

CASE REPORT

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# Intraoperative chlorhexidine-induced anaphylaxis suggesting an immunoglobulin-E-dependent mechanism indicated by basophil activation tests: two case reports

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## Abstract

**Background:** Although chlorhexidine allergy has been shown to be mediated by immunoglobulin (Ig) E, few reports investigated the mechanism of chlorhexidine-induced anaphylaxis using basophil activation tests (BATs).

**Case presentation:** A 79-year-old man underwent cholecystectomy under general anesthesia. Anaphylaxis was diagnosed based on the clinical symptoms and high serum tryptase and histamine levels. Skin tests showed positive results only for chlorhexidine. Subsequently, BATs demonstrated that the causative agent was likely chlorhexidine. The inhibitory effect of wortmannin, an inhibitor of phosphoinositide 3-kinase, on basophil activation suggested an IgE-dependent mechanism underlying chlorhexidine-induced anaphylaxis.

An 89-year-old man underwent inguinal hernioplasty under general anesthesia. Anaphylaxis was diagnosed based on the clinical symptoms and high serum tryptase and histamine levels. Skin tests and BATs with wortmannin were performed, showing similar results to case 1.

**Conclusions:** BATs suggested an IgE-dependent mechanism for chlorhexidine-induced anaphylaxis and might be useful for investigating the mechanisms underlying drug-induced anaphylaxis.

**Keywords:** Anaphylaxis, Chlorhexidine, Skin test, Basophil activation test, Specific IgE

## Background

Chlorhexidine is an antiseptic with a broad spectrum of activity and persistent effects on the skin. Since the widespread use of chlorhexidine, many cases of anaphylaxis have been reported [1]. The risk of chlorhexidine allergy has increasingly been recognized in recent years [2]. Chlorhexidine was the third-most common cause of

perioperative anaphylaxis after antibiotics and neuromuscular blocking agents [3]. The mechanisms underlying anaphylactic reactions to chlorhexidine have been investigated, and chlorhexidine allergy has been shown to be mediated by immunoglobulin (Ig) E [4]. To the best of our knowledge, this is the first report suggesting an IgE-dependent mechanism, as indicated by basophil activation tests (BATs).

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**Case presentation**

**Case 1**

A 79-year-old man (weight, 73 kg; height, 161 cm) underwent cholecystectomy under general anesthesia. Anesthesia was induced with propofol, rocuronium, fentanyl, and inhalation of sevoflurane. Anesthesia was maintained with sevoflurane and remifentanyl. He was given sulbactam and cefoperazone, and his skin was previously disinfected with chlorhexidine 1% (Hexak®; Yoshida Pharmaceutical Company, Tokyo, Japan). During surgery, frequent administrations of ephedrine and phenylephrine were required intraoperatively. Postoperatively, cutaneous wheals were observed, mainly over the abdomen. On suspicion of anaphylaxis, we administered 100 mg of hydrocortisone intravenously and 0.7 mg of adrenaline intramuscularly, stabilizing his general condition. He was transferred to the intensive care unit without extubation, and his trachea was extubated the next day. Serum tryptase levels measured at 30 min, 2 h, and 24 h after the reaction was identified were 9.6 µg/L, 10.0 µg/L, and

3.1 µg/L, respectively. Serum histamine levels measured at the same time points were 3.93 ng/L, 1.07 ng/L, and 1.27 ng/L, respectively. We diagnosed anaphylaxis based on the clinical symptoms and high serum tryptase and histamine levels.

Eighty-three days later, skin tests were performed with all suspected agents, showing positive results only for chlorhexidine (Hexak®) (Table 1). BATs with CD203c and CD63 (Beckman Coulter, Brea, USA) were subsequently performed using serial dilutions of chlorhexidine (Hexak® and Stericlone®; KENEI Pharmaceutical Company, Osaka, Japan). Hexak® contains alcohol, but Stericlone® does not. The methods of the BATs are detailed elsewhere [5]. Briefly, the basophils were incubated at 37 °C for 15 min with antibodies and drugs. Anti-IgE antibody (Beckman Coulter) and formyl-methionine-leucyl-phenylamine (fMLP; Sigma-Aldrich, St. Louis, USA) were used as positive controls. The isotype of the anti-IgE antibody is IgG1, and the host species is a mouse. Unlike anti-IgE, fMLP activates

**Table 1** Results of skin tests.

Drug	SPT				IDT			
	Concentration of stock solution (mg/mL)	Judgment	Wheal (mm)	Flare (mm)	Concentration of stock solution (mg/mL)	Judgment	Wheal (mm)	Flare (mm)
<b>Case 1</b>								
Saline	9	-			9	-		
Histamine	10	+	6	18	0.01	+	10	25
Propofol	10	-			1	-		
Rocuronium	10	-			0.05	-		
Remifentanyl	0.05	-			0.005	-		
Fentanyl	0.05	-			0.005	-		
Sulbactam and cefoperazone	10	-			1	-		
Latex	NA	-			NA	-		
Chlorhexidine	5	-			0.002	+	6	20
<b>Case 2</b>								
Saline	9	-			9	-		
Histamine	10	+	5	8	0.01	+	9	12
Propofol	10	-			1	-		
Rocuronium	10	-			0.05	-		
Remifentanyl	0.05	-			0.005	-		
Fentanyl	0.05	-			0.005	-		
Sulbactam and ampicillin	15	-			1.5	-		
Acetaminophen	10	-			1	-		
Hydroxyethyl starch	60	-			6	-		
Chlorhexidine	5	+	10	12	0.002	+	6	10

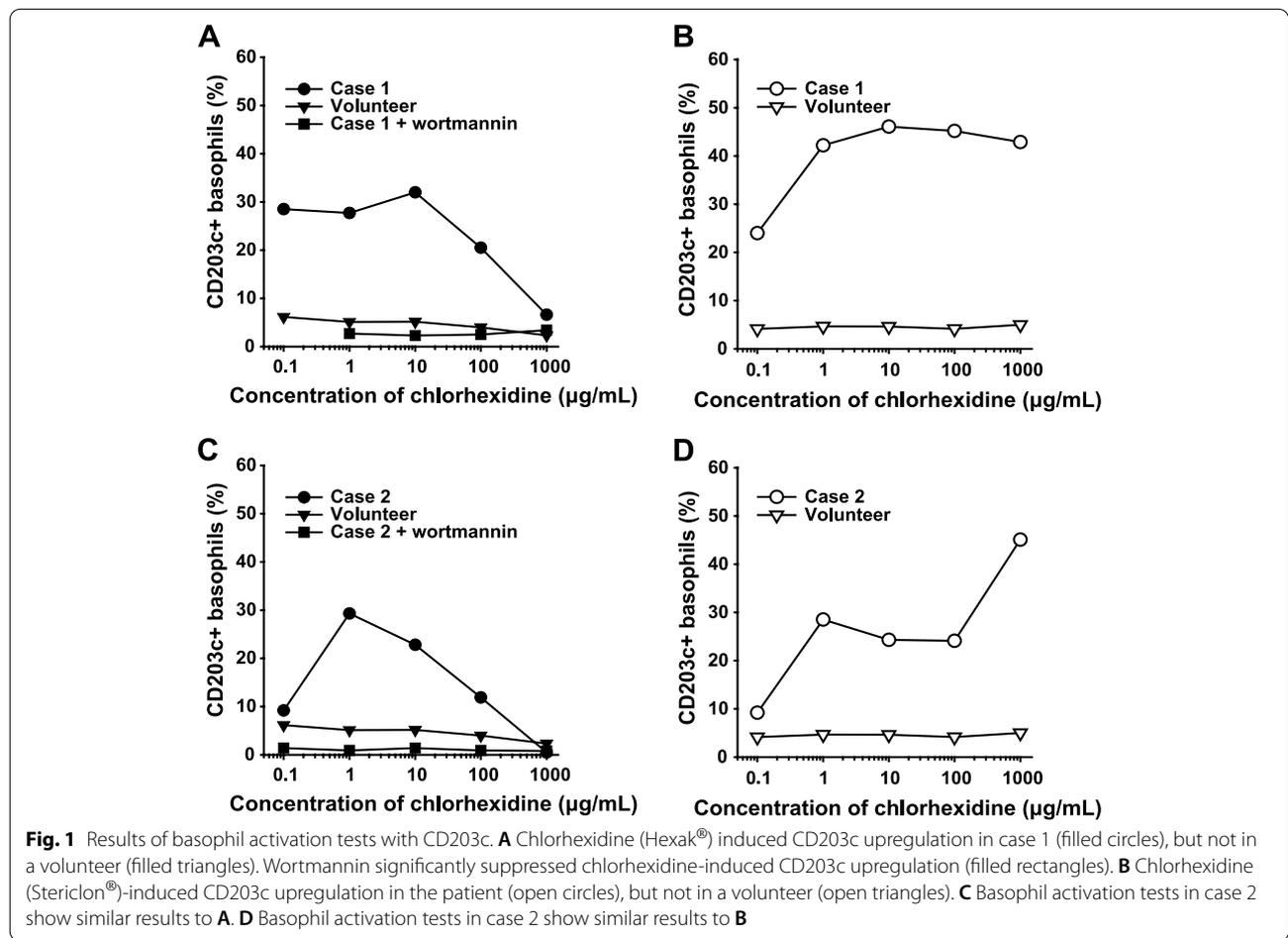
SPT reactions were deemed positive for wheal diameters ≥ 3 mm larger than that of the negative control or equal to at least half that in the positive control after 20 min. IDT reactions were deemed positive when the wheal diameter was equal to at least twice that of the post-injection wheal after 20 min [8]. SPT, skin prick test; IDT, intradermal test; NA, not applicable

basophils through an IgE-independent pathway [5, 6]. Wortmannin (Abcam, Cambridge, UK), an inhibitor of phosphoinositide 3-kinase (PI3-K), was used to investigate whether anaphylaxis was caused by an IgE-dependent or -independent mechanism [7]. We added one  $\mu\text{M}$  wortmannin at the same time as other drugs. Compared to CD203c+basophils from a healthy control, those in the patient were significantly elevated after adding Hexak<sup>®</sup> and Stericlon<sup>®</sup> (Fig. 1). Similar results were obtained for CD63+basophils. These results showed that the causative agent was likely chlorhexidine. The rates of CD203c+basophils that responded to negative control, anti-IgE at 10  $\mu\text{g}/\text{mL}$ , and fMLP at 1.3  $\mu\text{g}/\text{mL}$  stimulation were 5.0%, 22.9%, and 17.3%, and those with pretreatment wortmannin were 3.2%, 2.5%, and 7.7%, respectively. Pretreatment with wortmannin inhibited basophil activation induced by anti-IgE and chlorhexidine. However, only a relatively small change was observed when fMLP was used as a basophil stimulator. The inhibitory effect of wortmannin on basophil activation suggested an IgE-dependent mechanism underlying chlorhexidine-induced anaphylaxis (Fig. 1).

In addition, the level of specific IgE to chlorhexidine was measured at 6.63 UA/mL.

**Case 2**

An 89-year-old man (weight, 47 kg; height, 160 cm) underwent inguinal hernioplasty under general anesthesia. Anesthesia was induced with propofol, rocuronium, fentanyl, and inhalation of desflurane. Anesthesia was maintained with desflurane and remifentanyl. He was given sulbactam and ampicillin, and his skin had been previously disinfected with chlorhexidine 0.5% (Hexak<sup>®</sup>). Blood pressure fell to approximately 50 mmHg in the middle of the operation. Suspecting anaphylaxis, a total of 0.6 mg of adrenaline, chlorpheniramine 5 mg, and famotidine 20 mg were administered, resulting in stabilization of his general condition. Generalized erythema was observed when the surgery was concluded. Since he did not show any respiratory symptoms at the end of anesthesia, he was transferred to the high care unit after extubation. Serum tryptase levels measured at 30 min, 2 h, and 24 h after the reaction were 11.0  $\mu\text{g}/\text{L}$ , 18.9  $\mu\text{g}/\text{L}$ , and 4.9  $\mu\text{g}/\text{L}$ , respectively. Serum histamine



levels measured at the same time points were 67.1 ng/L, 2.94 ng/L, and 0.81 ng/L, respectively. Anaphylaxis was diagnosed based on the clinical symptoms and high serum tryptase and histamine levels.

Thirty-four days later, skin tests and BATs with wortmannin were performed, showing similar results to case 1 (Table 1, Fig. 1). Rates of CD203c+ basophils that responded to negative control, anti-IgE 10 µg/mL, and fMLP 1.3 µg/mL stimulation were 5.0%, 68.9%, and 59.1%, and those with wortmannin were 3.6%, 3.8%, and 58.6%, respectively. In addition, the level of specific IgE to chlorhexidine was 1.45 UA/mL.

## Discussion

We report two cases of chlorhexidine-induced anaphylaxis diagnosed by skin tests, BATs, and specific IgE quantification. BATs suggested an IgE-dependent mechanism for chlorhexidine-induced anaphylaxis.

Correct diagnosis is crucial in perioperative anaphylaxis, and skin tests remain the gold standard for detecting culprit drugs. However, skin tests carry a risk of inducing a recurrence of anaphylaxis, and the positive predictive value of skin tests is not 100%. Hence, there seems to be room for other in vitro tests to diagnose anaphylaxis [9]. The following in vitro tests are available to assess the diagnosis of chlorhexidine allergy: detection of specific IgE, histamine release test, and BATs. Although many patients have undergone quantification of specific IgE, few patients with chlorhexidine-induced anaphylaxis have undergone BATs [1]. We performed BATs in addition to specific IgE in our cases, with positive results in both. The positive reaction on BATs by chlorhexidine preparations with or without alcohol suggested that chlorhexidine itself is an allergen.

In recent years, BATs with wortmannin have become available to investigate the involvement of IgE. As PI3-K is an essential enzyme in the IgE-mediated pathway of basophil activation, suppression of basophil activation by wortmannin suggests IgE involvement [7, 10]. In the current cases, wortmannin almost completely suppressed basophil activation by chlorhexidine, suggesting an IgE-dependent mechanism of chlorhexidine-induced basophil activation.

## Conclusions

BATs suggested an IgE-dependent mechanism for chlorhexidine-induced anaphylaxis. BATs might be useful for investigating the mechanisms underlying drug-induced anaphylaxis.

## Abbreviations

BATs: Basophil activation tests; IgE: Immunoglobulin-E; PI3-K: Phosphoinositide 3-kinase; fMLP: Formyl-methionine-leucyl-phenylamine.

## Acknowledgements

Not applicable.

## Authors' contributions

MO and TT wrote the manuscript. TH, KN, NM, AT, and SS revised and edited the manuscript. The authors read and approved the final manuscript.

## Funding

The preparation of this manuscript was supported by the JSPS KAKENHI Grant Numbers 19K18286 and 18K08809.

## Availability of data and materials

Data relevant to this case report are not available for public access because of patient privacy concerns, but are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

We obtained the basophil activation test data in healthy volunteers according to a study protocol approved by the Ethics Committee of Gunma University Hospital (identification [ID]: 1259). The study protocol was registered with the University Hospital Medical Information Network Clinical Trials Registry (ID: UMIN000016761). Written informed consent for participation was obtained from the healthy volunteers.

### Consent for publication

Written informed consent for publication was obtained from the patient using a form approved by the ethics committee of Takasaki General Medical Center and JCHO Gunma Chuo Hospital.

### Competing interests

The authors declare that they have no competing interests.

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Received: 12 September 2022 Revised: 1 November 2022 Accepted: 7 November 2022

Published online: 22 November 2022

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