Three Cases of Ortho-phthalaldehyde-induced Anaphylaxis after Laryngoscopy: Detection of Specific IgE in Serum

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ABSTRACT
Background: Ortho-phthalaldehyde (OPA) has recently been used as a disinfectant for various medical apparatuses. OPA is not generally recognized as a potential allergen.

Case Summary: Subsequent to our recent report describing a patient presenting with OPA-induced anaphylaxis following laryngoscopy, we experienced two more such cases. In all three cases, the basophil histamine release test was useful for identifying the allergen as OPA. OPA-specific IgE was successfully detected in the serum of the patients by ELISA.

Discussion: Physicians and co-medical workers need to be aware of potential allergens to which patients may be exposed during routine medical procedures.

KEY WORDS
basophils, ELISA, histamine release, IgE-mediated anaphylaxis, skin test

INTRODUCTION
Ortho-phthalaldehyde (OPA) is a recently developed disinfectant.¹ Although glutaraldehyde is still widely used for disinfection of various medical apparatuses, increasing numbers of hospitals are adopting OPA, since this compound is not volatile and is much less irritable to medical workers.²,³ Here we report three cases of OPA-induced anaphylaxis after laryngoscopy. Our in vitro analyses clearly demonstrated that OPA-specific IgE was present in the serum of all three patients.

CLINICAL SUMMARY
CASE 1
The clinical course of this patient was already described elsewhere.⁴ In brief, a 25-year-old woman developed an anaphylactic reaction with dyspnea, runny nose, systemic urticaria, tachycardia and a mild decrease in the blood pressure following a laryngoscopic procedure for assessment of a vocal cord tumor in August 2005. She had undergone once-a-month checkups by laryngoscopy for the previous five months. Two weeks after her anaphylaxis had disappeared, skin tests and challenge tests were performed for latex and local anesthetics, since they had been used during the laryngoscopic procedure, but no allergic reactions were observed. However, a disinfectant solution containing 0.55% OPA, which was routinely used for pretreatment of the laryngoscope, showed a positive reaction in an intracutaneous test at a 1 : 1000 dilution. Her serum IgE level was 340 IU/ml.

CASE 2
In February 2006, a 36-year-old otolaryngologist de-
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**Fig. 1** Results of histamine release tests using basophils from patients 1 (A), 2 (B) and 3 (C). Basophils were exposed to serial dilutions of the OPA-containing disinfectant and pure OPA solution (original concentration of OPA in both solutions: 0.55%) for 45 minutes at 37°C, and histamine released in the supernatant was assayed. Anti-IgE antibody at 14 μg/ml was used as a positive control stimulus. Data are mean values of duplicate determinations. The result for patient 1 was already reported.4

He had previously performed such laryngoscopic procedures many times. His serum IgE level was 36 IU/ml.

developed an anaphylactic reaction including tachycardia, sneezing, runny nose and general urticaria immediately after a laryngoscopic demonstration of his vocal cord. Treatment with chlorpheniramine and hydrocortisone was effective in relieving his symptoms.
OPA-treated Laryngoscopy and Anaphylaxis

Table 1  ELISA for OPA-specific IgE.

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<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Control 1</th>
<th>Control 2</th>
<th>Control 3</th>
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<td>0.072</td>
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<td>0.008</td>
<td>0.087</td>
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<td></td>
<td>(0.007)</td>
<td>(0.009)</td>
<td>(0.006)</td>
<td>(0.008)</td>
<td>(0.008)</td>
<td>(0.078)</td>
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<tr>
<td>Exp 2</td>
<td>0.032</td>
<td>0.206</td>
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<td></td>
<td>(0.012)</td>
<td>(0.089)</td>
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<tr>
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The detailed assay method was as described in the text. The results of four representative experiments are presented. Other experiments using case 1 serum samples showed essentially similar OD levels. All experiments were performed in duplicate, and the data are the mean OD values measured at 450 nm. The OD values of samples loaded in wells without any HSA precoating step prior to OPA treatment are shown in parentheses.

CASE 3
In March 2006, a 58-year-old man developed an anaphylactic reaction immediately after laryngoscopic examination of a pharyngeal tumor. His symptoms, including tachycardia, runny nose and general urticaria, improved following treatment with chlorpheniramine and hydrocortisone. He had undergone several laryngoscopic examinations during the previous 10 months. His serum IgE level was 73 IU/ml. A disinfectant solution containing OPA at a 1:100 dilution gave a positive reaction at 15 minutes in an intracutaneous test (wheel of 14×10 mm and flare of 28×24 mm).

In all three cases, subsequent laryngoscopic examinations were performed using an OPA-disinfected, thoroughly rinsed (>15 minutes) fiberscope, and the patients never again manifested allergic symptoms.

PATHOLOGICAL FINDINGS

IN VITRO HISTAMINE RELEASE TESTS
After informed consent was obtained, venous blood was drawn from the patients, and in vitro histamine release tests were performed in a buffer containing 0.03% human serum albumin (HSA). All in vitro tests and skin tests were performed after an interval of at least two weeks after the anaphylactic episode. Also, basophils obtained from at least one or two control subjects were included in each experiment using the patients’ basophils. Importantly, the tests demonstrated that both the disinfectant and pure OPA (purchased from Wako Pure Chemical, Osaka, Japan) evoked obvious release of histamine not only from the basophils of case 1 (Fig. 1A), but also from the basophils of case 2 (Fig. 1B) and case 3 (Fig. 1C). On the other hand, the basophils from healthy control donors did not show histamine release in response to OPA. As already noted elsewhere, passive sensitization experiments revealed that the serum of case 1 contained an OPA-specific, heat-sensitive component capable of sensitizing basophils from control subjects, implying the presence of OPA-specific IgE in the serum.

DETECTION OF SPECIFIC IGE BY ELISA
We next tried to detect the specific IgE in the patients’ serum by ELISA. The procedures were as follows: 1) coating of a 96-well polystyrene plate (Nunc, Roskilde, Denmark) with 1% HSA in PBS at 4°C overnight; 2) additional coating with 0.55% OPA at 4°C for 1 hour; 3) blocking with 1% HSA in PBS at 4°C for 3 hours; 4) addition of 2× diluted serum samples, incubated at 37°C for 2.5 hours; 5) addition of biotin-anti-human IgE Ab (Biosource International, Camarillo, CA, USA) 2 μg/ml at 37°C for 1 hour; 6) addition of streptavidin-horseradish peroxidase (Amersham Bioscience Corp, Piscataway, NJ, USA) at 2×10^5 dilution at 37°C for 30 minutes; 7) color development with 3,3’,5,5’-tetramethylbenzidine (TMB) (Kirkgaard & Perry Laboratories Inc., Gaithersburg, MD, USA) at room temperature for 15 minutes; 8) addition of 3 N phosphate; and 9) measurement with a microtiter plate reader (Bio-Rad, Hercules, CA, USA). The tests demonstrated the presence of OPA-specific IgE in the sera from all three patients (Table 1), as indicated by higher OD levels compared to the control serum samples. In addition, it was possible to detect the difference in OD values between the patients and normal controls even if the serum samples were diluted up to 10-fold. Precoating of the plates with albumin prior to treatment with OPA was essential; when the precoating step was omitted, detection of OPA-specific IgE was unsuccessful. These results showed that specific IgE in the serum of OPA-induced anaphylactic subjects can be detected by our ELISA system, and that conjugation of OPA to proteins is critical for this assay method.

DISCUSSION
After we first reported a case of anaphylaxis following an OPA-disinfected laryngoscopic procedure, we ex-
experienced two additional similar patients, described here as cases 2 and 3. Both in vitro histamine-release tests and ELISA were useful methods for identifying the allergen and the specific IgE. The interaction of OPA and OPA-specific IgE antibody may be directly involved in the pathogenesis of anaphylaxis in the patients. Presumably these patients had been sensitized by repeated exposure to OPA remaining on the fiberscope.

Demonstration of the involvement of specific IgE is often difficult for anaphylactic reactions induced by low-molecular-weight substances. In fact, immobilization of such small substances often destroys their antigenicity, making it difficult to detect specific IgE binding. The Prausnitz-Küstner reaction is a traditional method for definitively demonstrating the presence of specific IgE, but it is not feasible in many situations since infection by unknown pathogens may occur. In vitro assay methods such as ELISA depend on the binding property of the molecule.⁷,⁸ In this regard, OPA (molecular weight 134.1) may be an ideal molecule for establishing such an in vitro test system, since this molecule binds to carrier proteins. It could be said that OPA acts as a hapten when it induces an anaphylactic reaction in vivo, and that in vitro hapteneated OPA may be antigenically potent, capable of eliciting basophil histamine release and ELISA detection. The present cases, in addition to Sokol’s report,⁹ collectively suggest that repeated exposure to OPA-disinfected equipment can sensitize some patients due to production of specific IgE, and that OPA may be a clinically important allergen that induces type I allergic reactions. Thus, it is important for us to be aware of such potential allergens to which patients may be exposed during routine medical procedures.

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REFERENCES