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Association between mutated Mas-related G protein-coupled receptor-X2 and rocuronium-induced intraoperative anaphylaxis

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Keywords: allergy testing; anaphylaxis; MRGPRX2; neuromuscular blocking agent; non-IgE-mediated allergic reaction; rocuronium

Editor—Unexpected perioperative anaphylaxis is one of the most challenging clinical scenarios for anaesthesiologists.[1] The perioperative drugs most likely to cause anaphylaxis are antibiotics, neuromuscular blocking agents (NMBAs) and opioids. Recent studies have shown that NMBAs and opioids directly stimulate the Mas-related G protein-coupled receptor b2 (Mrgprb2), which is specifically expressed by mouse mast cells and which induces mast cell histamine release.[2] This immunoglobulin E (IgE)-independent pathway is referred to as a non-IgE-mediated allergic reaction. Some anaphylaxis cases caused by NMBAs have a negative basophil activation test (BAT), an IgE-mediated allergic reaction test, despite having a positive skin test.[3] We postulated that the Mas-related G protein-coupled receptor X2 (*MRGPRX2*), which is the human orthologue of mouse *Mrgprb2* and is expressed in mast cells, peripheral blood basophils, and eosinophils,[4] plays a key role in non-IgE-mediated perioperative anaphylaxis in humans.

We present a case of perioperative anaphylactic reaction to rocuronium, a widely used NMBA. Informed consent was obtained from the patient for the publication of this case report. All research protocols were approved by the Ehime University Committee for clinical research and Saiseikai Matsuyama Hospital Committee for Clinical Research, and registered as UMIN000032695 on UMIN-CTR Clinical Trial. As specific IgE antibodies against rocuronium were not detected, this reaction was diagnosed as a non-IgE-mediated allergic reaction. Furthermore, we identified specific mutations in the *MRGPRX2* DNA sequence likely associated with the development of hypersensitivity to rocuronium.

A 30-yr-old male (59 kg, 173 cm) was scheduled for open reduction and internal fixation of a clavicle fracture under general anaesthesia. He had a history of bullectomy under general anaesthesia 10 years ago with propofol, remifentanil, and vecuronium and cefazoline as a preoperative antibiotic. Upon arrival in the operating room, his arterial BP, HR, and peripheral oxygen saturation (SpO_2) were 120/62 mm Hg, 60 beats min^{-1}, and 100%, respectively. Anaesthesia was induced i.v. with propofol (target-controlled infusion mode at 3.0 μg ml^{-1}), remifentanil 0.25 μg kg^{-1} min^{-1}, lidocaine 50 mg, fentanyl 100 μg, and rocuronium 50 mg. At 2 min after rocuronium infusion, HR increased to 143 beats min^{-1} and subsequently BP decreased to 60/28 mmHg together with appearance of erythema and wheals over his entire body. After tracheal intubation, SpO_2 decreased to 89% with wheezing while receiving mechanical ventilation (fraction of inspired oxygen [FiO2]: 0.4).

Incremental doses of ephedrine (8 mg, three doses) and phenylephrine (0.1 mg, five doses) were administered i.v., but BP and HR failed to respond to these interventions, and SpO_2 did not recover even with inspired oxygen concentration at 100%. Suspecting an anaphylactic reaction to anaesthetic drugs, we stopped the infusion of propofol, and 4 vol% desflurane was administered and methylprednisolone 125 mg i.v. was administered with a continuous infusion of norepinephrine (0.1 μg kg^{-1} min^{-1}), which increased BP to 110/58 mmHg and decreased his HR to 85 beats min^{-1}. Respiratory status

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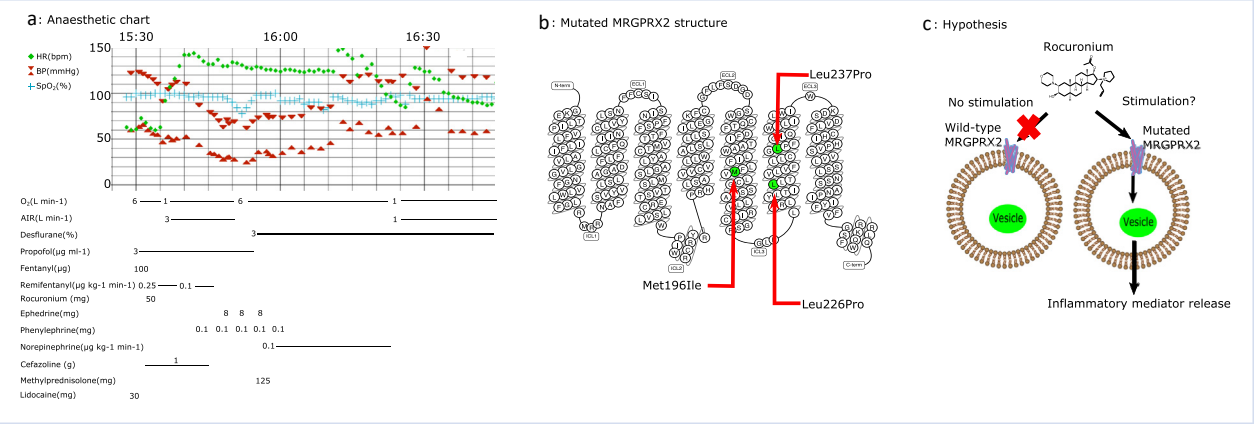

Fig. 1. Summary of a case of perioperative anaphylaxis. (a) Anaesthetic chart of this case recorded by PaperChart (http://paperchart.net/pcarchive.html). (b) Reconstructed secondary protein structure of human MRGPRX2 predicted using GPCRdb (https://www.gpcrdb.org/protein/mrgx2_human/). The red shading represents the mutated amino acids. (c) Our hypothesis for the relationship between rocuronium and mutated MRGPRX2. Specific mutations of MRGPRX2 might cause anaphylaxis related to rocuronium. ECL, extracellular loop; ICL, intracellular loop; MRGPRX2, Mas-related G protein-coupled receptor X2.

improved with no wheezing, and SpO_2 increased to 100% (FiO_2: 0.4). The surgery was aborted, and the trachea was extubated 1 h after he was haemodynamically stable.

Four weeks after general anaesthesia, an intradermal allergy test with propofol, xylocaine, cefazoline, and rocuronium was performed. All drugs showed a negative reaction at 1:10 and 1:100 dilutions. However, the undiluted rocuronium resulted in a positive reaction. Total IgE and specific IgE to rocuronium were 92.2 IU ml^{-1} and <0.10 UA ml^{-1} (ImmunoCAP Specific IgE system; Phadia AB, Uppsala, Sweden),[5] respectively, with normal values of 170 IU ml^{-1} or less for total IgE, and 0.35 UA ml^{-1} or less for specific IgE. We considered that he had no sensitisation owing to the cross-reaction between rocuronium and the vecuronium used in the previous operation because we detected no specific IgE antibody to rocuronium. The allergic reaction was diagnosed as rocuronium-induced non-IgE-mediated anaphylaxis.

We then analysed the full-length DNA of *MRGPRX2* using the primer walking sequencing method. For *MRGPRX2* (NM_001303615.1) target sequencing, genomic DNA was extracted from whole-blood samples and the targeted fragments of *MRGPRX2* were amplified using a *Taq* polymerase. The polymerase chain reaction (PCR) products were purified and cloned into a pCR™4-TOPO® TA vector (Thermo Fisher Scientific, Tokyo, Japan). The inserts were then sequenced using an Illumina HiSeq 2000 sequencer. The sequencing results revealed three amino acid mutations (Met196Ile, Leu226Pro, and Leu237Pro) located in the transmembrane domains in the *MRGPRX2* sequence (Fig 1).

In ~50% of perioperative anaphylaxis cases, no specific IgE antibodies are detected.[6] Owing to a lack of a specific identifiable cause, these are often diagnosed as non-IgE-mediated anaphylaxis. We posit that MRGPRX2 plays a key role in the non-IgE-mediated allergic pathway. MRGPRX2 has been implicated in various allergic diseases, such as asthma and chronic pruritus.[7,8] Although some studies suggest that rocuronium stimulates mast cells via the MRGPRX2 pathway, others show a lack of effect of rocuronium on wild-type MRGPRX2.[9] Indeed, rocuronium-induced perioperative anaphylaxis is rare. In addition, rocuronium failed to stimulate rat basophilic RBL-2H3 cells expressing wild-type MRGPRX2 in our *in vitro* pilot study. Of interest, Lansu and colleagues[10] identified an MRGPRX2 mutation that alters its affinity for anaesthetics.

Planned follow-up studies include expanding the *MRGPRX2* gene sequence analysis to additional perioperative anaphylaxis patients and measurement of histamine release from RBL-2H3 cells expressing mutated MRGPRX2 in response to rocuronium. These studies will help reveal the mechanism of perioperative anaphylaxis and inform a preventive strategy against this potentially lethal clinical syndrome.

Declarations of interest

The authors declare that they have no conflicts of interest.

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Association between mutated Mas-related G protein-coupled receptor-X2 and rocuronium-induced intraoperative anaphylaxis. Comment on *Br J Anaesth* 2020; 125: e446–e448

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Keywords: immediate drug hypersensitivity; MRGPRX2; neuromuscular blocking agent; perioperative anaphylaxis; rocuronium

Editor—We read with great interest the paper by Suzuki and colleagues[1] about the association between the Mas-related G protein-coupled receptor-X2 (MRGPRX2) and rocuronium-induced perioperative anaphylaxis. The authors describe a patient who experienced severe perioperative anaphylaxis. Based on a positive intradermal skin test (IDT) and a negative serum-specific IgE (sIgE) antibody result to rocuronium, the authors posit this reaction to have resulted from occupation of MRGPRX2 by the aminosteroid-derived neuromuscular blocking agent (NMBA) rocuronium. However, we would like to express some comments and concerns about their diagnosis and interpretation of the data. Perioperative anaphylaxis constitutes a significant condition with serious consequences of diagnostic mismanagement.[2] In this respect, firstly establishing the correct cause of the reaction and secondly resolving between IgE- and MRGPRX2-dependent reactions are critical for NMBA stewardship.

In this case, the diagnosis of rocuronium hypersensitivity relied upon a positive IDT with undiluted rocuronium (10 mg ml^{-1}) without details about injection volume, injection bleb, or final wheal and flare reaction. Most importantly, the test concentration is 200-fold the maximum non-irritative concentration of 0.05 mg ml^{-1} as recommended for IDT in the recent *European Academy of Allergy and Clinical Immunology* position paper on the investigation of perioperative immediate hypersensitivity reactions.[3] Therefore, a false-positive result of IDT cannot be excluded, as this concentration is likely to induce wheal and flare responses in healthy control individuals.[4]

Moreover, it seems that not all compounds that the patient was exposed to were tested (e.g. remifentanil); other important compounds (e.g. latex and chlorhexidine) were simply not investigated, and for lidocaine and cefazolin, investigations should have included a challenge test.[5] Furthermore, a negative rocuronium sIgE result does not rule out an IgE-mediated rocuronium allergy. We have shown the negative predictive value for sIgE rocuronium to be 72%, compared with 95% for skin testing and 75% for basophil activation testing (BAT).[6] Therefore, the basis for confirming rocuronium as cause is inadequate, and the anaphylaxis in this patient may have resulted from another cause.

We also respectfully disagree with the statement that the negative rocuronium sIgE result excludes cross-reactivity with vecuronium, another aminosteroid-derived NMBA that was used earlier in this patient. A morphine-based sIgE assay could have been a more sensitive method to detect an eventual sensitisation to substituted tertiary and quaternary ammonium structures, the major antigenic determinant of NMBAs.[7,8] Moreover, potential cross sensitisation could have been explored by skin testing or BAT, as the latter more closely mirrors the *in vivo* situation than traditional sIgE binding assays,[6,9–11] as described in a recent review on the value and

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