



Postmortem detection of antibiotic-specific immunoglobulin E in the case of anaphylactic death



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ABSTRACT

An allergic anaphylactic reaction is a potentially fatal cascade consisting of an initial sensitization, antigen exposure, crosslinking of immunoglobulin E (IgE) specific for the antigen, activation of mast cells, and release of bioactive substances. Postmortem diagnosis of anaphylaxis is challenging because of the limited availability of antemortem history and minimal macroscopic evidence at autopsy. The elevated activity of a neutral proteinase, such as tryptase, can be a surrogate marker for the activation and degranulation of mast cells. However, it does not directly indicate the involvement of antigen-specific IgE, which has an important role in IgE-mediated allergic anaphylaxis.

In the present study, we examined blood from a case of death following infusion of the antibiotic ceftriaxone (CTRX), with a control case for comparison. The aim was to detect IgE specific for CTRX. A pull-down assay with *N*-hydroxysuccinimide-activated sepharose identified IgE specific for CTRX only in the serum obtained from the case of CTRX exposure, and not in the control case. The specificity of IgE was confirmed by adsorption to an excess of CTRX, which resulted in the signal for IgE disappearing in the pull-down assay. This antigen-specific IgE is a key molecule in the IgE-mediated allergic anaphylaxis and seldom investigated in postmortem examinations. Its detection can provide support for the postmortem diagnosis of allergic anaphylaxis, especially when combined with an antemortem history of allergen exposure and elevated neutral proteinase levels in serum.

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1. Introduction

Ceftriaxone (CTRX) is a third-generation cephalosporin and a broad-spectrum antibiotic. Allergic anaphylaxis is a rare adverse event triggered by CTRX [1], but several deaths related to this antibiotic have been reported [2–4].

The antigen-specific immunoglobulin E (IgE) is a key molecule in IgE-mediated allergic anaphylaxis. As the antigen crosslinks with antigen-specific IgEs bound to Fcε receptors on the surfaces of mast cells, activated mastocytes release preformed (e.g. histamine and tryptase) and newly generated (e.g. prostaglandin D2 and leukotriene C4) mediators that shape the host response of immediate hypersensitivity reactions.

Postmortem diagnosis of anaphylaxis is challenging because of the limited availability of antemortem history and minimal

macroscopic findings at autopsy. Several investigators have reported diagnostic clues for detecting local mastocytosis and eosinophilia in the spleen and upper airways [5,6]. Diagnostic postmortem biochemistry values, such as the levels of tryptase and chymase in blood, have also been reported [7–10]. Although the elevated activities of these neutral proteinases in blood can be used as surrogate markers for the activation and degranulation of mast cells, they do not directly indicate the involvement of antigen-specific IgE, which is seldom examined in the medico-legal autopsy setting. In this study, we adapted a pull-down assay [11,12] to detect IgE proteins specific for a low molecular weight antibiotic.

2. Materials and methods

2.1. Case history

2.1.1. Case 1 (CTRX-exposure case)

A Japanese man in his 60s suffered from a bacterial infection of undiagnosed origin and was given a drip infusion of CTRX. The decedent had previously been exposed several times to unknown antibiotics without any allergic reactions. Within minutes of the

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start of infusion, he collapsed and was transferred to an emergency hospital. His death was confirmed after 80 min of attempted resuscitation.

An autopsy was performed at 24 h postmortem. Macroscopic examination showed no remarkable injuries or obvious disease. Glottis edema was not mentioned in the autopsy records. Histopathological examinations with Giemsa staining revealed an increased number of eosinophils in the spleen. Immunohistochemistry with anti-mast cell tryptase antibody (Leica Biosystems, Nussloch, Germany) revealed mast cells with a starry-sky pattern in the lung and other organs. Tryptase and total IgE in the femoral vein blood were quantified at 284.0 ng/mL and 113 IU/mL, respectively (LSI Medience Corporation, Tokyo, Japan). Toxicological examinations were conducted for ethanol, drugs of abuse and prescription medications using head-space gas chromatography and liquid chromatography with tandem mass spectrometry. These analyses gave negative results except for lidocaine, which was administered during tracheal intubation. The blood concentration of CTRX was below the detection limit. The cause of death was assumed to be anaphylaxis induced by CTRX.

2.1.2. Case 2 (control case)

For a control, a blood sample was obtained from a victim with no prior history of allergic reactions. The total IgE level in the blood measured by a fluorescent enzyme immunoassay was at 17 IU/mL (LSI Medience Corporation). There was no biochemical evidence of mast cell activation, as the tryptase level in the blood was only 2.7 ng/mL. In addition, histological and immunohistochemical examinations revealed a small number of mast cells without degranulation.

2.2. Reagents

N-Hydroxysuccinimide (NHS)-activated sepharose (GE Healthcare Japan, Tokyo, Japan), a CTRX disodium salt hemiheptahydrate standard (Wako Pure Chemical Industries, Osaka, Japan), and peroxidase-labeled goat anti-human IgE(ϵ) (KPL, Gaithersburg, MD) were purchased from commercial sources.

2.3. Coupling reaction

To detect the IgE specific for CTRX, this low molecular weight antibiotic must be bound to a solid phase, otherwise CTRX will be removed from the column during the washing procedure and no signal from the IgE will be detected. In this study, we bound the CTRX to NHS-activated sepharose beads via amine coupling (Fig. 1).

The coupling reaction between NHS-activated sepharose and CTRX was performed following the manufacturer's instructions [13]. Briefly, CTRX was diluted to a concentration of 2 mg/mL in the coupling buffer (0.2 mol/L sodium hydrogen carbonate and 0.5 mol/L sodium chloride). After washing with 1 mmol/L hydrochloric acid (HCl), NHS-activated sepharose was gently mixed with diluted CTRX for 16 h at 4 °C. Excess active groups on the sepharose beads were quenched with 2-aminoethanol. After washing four times with the washing buffer (0.1 mol/L sodium acetate and 0.5 mol/L sodium chloride, pH adjusted to 4.0 using HCl), the beads were equilibrated using phosphate-buffered saline.

2.4. Pull-down assay

Blood samples were obtained at autopsy for cases 1 and 2. This study was approved by the Institutional Review Board at Kobe University Graduate School of Medicine.

Serum was stored at –80 °C until analysis, and then incubated with CTRX-conjugated sepharose beads at 4 °C. After washing the beads with phosphate-buffered saline, substances bound to the beads via CTRX were eluted with elution buffer (0.1 mol/L glycine, pH adjusted to 3.0 with HCl). The supernatant was mixed with Laemmli buffer, and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blotting (WB).

2.5. CTRX adsorption assay

To confirm the specificity of the detected IgE for CTRX, the adsorption of IgE by excess CTRX was investigated as follows.

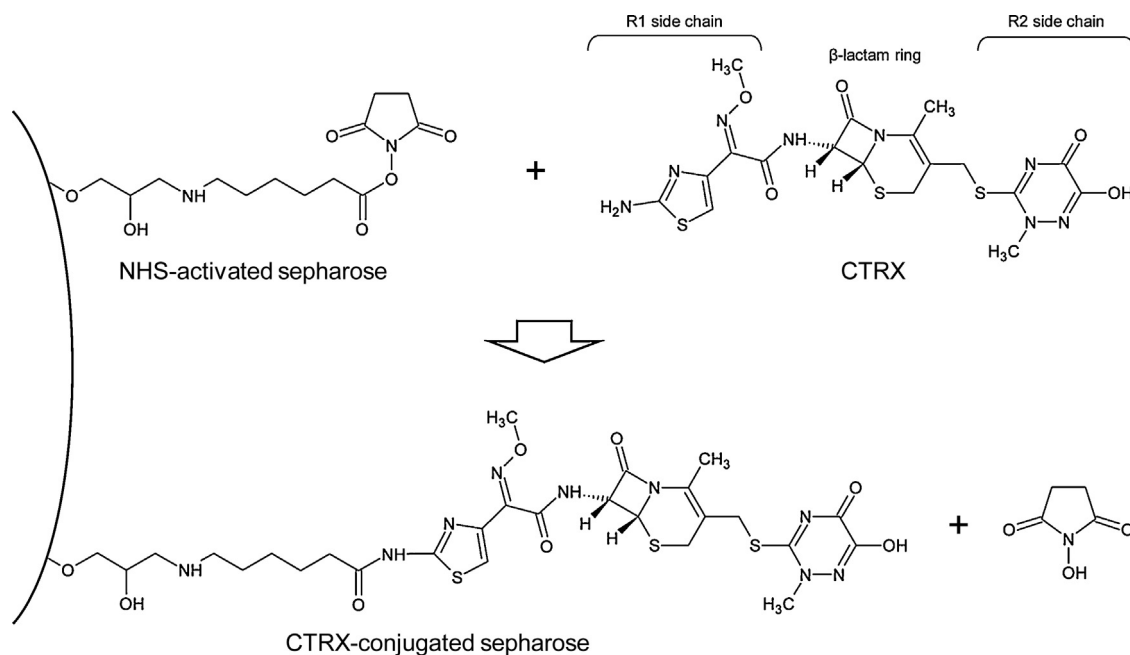


Fig. 1. Schematic showing the coupling reaction of ceftriaxone (CTRX) to a solid phase. CTRX is bound to *N*-hydroxysuccinimide (NHS)-activated sepharose beads by amine coupling. CTRX has three important structural components, including a β -lactam ring and R1 and R2 side chains. In this case, the R2 side chain of CTRX was preserved throughout the reaction.

Serum was pre-treated with 2 mg/mL CTRX for 7 h at 4 °C, and then subjected to the pull-down assay described above.

2.6. Total IgE detection by WB

For the detection of non-specific total IgE in blood, serum was mixed with Laemmli buffer and subjected to SDS-PAGE followed by WB.

2.7. SDS-PAGE and WB

The samples were analyzed by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Internal peroxidases were consumed during incubation with 3% hydrogen peroxide for 15 min. The membranes were blocked in 5% nonfat dry milk, and then incubated with peroxidase-labeled goat anti-human IgE antibody. After incubation with Chemi-Lumi One Super (Nacalai Tesque, Kyoto, Japan), signals were detected using a LAS-3000 imager (Fuji Film, Tokyo, Japan).

3. Results

IgE specific for CTRX was detected in the allergic anaphylactic death case associated with CTRX exposure but not in the control case (Fig. 2). To confirm the CTRX-specificity of the IgE in the serum, we submitted it to the adsorption assay (Section 2.5) and found that the IgE was adsorbed during pre-treatment with CTRX so that no signal for CTRX-specific IgE was detected in the subsequent pull-down assay. The signal intensities for total IgE in the WB were consistent with the blood concentrations measured by a fluorescent enzyme immunoassay (Sections 2.1.1 and 2.1.2).

4. Discussion

For postmortem diagnosis of anaphylaxis, recent reports have shown that postmortem biochemical analyses, such as the finding of elevated tryptase level in blood, are useful [7–9]. However, postmortem serum tryptase levels can also be elevated in cases of death not related to anaphylaxis [14–16], and in cases with long postmortem intervals [17,18]. For these reasons, an upper threshold of 45 ng/mL has been proposed for postmortem tryptase [19], which is higher than accepted antemortem clinical criteria. Moreover, elevation of the serum tryptase level indicates only that activation of mast cells has occurred but does not confirm the involvement of antigen-specific IgE, which has an important role in IgE-mediated allergic anaphylaxis.

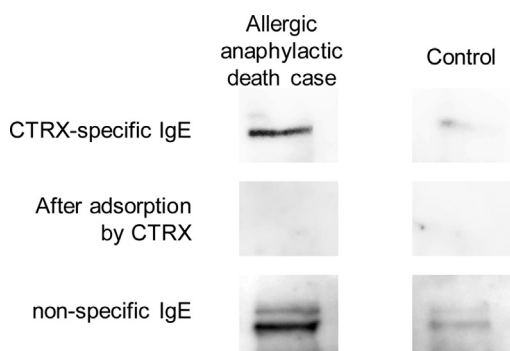


Fig. 2. Results of the pull-down assay showing detection of ceftriaxone (CTRX)-specific immunoglobulin E (IgE). CTRX-specific IgE was detected in a case of death following allergic anaphylaxis after exposure to CTRX. CTRX-specific IgE was not detected in the control case (top row). The IgE antibody, which bound to the CTRX-conjugated sepharose in the assay, was not detected after pre-treatment of the serum with CTRX (middle row). Total IgE was detected in both cases (bottom row).

Serum IgE measurement is useful in the clinical diagnosis of allergic reactions. It has been noted that high levels of IgE are only indicative of an individual's degree of sensitization and not of the likelihood of acute allergic anaphylaxis as a cause of death [7,8]. However, the existence of antigen-specific IgE suggests that the patient has had an allergic response to an antigen, and detection of IgE may be useful for supporting a diagnosis of IgE-mediated allergic anaphylaxis [17]. In Case 1, the history of antemortem exposure to CTRX, detection of CTRX-specific IgE, and mast cell activation demonstrated by elevated serum tryptase levels and immunohistochemistry with anti-tryptase antibody were all indicative of an IgE-mediated allergic anaphylactic cascade. Therefore, the cause of death was diagnosed as an allergic anaphylactic reaction to CTRX.

Some studies have reported the detection of IgEs specific for high molecular weight antigens, such as honey bee venom, food, and a molecular-targeted drug, and used these results to support diagnoses of allergic anaphylaxis [8,20]. Today, the levels of IgEs specific for several antigens can be measured in clinical practice. A panel approach for the detection of IgEs specific to an array of antigens is also available, but the antigens in the array are limited and do not represent the full range of allergens. Another technical limitation is the molecular weight of the target antigen. As mentioned above, low molecular weight substances such as CTRX (molecular weight of 661.60) need to be chemically bound to the solid phase so they are not removed from the column during washing. Because of these difficulties, antigen-specific IgEs are usually not examined in postmortem investigations.

Cephalosporin has three important structural components, including a β -lactam ring and R1 and R2 side chains (Fig. 1). Each of these components can act as an allergen [2]. Although some of these structural components are common between CTRX and other cephalosporin antibiotics (e.g. cefotaxime and cefepime), CTRX has a unique R2 side chain. Therefore the IgE specific to this side chain would not be expected to cross-react with other antibiotics [21–23].

In Case 1, the patient had not had previous allergic episodes with other antibiotics but collapsed immediately after CTRX infusion. Therefore we suspected a hypersensitivity reaction to the unique R2 side chain of CTRX. To maintain the structure of the R2 side chain of CTRX during the coupling reaction, we used NHS-activated sepharose and bound the R1 side chain to the solid phase sepharose beads by amine coupling.

When no signal is detected in a WB assay, there are several possible reasons. These include impairment of the detection system because of transfer efficiency or an inactivated enzyme, and a time-dependent decrease of the IgE levels in blood [24]. Deaths from IgE-independent nonallergic reactions [3] and from other causes, such as sudden cardiac death, would also give a negative result with this method.

Another factor is the structural modification of an allergen through chemical coupling. Although our method using NHS-activated sepharose could detect IgE specific for the R2 side chain of CTRX, it should not be applied in cases where the R1 side chain-specific IgE is of interest. In the present study, the amine coupling process resulted in masking of the end of the R1 component and R1-specific IgE would not react with the CTRX-conjugated beads. For the detection of IgE specific to the R1 side chain of CTRX, other coupling methods will be necessary. Epoxy-activated sepharose, which has different active groups, has been applied in previous studies to detect IgEs specific for cephalosporins [25] and quinolones [26]. An appropriate choice of solid phase and coupling method will allow the detection of a variety of allergen-specific IgEs and identification of the offending allergen, including those of low molecular weight.

5. Conclusion

We detected antibiotic-specific IgE, which is a key molecule in IgE-mediated allergic anaphylaxis. Although the diagnosis of death by anaphylaxis is usually based on a clinical history of allergen exposure and the observation of mast cell activation in postmortem analysis, detection of the antigen-specific IgE provides evidence in support of this diagnosis.

Conflict of interest

The authors have no conflict of interest to declare.

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References

- [1] R.Y. Lin, A perspective on penicillin allergy, *Arch. Intern. Med.* 152 (1992) 930–937.
- [2] P.S. Kelkar, J.T. Li, Cephalosporin allergy, *N. Engl. J. Med.* 345 (2001) 804–809.
- [3] M.R. Ernst, P.J. van Dijken, P.J. Kabel, J.M. Draaisma, Anaphylaxis after first exposure to ceftriaxone, *Acta Paediatr.* 91 (2002) 255–256.
- [4] I. Riezzo, S. Bello, M. Neri, E. Turillazzi, V. Fineschi, Ceftriaxone intradermal test-related fatal anaphylactic shock: a medico-legal nightmare, *Allergy* 65 (2010) 130–131, <http://dx.doi.org/10.1111/j.1398-9995.2009.02088.x>.
- [5] E. Edston, Accumulation of eosinophils, mast cells, and basophils in the spleen in anaphylactic deaths, *Forensic Sci. Med. Pathol.* 9 (2013) 496–500, <http://dx.doi.org/10.1007/s12024-013-9468-9>.
- [6] N. Trani, L.R. Bonetti, G. Gualandri, G. Barbolini, Immediate anaphylactic death following antibiotics injection: splenic eosinophilia easily revealed by pagoda red stain, *Forensic Sci. Int.* 181 (2008) 21–25, <http://dx.doi.org/10.1016/j.forsciint.2008.08.011>.
- [7] C. Palmiere, P. Mangin, Postmortem chemistry update part 2, *Int. J. Leg. Med.* 126 (2012) 199–215.
- [8] J.W. Yunginger, D.R. Nelson, D.L. Squillace, R.T. Jones, K.E. Holley, B.A. Hyma, et al., Laboratory investigation of deaths due to anaphylaxis, *J. Forensic Sci.* 36 (1991) 857–865, <http://dx.doi.org/10.1007/s00414-011-0614-1>.
- [9] L.B. Schwartz, D.D. Metcalfe, J.S. Miller, H. Earl, T. Sullivan, Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis, *N. Engl. J. Med.* 316 (1987) 1622–1626.
- [10] H. Nishio, S. Takai, M. Miyazaki, H. Horiuchi, M. Osawa, K. Uemura, et al., Usefulness of serum mast cell-specific chymase levels for postmortem diagnosis of anaphylaxis, *Int. J. Leg. Med.* 119 (2005) 331–334.
- [11] M. Takahashi, Y. Rikitake, Y. Nagamatsu, T. Hara, W. Ikeda, K. Hirata, et al., Sequential activation of Rap1 and Rac1 small G proteins by PDGF locally at leading edges of NIH3T3 cells, *Genes Cells* 13 (2008) 549–569, <http://dx.doi.org/10.1111/j.1365-2443.2008.01187.x>.
- [12] H. Tawa, Y. Rikitake, M. Takahashi, H. Amano, M. Miyata, S. Satomi-Kobayashi, et al., Role of afadin in vascular endothelial growth factor- and sphingosine 1-phosphate-induced angiogenesis, *Circ. Res.* 106 (2010) 1731–1742, <http://dx.doi.org/10.1161/CIRCRESAHA.110.216747>.
- [13] GE Healthcare Life Sciences, NHS-Activated Sepharose 4 Fast Flow, 2011 https://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1314823637792/litdoc71500014AD_20110831230521.pdf (retrieved 21.08.15).
- [14] B. Randall, J. Butts, J.F. Halsey, Elevated postmortem tryptase in the absence of anaphylaxis, *J. Forensic Sci.* 40 (1995) 208–211.
- [15] E. Edson, M. van Hage-Hamsten, beta-Tryptase measurements post-mortem in anaphylactic deaths and in controls, *Forensic Sci. Int.* 93 (1998) 135–142.
- [16] H. Nishio, K. Suzuki, Three cases of suspected hyperthermia with remarkable elevation of serum mast cell tryptase, *Forensic Sci. Int.* 149 (2005) 51–55.
- [17] K.D. Horn, J.F. Halsey, R.E. Aumwalt, Utilization of serum tryptase and immunoglobulin E assay in the postmortem diagnosis of anaphylaxis, *Am. J. Forensic Med. Pathol.* 25 (2004) 37–43.
- [18] L. Comment, L.R. Bonetti, P. Mangin, C. Palmiere, Measurement of beta-tryptase in postmortem serum, pericardial fluid, urine and vitreous humor in the forensic setting, *Forensic Sci. Int.* 240 (2014) 29–34, <http://dx.doi.org/10.1016/j.forsciint.2014.04.006>.
- [19] D.E. Mayer, A. Krauskopf, W. Hemmer, K. Moritz, R. Jarisch, C. Reiter, Usefulness of post mortem determination of serum tryptase, histamine and diamine oxidase in the diagnosis of fatal anaphylaxis, *Forensic Sci. Int.* 212 (2011) 96–101, <http://dx.doi.org/10.1016/j.forsciint.2011.05.020>.
- [20] C.H. Chung, B. Mirakhur, E. Chan, Q.T. Le, J. Berlin, M. Morse, et al., Cetuximab-induced anaphylaxis and IgE specific for galactose- α -1,3-galactose, *N. Engl. J. Med.* 358 (2008) 1109–1117, <http://dx.doi.org/10.1056/NEJMoa074943>.
- [21] A. Romano, D. Quarantino, L. Venemalm, M.J. Torres, A. Venuti, M. Mlanca, A case of IgE-mediated hypersensitivity to ceftriaxone, *J. Allergy Clin. Immunol.* 104 (1999) 113–114.
- [22] A. Romano, C. Mayorga, M.J. Torres, M.C. Artesani, R. Suau, F. Sanchez, et al., Immediate allergic reactions to cephalosporins: cross-reactivity and selective responses, *J. Allergy Clin. Immunol.* 106 (2000) 1177–1183.
- [23] S.A. Poston, H.R. Jennings, K.L. Poe, Cefazolin tolerance does not predict ceftriaxone hypersensitivity; unique side chains precipitate anaphylaxis, *Pharmacotherapy* 24 (2004) 668–672.
- [24] A. Romano, E. Piunti, M. Di Fonso, M. Viola, A. Venuti, L. Venemalm, Selective immediate hypersensitivity to ceftriaxone, *Allergy* 55 (2000) 415–416.
- [25] D.G. Harle, B.A. Baldo, Drugs as allergens: an immunoassay for detecting IgE antibodies to cephalosporins, *Int. Arch. Allergy Appl. Immunol.* 92 (1990) 439–444.
- [26] M. Manfredi, M. Severino, S. Testi, D. Macchia, G. Ermini, W.J. Pichler, et al., Detection of specific IgE to quinolones, *J. Allergy Clin. Immunol.* 113 (2004) 155–160.