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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.

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.

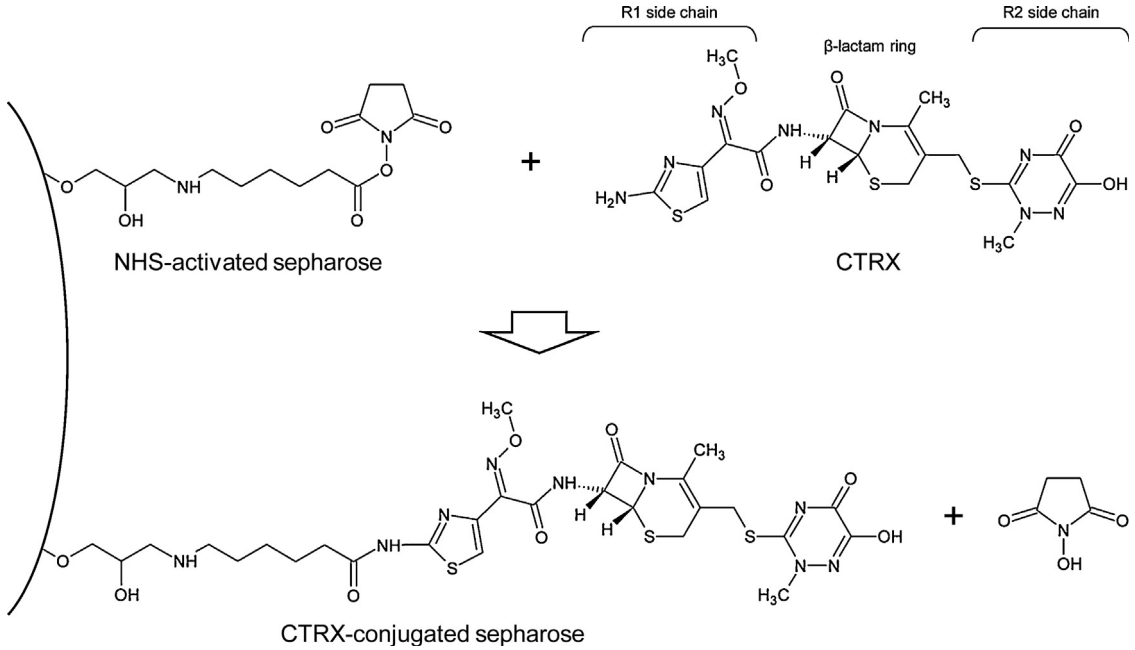

Fig. 1. Schematic showing the coupling reaction of ceftriaxone (CTRX) to a solid phase. CTRX is bound to *N*-hydroxysuccinimide (NHS)-activated sepharose beads by amine coupling. CTRX has three important structural components, including a β-lactam ring and R1 and R2 side chains. In this case, the R2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