anaphylactic death group (n=30) and control group (who died due to sudden cardiac death, falling from a height, or traffic accidents, n=30), and the significance of FcεRIα and tryptase in the forensic diagnosis of drug-induced anaphylactic death was explored.

Materials and methods

Samples

Sixty anatomical cases of death from 2007 to 2016 at the CMU Center of Forensic Investigation were collected (elapsed time after death < 48 h or bodies frozen within 6 h after death). Patients were grouped according to the cause of death: the drug-induced anaphylactic death group (n=30) or the control group (who died due to sudden cardiac death, falling from a height, or traffic accidents, n=30). The causes of death in the selected patients were diagnosed based on systematic and comprehensive forensic anatomy, histopathological examination, toxicological examination and biochemical examination; cases of other systemic diseases or injuries unrelated to the cause of death were excluded. The diagnosis of drug-induced anaphylactic death in this study was mainly based on a history of drug exposure. There were clear clinical records of intravenous drugs. Autopsies that showed throat, epiglottis, pulmonary edema and congestion, eosinophil infiltration, and other causes of death (such as poisoning, mechanical asphyxia, etc.) were strictly excluded. By consulting the original case records, qualifying cases were individually screened according to the above

criteria. This study was reviewed and approved by the Ethics Committee of China Medical University.

By carefully checking the archival identification data, qualifying cases were strictly screened, and the corresponding numbered wax blocks were obtained for sectioning. The details of drug-induced anaphylactic death cases are shown in Table 1. All the selected cases were exposed to intravenous drip, and there was a short-term rescue process after allergic reaction. Single drugs were used for rescue, and only dexamethasone or adrenaline was injected intramuscularly. Among the drug-induced anaphylactic death group, 16 patients were male (53.3%), and 14 patients were female (46.7%). The minimum age was 4 years old, and the maximum age was 78 years old.

Reagents and instruments

A mouse anti-FcεRIα monoclonal antibody (MA1-499, Thermo Fisher, USA), mouse anti-Mast Cell tryptase monoclonal antibody (ab2378, Abcam, UK), rabbit anti-FcεRIα polyclonal antibody (A1751, Beijing ABclonal, China), goat anti-mouse IgG antibody (ZB-2305), SP kit (9002) and DAB kit (ZLI9032) (Beijing Zhongshan Jinqiao, China), Alexa Fluor®488 donkey anti-rabbit IgG and Alexa Fluor®594 donkey anti-mouse IgG antibodies (A21206 and A21203, Life, USA), PBS and citrate buffer (Wuhan Boshide, China), optical microscope (Olympus, Japan) and inverted fluorescence microscopy (Leica, Germany) were used.

Methods

Immunohistochemical staining

Paraffin-embedded blocks with lung tissues from the drug-induced anaphylactic death group and the control group were selected. Continuous Sects. (4-μm thick) were deparaffinized with xylene and rehydrated with gradient ethanol, followed by antigen retrieval in a microwave in 10 mM citrate buffer. The slices were incubated in 3% hydrogen peroxide for 30 min to block the activity of endogenous peroxidase, and nonspecific binding was blocked with goat serum for 2 h. Sections from the experimental group were incubated overnight at 4 °C with a mouse anti-FcεRIα monoclonal antibody (1:800) and mouse anti-tryptase monoclonal antibody (1:6400). The next day, the sections were incubated with a goat anti-mouse secondary antibody for 30 min and then incubated with horseradish enzyme-labeled streptavidin working solution for 30 min. DAB staining, hematoxylin staining of the nuclei, neutral balsam sealing and optical microscopy were used to observe and obtain images. PBS

Table 1 General conditions of drug-induced anaphylactic death

Number	Age (year)	Gender	Exposure to drugs	Time from medication to allergy (min)	Survival time after allergy (min)	Storage Conditions of corpse	Postmortem autopsy time (day)
1	60	male	Ceftriaxone sodium	1 min	5 min	frozen	6d
2	63	female	Safflower injection	3 min	10 min	frozen	5d
3	30	female	Azithromycin	7 min	20 min	frozen	4d
4	5	male	Ambroxol Hydrochloride for Injection	5 min	40 min	frozen	7d
5	36	male	Sulbactam and Cefoperazone	10 min	30 min	frozen	6d
6	26	female	Midazolam Injection	15 min	40 min	refrigeration	1d
7	62	female	Ceftriaxone sodium	3 min	30 min	frozen	5d
8	43	male	Sulbactam and Cefoperazone	10 min	1 h	frozen	5d
9	54	male	Sulbactam and Cefoperazone	1 min	1 h	frozen	5d
10	49	female	Ceftriaxone sodium	5 min	30 min	frozen	3d
11	49	male	Sulbactam and Cefoperazone	2 min	1 h	refrigeration	1d
12	50	male	Sanqi Panax Notoginseng for Injection	6 min	40 min	refrigeration	2d
13	31	male	Sulbactam and Cefoperazone	5 min	20 min	frozen	4d
14	65	female	Sulbactam and Cefoperazone	5 min	7 min	frozen	7d
15	50	male	Safflower injection	15 min	40 min	frozen	3d
16	72	female	Sulbactam and Cefoperazone	5 min	16 min	frozen	4d
17	45	female	Sulbactam and Cefoperazone	30 min	20 min	refrigeration	2d
18	42	male	Sulbactam and Cefoperazone	3 min	20 min	refrigeration	1d
19	44	male	Ceftriaxone sodium	10 min	40 min	frozen	4d
20	4	male	Cefuroxime	10 s	20 min	frozen	2d
21	78	female	Sulbactam and Cefoperazone	1 min	30 min	frozen	3d
22	45	female	Ambroxol	3 min	30 min	frozen	5d
23	52	female	Ceftriaxone sodium	a few seconds	20 min	frozen	4d
24	51	male	Ceftriaxone sodium	1 min	10 min	frozen	5d
25	43	male	Ceftriaxone sodium	1 min	20 min	frozen	5d
26	16	female	Ceftriaxone sodium	1 min	40 min	frozen	4d
27	23	female	Ambroxol Hydrochloride for Injection	5 min	30 min	frozen	3d
28	44	female	Sulbactam and Cefoperazone	1 min	40 min	frozen	6d
29	69	male	Ceftazidime Pentahydrate	10 min	30 min	frozen	4d
30	56	male	Ceftazidime Pentahydrate	3 min	20 min	frozen	3d

was used instead of a primary antibody as a negative control in the experiment.

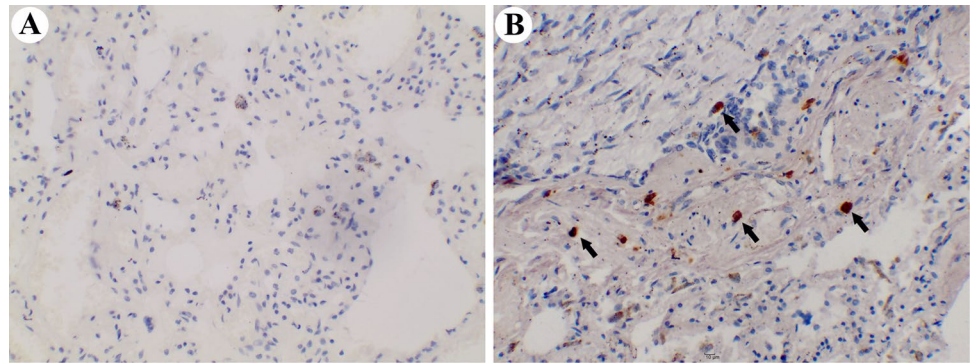
Judgment criteria for the results: All sections were measured by one blinded observer. The staining of each section was observed under a microscope at $\times 200$ magnification and $\times 400$ magnification, and microscopic image analysis technology (Motic Images Advanced 3.2) was used to examine the expression of Fc ϵ RI α and tryptase. The appearance of brownish-yellow or brownish-red particles in the cell membrane was considered positive staining for Fc ϵ RI α ; brown particles in the cytoplasm or membrane were considered positive staining for tryptase. The lung tissue of each group was examined in 5 random visual fields under a microscope at $\times 200$ magnification, and the number of Fc ϵ RI α - and tryptase-positive cells in each visual field was calculated by Olympus Stream software. All data are expressed as the

mean $\pm$ standard deviation (mean $\pm$ SD). $p < 0.05$ was considered statistically significant.

Double immunofluorescence staining

Double immunofluorescence staining for Fc ϵ RI α and the mast cell marker tryptase was performed on paraffin lung specimens. The Sects. (4- μ m thick) were dewaxed and rinsed in water, antigen was retrieved with citrate buffer, and blocking was performed with BSA. Sections were then exposed to a rabbit anti-Fc ϵ RI α (1:200 dilution) and mouse anti-tryptase (1:800 dilution) antibody overnight. Alexa Fluor® 488 donkey anti-rabbit IgG and Alexa Fluor® 594 donkey anti-mouse IgG (1:200 dilution) antibodies were used as secondary antibodies. Nuclei were counterstained with DAPI,

Fig. 1 Tryptase IHC of human lung tissue ($\times 200$). In the control group, positive mast cells (black arrow) were occasionally observed in the lung interstitium with clear cell boundaries **A**. In the drug-induced anaphylactic death group, there were more positive mast cells (black arrow) stained with tryptase **B** in the lung tissue



and the results were observed under a fluorescence microscope and imaged.

Judgment criteria for the results: Blue fluorescence indicated the nucleus, green fluorescence indicated positive immunofluorescence staining for tryptase, red fluorescence indicated positive immunofluorescence staining for FcεRIα, and yellow fluorescence indicated concurrent immunofluorescence staining of tryptase and FcεRIα.

Statistical analysis

All data are expressed as the mean $\pm$ SD, and statistical analysis was performed using GraphPad Prism 6.0 (GraphPad software, La Jolla, CA, USA). $p < 0.05$ was considered statistically significant.

Results

Immunohistochemical staining of tryptase

A large number of tryptase-positive mast cells were found in the lung tissues of the drug-induced anaphylactic death group, which were mainly located around the bronchioles, bronchi and blood vessels in the lung tissues, and there were occasionally positively staining cells in the lung tissues of

the control group (Fig. 1). There were significant differences between the drug-induced anaphylactic death group (6.40 ± 0.93) and the control group (3.00 ± 0.71) ($p < 0.05$).

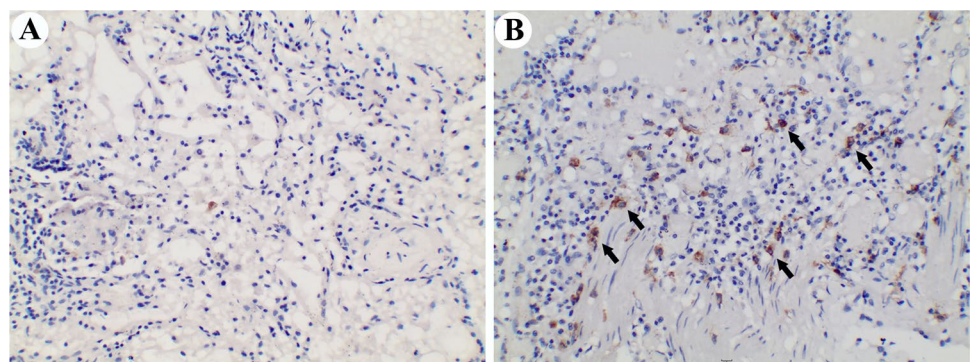
Immunohistochemical staining of FcεRIα

A large number of FcεRIα-positive cells were seen in the lung tissue of the drug-induced anaphylactic death group, and there were a large number of brown particles on the cell membrane. Cells with positive staining were mainly located near the bronchiole and small blood vessels. In the control group, cells with positive staining were occasionally found in the lung tissue (Fig. 2). Compared with the control group (1.20 ± 0.37), the drug-induced anaphylactic death group (12.00 ± 1.34) was significantly different ($p < 0.05$).

Double immunofluorescence staining of FcεRIα and tryptase

Tryptase is the most abundant in the cytoplasm of mast cells and is highly selective, so it is used as a marker protein for identifying mast cells. In the results, blue fluorescence indicated nuclei, green fluorescence indicated positive tryptase staining, red fluorescence indicated positive FcεRIα

Fig. 2 FcεRIα IHC of human lung tissue ($\times 200$). The lung tissue of the control group occasionally had positively stained cells (black arrow) **A**; the lung tissue of the drug-induced anaphylactic death group showed a large number of FcεRIα-positive cells (black arrow) with brown particles on the cell membrane **B**



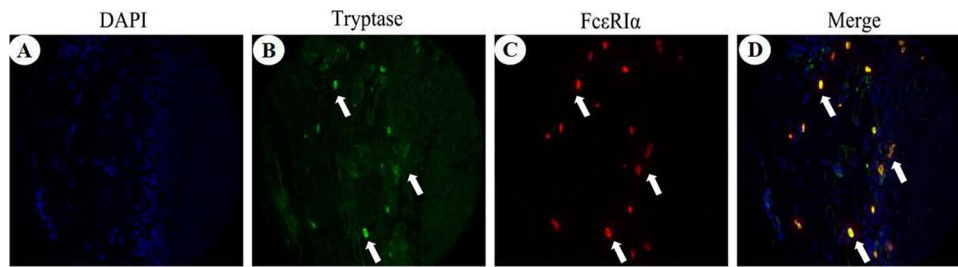


Fig. 3 Double immunofluorescence staining of FcεRIα and tryptase in lung tissues during drug-induced anaphylactic death (×200). Blue color: DAPI staining of the nucleus **A**, green color: positive staining for tryptase (white arrows) **B**, red color: positive staining for

FcεRIα (white arrows) **C**, and yellow color: costaining of tryptase and FcεRIα (white arrows) **D**, indicating that cells staining positive for FcεRIα are mast cells

staining, yellow fluorescence indicated tryptase and FcεRIα costaining (Fig. 3).

Discussion

Drug-induced anaphylactic death is a potential, fatal, and cumulative multisystemic adverse reaction that is unpredictable; it is usually not related to the dosage of medication and often occurs suddenly after exposure to the pathogenic drug. Drug-induced anaphylactic death is more common in forensic identification, but its diagnostic method is a major problem in forensic pathology. At present, due to the lack of specific morphological findings, the forensic identification of deaths caused by anaphylactic shock must rely entirely on exclusion or indirect evidence. Although the cause of death can be confirmed by examining the medical history and clinical symptoms, as well as the results of autopsy and pathology, in many cases, due to the lack of a history of exposure to allergens or clinical conditions, some biochemical indicators used as auxiliary examinations are limited by specificity and stability. It is difficult to make a forensic diagnosis of drug-induced anaphylactic death to a certain extent [12]. In recent years, scholars and experts have studied the contents of serum IgE, histamine, mast cell tryptase and other substances in drug-induced anaphylactic death as well as the immunohistochemical expression in various tissues to provide specific and objective morphological evidence for the forensic identification of drug-induced anaphylactic death [13–16]. This study has performed some basic research on this basis.

IgE and FcεRI are the most important proteins in allergic reactions, and their expression levels are directly related to the occurrence of allergic diseases. The accumulation of FcεRI on the surface of effector cells is key to activating biological signals in target cells. The FcεRIα subunit is the key site that triggers allergic reactions, and the increased expression of the α subunit on the surface

of the target cell membrane will promote the occurrence of IgE-mediated allergic reactions [17]. It is not clear how FcεRIα is expressed and whether it is abnormally expressed in anaphylactic shock death.

Immunohistochemical staining of FcεRIα and tryptase was carried out in this study, and the expression of FcεRIα increased in the lung tissue of the drug-induced anaphylactic death group and was mainly distributed around the bronchial mucosa, alveolar septum, and small blood vessels in the lung tissue. The staining results of tryptase were consistent with this finding. A double immunofluorescence staining experiment confirmed that all FcεRIα-positive cells observed in lung tissue were mast cells. These findings support that the high expression of FcεRIα plays a very important role in drug-induced anaphylactic death.

In conclusion, although the detection of tryptase elevation after death is an important index to support the diagnosis of drug-induced anaphylactic death, in addition to drug allergic reactions, tryptase expression can also increase in other diseases and environmental conditions, such as myocardial infarction, sudden infant death syndrome (SIDS), heroin-related death, fatal traumas, etc. [18–20]. Moreover, under postmortem autolysis conditions, tryptase, as a neutral protein particle of the mast cell cytoplasm, is released in large amounts into the interstitial space with the autolysis of cells, which leads to the increased expression of tryptase in nonallergic tissues. Thus, it cannot be used as the sole criterion for diagnosis. Comparatively, as a membrane protein of mast cells, FcεRIα is less affected by autolysis after death, and the immunohistochemical detection of FcεRIα can be used as an objective morphological reference index for the forensic diagnosis of drug-induced anaphylactic death, but caution must be used. Therefore, it is suggested that for individuals with suspected drug-induced anaphylactic death, the corpse should be preserved in an appropriate way or autopsied as soon as possible.

Key points

1. FcεRIα is a high affinity receptor for IgE that is present in the membrane of mast cells.
2. As a key receptor for IgE, FcεRIα participates in the process of anaphylaxis and may play a positive regulatory role in the pathogenesis of anaphylactic shock.
3. Immunostaining of lung tissue showed staining for tryptase corresponded with staining for FcεRIα.
4. More immunostaining for FcεRI was observed in lung tissue following death from drug-related anaphylaxis than death from other causes.
5. The membrane protein FcεRI would be expected to be more stable than tryptase in the post mortem period with its immunohistochemical detection providing a useful tool for autopsy diagnosis of anaphylaxis.

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Declarations

Ethics approval Samples were obtained and analyzed after approval from the Ethics Committee of China Medical University (no. [2018] 065).

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