
Case report

Heterogeneity of the IgE response to allergenic determinants of cefaclor in serum samples from patients with cefaclor-induced anaphylaxis

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Background: β -Lactam antibiotics, such as cefaclor, may cause IgE-mediated anaphylactic reactions. However, the clinically available serologic test has not been widely accepted, and the antigenic determinants of these drugs are unclear.

Objective: To describe 4 cases of anaphylaxis caused by cefaclor in which a specific IgE response to cefaclor was demonstrated.

Methods: Four patients with anaphylaxis to cefaclor and 35 nonatopic controls never exposed to cefaclor were studied. Skin tests and oral challenges with this drug were performed. The specific IgE response to the antigenic determinant of cefaclor-human serum albumin (HSA) conjugate was compared in each patient. The serum specific IgE to cefaclor-HSA conjugate was detected using enzyme-linked immunosorbent assay (ELISA). Also, ELISA inhibition studies using various concentrations of cefaclor-HSA, HSA alone, and free cefaclor were performed, as were hapten inhibition studies using cefaclor, cephalixin, cefadroxil, ampicillin, ceftriaxone, and cefotaxime.

Results: Three patients showed high levels of serum specific IgE to cefaclor-HSA and marked inhibition patterns to free cefaclor and cefaclor-HSA conjugate on ELISA inhibition testing. Hapten inhibition testing in 3 individual serum samples showed 2 different patterns. In patient 3, significant dose-dependent inhibitions (up to 92%) were noted with additions of free cefaclor and cefaclor-HSA conjugate, and lesser inhibitions (up to 74%) were noted with cephalixin, which shares the aminobenzyl side chain. In patients 1 and 2, marked dose-dependent inhibitions were noted only with additions of cefaclor-HSA conjugate and free cefaclor, whereas minimal inhibitions were noted with the other 5 compounds.

Conclusions: The specific IgE response to cefaclor-HSA conjugate in patients with cefaclor anaphylaxis occurs against the hapten, in which heterogeneity of the antigenic determinant was noted to depend on the individual.

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INTRODUCTION

Cefaclor is an oral second-generation cephalosporin that has been widely used against various respiratory tract infections. Shortly after the introduction of this cephalosporin in the clinical setting, allergic reactions to this drug, including anaphylaxis, were reported.¹ A life-threatening anaphylactic reaction to cefaclor may be caused by an immediate hypersensitivity mechanism correlated with the presence of specific IgE antibodies.^{2,3}

Low-molecular-weight β -lactam antibiotics have to be covalently bound to high-molecular-weight carrier proteins, such as tissue or serum albumin, and then the newly developed drug-protein complexes can elicit an immunologic re-

sponse.⁴ Unlike penicillin, the immunogenic determinants that are produced by the degradation of the cephalosporins are unknown. In vitro studies^{5,6} with penicillin and cephalosporins have shown a high degree of immunologic cross-reactivity, which is to be expected owing to the presence of a common β -lactam ring. However, there is only infrequent clinically relevant cross-reactivity between penicillin and the cephalosporins,⁷ and the case reports^{8–11} of patients who are allergic to a particular cephalosporin tolerating other cephalosporins suggest that the antibodies to R-side chains rather than to the common ring structure are more important for the immune response to cephalosporins. This study describes 4 patients with anaphylaxis induced by cefaclor in whom a specific IgE molecule to cefaclor-human serum albumin (HSA) conjugate was demonstrated by using enzyme-linked immunosorbent assay (ELISA). The antigenic determinant of cefaclor was compared by using the ELISA inhibition test.

METHODS

Study Participants

Four patients with anaphylactic reactions to cefaclor participated in this study. All 4 patients had a suspected history of

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anaphylactic reactions to cefaclor, and they all had a positive skin test reaction to this drug. In addition, if any other drugs were also suspected as the cause of the anaphylactic reaction, the diagnosis had to be confirmed by an oral challenge test using increasing doses of cefaclor (25, 50, 100, and 250 mg) until there was a response. Skin prick and intradermal tests were performed using cefaclor (3 mg/mL) on the volar side of the forearm. All the patients were initially tested using the skin prick method, and reactions were read after 15 minutes. A reaction was considered positive when the mean wheal diameter was 3 mm greater than the negative control. When the skin prick test responses were negative, 0.01 mL of serially diluted solutions (from 1:1,000 to 1:10 dilutions) were administered using the intradermal method. Histamine and isotonic sodium chloride solution (Allergopharma Co, Hamburg, Germany) were also used as positive and negative controls, respectively. The total serum IgE level was measured by radioimmunoassay. Commercially available assays for cefaclor specific IgE (CAP system; Pharmacia & Upjohn, Uppsala, Sweden) were performed according to the manufacturer's instructions. Serum samples from the 4 patients and from the 35 nonatopic controls who had never received cefaclor were collected and stored at -20°C . The present study was approved by the institutional review boards of Ajou University Hospital and Eulji Hospital, and written informed consent was obtained from all the study participants.

Preparation of Cefaclor-HSA Conjugates

Cefaclor was conjugated to HSA using a cross-linker, Sulfo-SMPB (Pierce Biotechnology Inc, Rockford, IL). Albumin (20 mg) was dissolved in 2 mL of conjugation buffer, and 8 mg of Sulfo-SMPB in 4 mL of conjugation buffer was then added. The resulting mixture underwent reaction for 1 hour at room temperature. The desalting column was equilibrated with 15 mL of conjugation buffer. The reaction volume was applied onto the column and then was eluted using 0.5-mL aliquots of the conjugation buffer. Protein elution was monitored by the absorbance at 280 nm. Cefaclor in conjugation buffer (1:5 wt/vol diluted) was added to the pooled fractions containing HSA, and this was incubated for 2 hours at room temperature. The final mixed solution was dialyzed against phosphate-buffered saline solution (PBS) for 72 hours before use.

ELISA and ELISA Inhibition Assays

Cefaclor specific IgE antibodies were detected by using the following method. Microplates (Costar, New York, NY) were coated with 100 μL per well of the cefaclor-HSA conjugates (10 $\mu\text{g/mL}$), and these were incubated for 2 hours at 37°C and then left at 4°C overnight. Each well was washed 4 times with 0.05% Tween PBS (PBS-T), and the remaining binding sites were blocked by incubation with 1% bovine serum albumin-PBS-T for 1 hour at room temperature. After washing, the wells were incubated for 1 hour at room temperature with 50 $\mu\text{L/mL}$ of serum from the patient or the control (1:5

vol/vol diluted). After washing 4 times with PBS-T, goat anti-human IgE (1:1,000 vol/vol diluted) was added to each well and incubated with shaking for 1 hour at room temperature. After washing, the wells were incubated with 1:200 vol/vol enzyme-labeled IgE for 1 hour at room temperature. The colorimetric reaction was developed with B (3,3',5,5'-tetramethylbenzidine) substrate solution for 15 minutes at room temperature. The absorbance was read at 405/450 nm in an automated microplate reader (Benchmark; Bio-Rad Laboratories Inc, Hercules, Calif). All the assays were performed in duplicate. The positive cutoff value was derived from the mean $+3$ SDs of the absorbance value from the 35 control subjects. A competitive ELISA inhibition of IgE binding in 3 of the cefaclor-reactive patients' serum samples to the drug-HSA conjugate was performed by preincubation with 5 concentrations (0–100 $\mu\text{g/mL}$) of cefaclor-HSA conjugates, HSA, and free cefaclor. Serum samples with albumin were considered controls, and the inhibition percentage of the specific IgE binding was expressed as $100 - [(\text{absorbance of samples preincubated with inhibitor} / \text{absorbance of samples without inhibitor}) \times 100]$.

Hapten Inhibition Tests

Hapten inhibition testing was performed to discover the antigenic determinant of the cefaclor molecule. The serum samples were preincubated with 5 concentrations (0–100 $\mu\text{g/mL}$) of free cefaclor, ampicillin, cephalixin, cefadroxil, ceftriaxone, and cefotaxime overnight at 4°C . The structures of the compounds used in these inhibition studies are given in Figure 1.¹² Then, the same steps of the experiment were continued as described previously herein.

RESULTS

Clinical Characteristics of the Study Patients

The clinical characteristics of patients with cefaclor-induced anaphylaxis are summarized in Table 1. Two patients showed positive reactions on skin prick testing, and the other 2 had positive intradermal test results using serial concentrations of cefaclor. In all 4 patients, the interval between intake of the drug and the onset of symptoms was 30 minutes or less, and

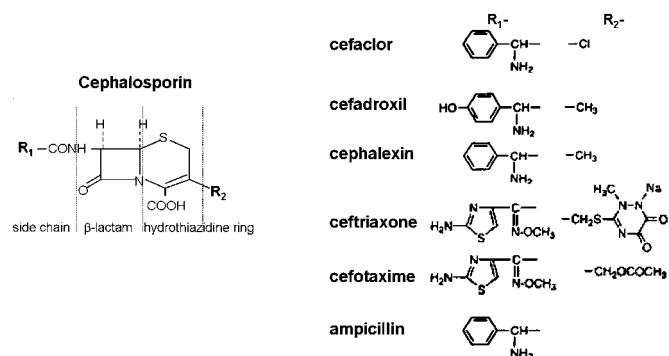


Figure 1. Chemical structures of the β -lactam antibiotics used in the hapten inhibition studies.

Table 1. Clinical Characteristics of the Study Patients

Patient No./ Age, y/Sex	Onset, min*	Clinical symptoms	Interval†	Episode‡	Drug§	Atopy	IgE, IU/mL	Cefaclor skin test, mm	Cefaclor specific IgE, kU/L	OPT
1/40/M	30	U/H/B	1 d	1	N	P	134	Prick, 5×4/27×23	0.57	P
2/15/F	15	A/B	1 wk	1	N	P	197	Intradermal, 7×5/31×20	2.33	P
3/40/F	10	U/H/B	4 wk	4	N	P	240	Intradermal, 6×5/20×12	4.14	P
4/37/M	30	U/H	1 wk	1	P	P	514	Prick, 3×3/20×17	N	P

Abbreviations: A, angioedema; B, bronchial spasm; H, hypotension; N, negative; OPT, oral provocation test with cefaclor; P, positive; U, urticaria.

*Defined as the interval between intake of the drug and the appearance of the first symptoms.

†Defined as the interval between the allergic event and the physician visit.

‡Defined as the number of episodes of anaphylaxis.

§Defined as a medical history of drug allergy.

the interval between the occurrence of the anaphylactic reaction to cefaclor and the study was 4 weeks or less. One patient had 4 episodes of anaphylactic reaction, and the other patients each had a single episode. None of the patients had a positive history of penicillin allergy. One patient had a history of anaphylactoid reaction caused by an intramuscular injection of diclofenac 3 years earlier. Two patients showed elevated total IgE levels. All the patients had a positive response to oral challenge with cefaclor, and 3 patients showed a systemic reaction at a lower dose during clinical use. Three patients showed positive levels of cefaclor specific IgE using CAP-FEIA (Pharmacia & Upjohn).

ELISA and ELISA Inhibition Studies

Three patients showed high serum levels of specific IgE to cefaclor-HSA when the positive cutoff value was determined from the mean +3 SDs of the absorbance values of the negative control (Fig 2). The ELISA inhibition tests were performed on these 3 patients. All 3 patients showed similar inhibition patterns to free cefaclor and cefaclor-HSA conjugate (Fig 3). When we conducted hapten inhibition tests with cefaclor, ampicillin, cephalixin, cefadroxil, ceftriaxone, and cefotaxime, the patients showed 2 different inhibition patterns. In patient 3, significant dose-dependent inhibitions (up to 92%) were noted with additions of cefaclor, and lesser inhibitions were noted with additions of cephalixin (up to 74%), which shares the same aminobenzyl side chain; cefadroxil, with a similar aminobenzyl side chain; and cefotaxime, with a different side chain. Minimal inhibitions were noted with the other 2 compounds. In patients 1 and 2, marked dose-dependent inhibitions were noted only with additions of cefaclor, whereas minimal inhibitions were noted with the other 5 compounds (Fig 4).

DISCUSSION

In this study, we confirmed an IgE-mediated mechanism for patients with cefaclor-induced anaphylaxis using the in vitro ELISA method. In addition, the hapten inhibition results demonstrated the heterogeneity of antigenic determinants, and this was recognized by the specific IgE antibodies to cefaclor-HSA conjugate. By using the ELISA system with the newly manufactured drug-HSA conjugates, we detected

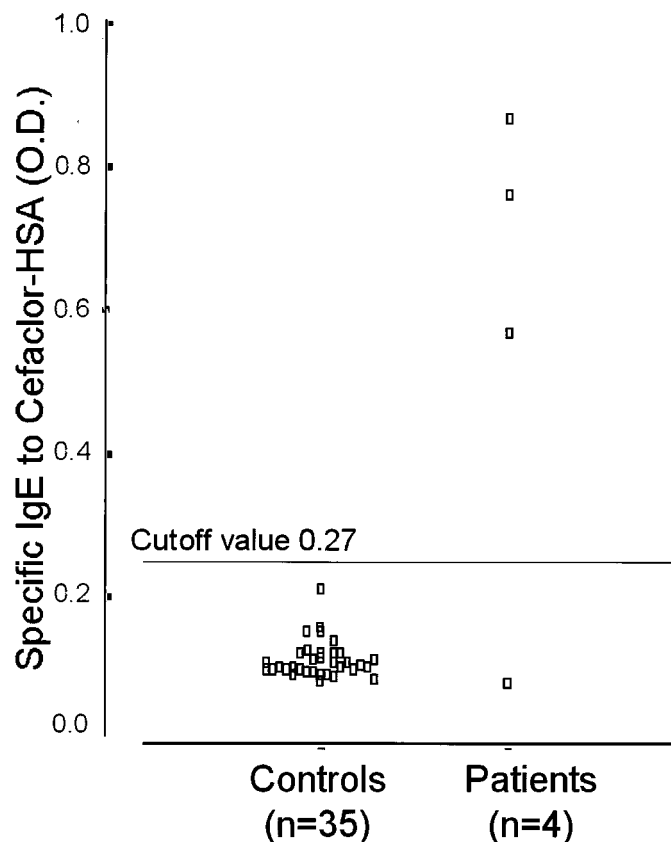


Figure 2. Specific IgE levels to cefaclor-human serum albumin (HSA) conjugate in serum samples from 4 patients and 35 controls. The horizontal line represents the positive cutoff value that was derived from the mean +3 SDs of the absorbance value of the controls.

drug-specific IgE antibodies in serum samples from patients who experienced an anaphylactic reaction to this antibiotic, whereas specific IgE was not demonstrated in the control serum samples. The specificity of this assay is supported by the dose-dependent inhibition pattern of IgE antibody binding in the patients' serum samples to cefaclor-HSA conjugate by preincubation with cefaclor-HSA conjugate and the free drug

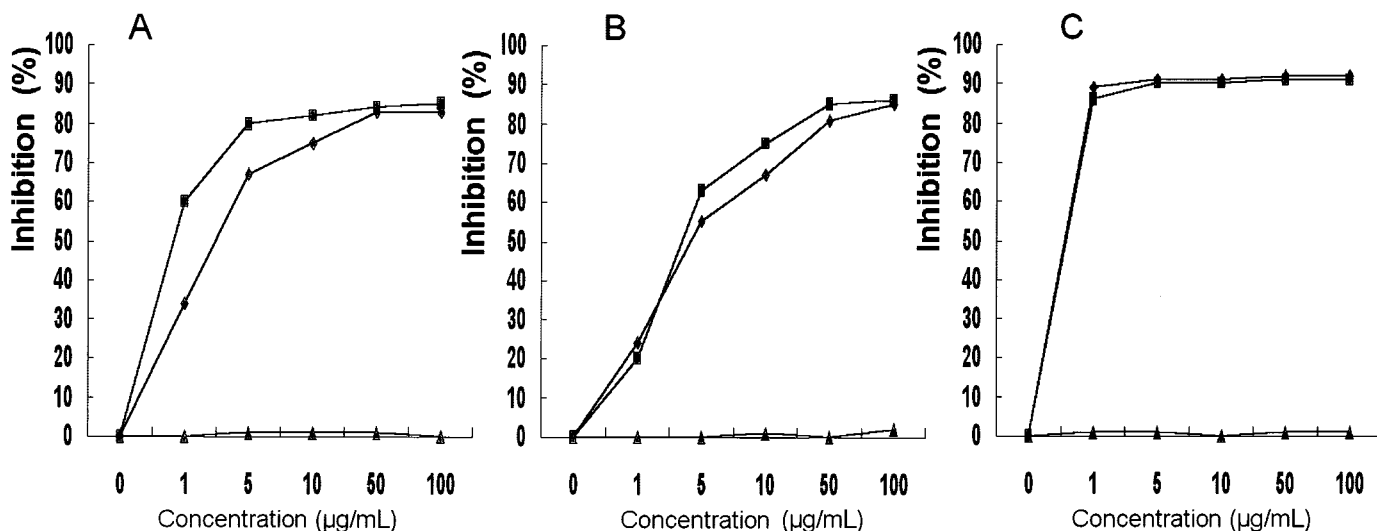


Figure 3. Inhibition of IgE binding to cefaclor-human serum albumin (HSA) in patients 1 (A), 2 (B), and 3 (C), who had serial additions of cefaclor-HSA conjugate (■), free cefaclor (◆), and HSA alone (▲).

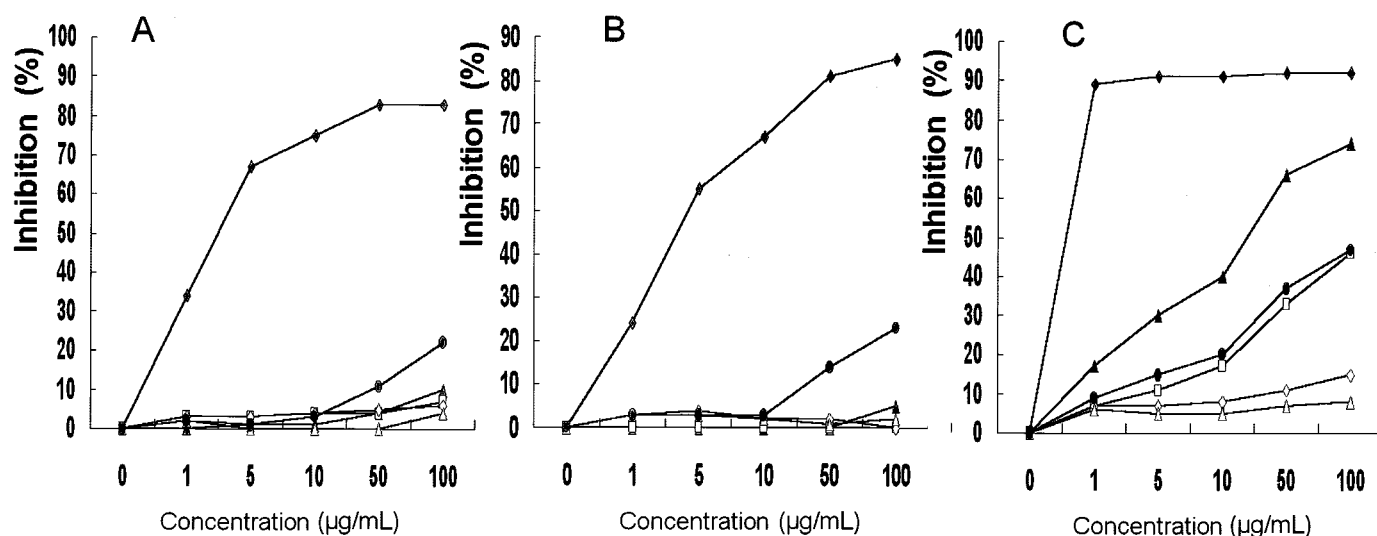


Figure 4. Hapten inhibition tests using cefaclor (◆), ampicillin (◇), cephalixin (▲), cefadroxil (□), ceftriaxone (△), and cefotaxime (●).

and the lack of inhibition with a structurally unrelated drug. However, the ELISA method is not as sensitive as skin testing.

As our results show, 1 patient had a negative specific IgE titer below the cutoff value even though he had a positive skin test result. It is well-known that allergic skin testing is considered to be a valuable procedure, yet there is currently no widespread agreement on the selection of drug antigens and the concentrations that should be used.¹³ In addition, sensitization after skin testing occurs, and the rarely reported serious reactions are also to be considered.^{14,15} No single test is satisfactory for the clinical evaluation of cephalosporin-allergic patients. In patient 4, we also checked the serum specific

IgE levels to other cephalosporins, including cefadroxil, cephalixin, ceftriaxone, and ampicillin, but specific IgE antibodies were not detected to these antibiotic-HSA conjugates using our ELISA system. Further studies are needed to try to detect a serum specific IgE to a new type of conjugate bound with other tissue protein conjugates in these patients.

Little is known about the extent of cross-reactivity among different cephalosporins, and, thus, not much is known about the safety of administering a particular cephalosporin to patients who previously experienced an allergic reaction to another cephalosporin.¹⁶ Case reports⁸⁻¹¹ of patients who are allergic to a particular cephalosporin tolerating other cephalosporins seem to suggest that the immune response is di-

rected against the R-group side chains rather than to the core portion of the molecules.

In this study, patients 1 and 2 displayed negative inhibitory potency with all the other β -lactams on the hapten inhibition tests. Thus, these patients' inhibition patterns seemed to involve the entire cefaclor molecule, as was previously demonstrated by Pham and Baldo.¹⁷ However, the other patient showed inhibitions to cephalexin and cefaclor, which share the same aminobenzyl (R1) side chain. These results suggest that the specific IgE response to the hapten portion of cefaclor and its cross-reactivity with other cephalosporins can be different depending on the sensitized individuals. Therefore, this study provides more data on the heterogeneity of cephalosporin antigenic determinants and secondary affirmation of previous works.^{17,18} In summary, we demonstrated the IgE-mediated reactions to cefaclor. In vitro diagnosis of these reactions can be successfully performed using the ELISA method with drug-HSA conjugates. In addition, we believe that this method could be applied to detect serum specific IgE for patients who are suspected of having an immediate hypersensitivity reaction to other cephalosporins.

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