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Short Communication

Detection of serum IgE antibody directed to aminothiazole using immobilized cephalosporins without protein conjugation

Akihito Yokoyama, Nobuoki Kohno, Kimiko Sakai, Hitoshi Katayama, Kazunori Irifune, Keiichi Kondo, Yutaka Hirasawa and Kunio Hiwada

Second Department of Internal Medicine, Ehime University School of Medicine, Ehime, Japan

Abstract

It is well known that allergic reactions may sometimes occur in patients under treatment with β-lactam antibiotics. For the detection of antidrug antibodies *in vitro*, conjugation with human serum albumin has been considered to be essential. In this study, we found that cefotiam, cefpirome, and ceftazidime could be immobilized without conjugation to carrier protein to construct a solid-phase enzyme-linked immunosorbent assay (ELISA) system. We describe a patient (26-year-old female nurse) with contact urticaria induced by antibiotics. Using the serum of this patient, we successfully detected IgE antibody directed to the aminothiazolyl group of cephalosporins, which has not previously been reported. Results suggest that the simple ELISA using unconjugated antibiotics could be applicable to patients with allergy to some cephalosporins and the aminothiazole side chain of the cephalosporins could cause an IgE-mediated allergic reaction.

Key words: antibiotics, contact dermatitis, contact urticaria, drug allergy, enzyme-linked immunosorbent assay, immunoglobulin E.

Correspondence: Akihito Yokoyama, Second Department of Internal Medicine, Ehime University School of Medicine, Onsen-gun, Ehime 791-0295, Japan.
Email: <yokoyan@m.ehime-u.ac.jp>

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Introduction

It is well known that allergic reactions may sometimes occur in patients being treated with β-lactam antibiotics. There is no doubt that detection of IgE antibody by *in vitro* methods is rather more desirable than *in vivo* methods such as Prausnitz-Küstner reaction for clinical investigation of causal compounds. However, new antigenic determinants formed by binding with serum proteins that are considered to be mostly responsible for the allergic reaction appear *in vivo*. In this context, conjugation with human serum albumin has been considered to be essential to detect antibodies in immunoassays. Using such conjugated compounds, the fine structural recognition pattern has been studied in β-lactam drugs such as cefaclor and cephalothin.[1,2] Similarly, extensive cross-reactivity is well known between penicillin and cephalosporins in bicyclic nuclear structure (the β-lactam ring). On this molecular basis, cephalosporins should not be administered to patients with penicillin allergy.[3] The allergic determinants of the drugs are on the cephalosporin molecule itself rather than on newly formed sites following conjugation with albumin. These observations are compatible with the findings of the basic immunology. Since most drugs are small molecules and haptens, conjugation with carrier protein is necessary to induce immunologic response. In such immunologic response, T cells will recognize carrier protein but antibodies will recognize determinants on hapten. Therefore, if drugs could be coated on the plates without conjugation with protein, IgE antibody could be expected to react with the drugs.

In the present study, we tried to immobilize antibiotics without conjugation to carrier protein and construct a solid-phase enzyme-linked immunosorbent assay (ELISA)

system. We also investigated a patient with contact urticaria induced by a variety of cephem antibiotics. Using the serum of this patient, we successfully identified the IgE antibody directed to the common side chain of cephalosporins.

METHODS

Materials

Samples of antibiotics were obtained from two companies. The compounds, 2-aminothiazole (AT), 1H-tetrazole (TZ) and 2-amino 4-thiazoleacetic acid (ATA) were purchased from WAKO Pure Chemicals (Osaka, Japan). Serum was obtained under informed consent from the patient described below. As control samples, sera from two patients with bronchial asthma (37-year-old male with 1383 U/mL of serum IgE, and 41-year-old female with 4370 U/mL of IgE) and a normal volunteer (26-year-old male) were also obtained.

Profile of patient with contact urticaria

A 25-year-old nurse was referred to our hospital in July 1997, for further investigation of her contact urticaria. During the year prior to her visit, she sometimes developed redness, itching and swelling in her hand and forearm at the site of contact with antibiotic solutions that she prepared in the course of her work. The symptoms developed 5–10 min after contact with the solution, and disappeared in 4–5 h. More recently, they had extended to her face, especially around her eyes. She had hand eczema and had been treated by a dermatologist 2–3 years previously. She had not taken cephem antibiotics for at least 10 years. For 9 years she had had an allergy to metal, such as a necklace or a watch, and an allergy to Japanese cedar pollen. Her height was 148 cm and her weight was 44 kg. Physical examination showed nothing unusual, except a few rice-sized lymph node swellings on the right side of her neck. Laboratory examination showed no abnormality, except increased serum IgE levels of 460 IU/mL with more than 100 U_A/mL of IgE specific to Japanese cedar (by CAP-RAST). She was notified that contact with antibiotics could endanger her life. She was advised to keep away from preparing antibiotic solutions and to wear latex gloves when she could not avoid preparing these solutions. Since then, her symptoms have not reappeared.

Determination of specific antibody levels

Specific IgE antibody titers were determined by an enzyme-linked immunosorbent assay (ELISA).[4] Plates (Costar, Cambridge, MA, USA) were coated with 300 μg/mL antibiotics (without conjugation to protein) diluted in saline or 0.1 mol/L $NaHCO_3$ (pH 8.3).

Following overnight incubation at 4°C, the plates were washed three times and blocked with 1% bovine serum albumin (BSA; Sigma, St Louis, MO, USA) in PBS for 2 h at 37°C. Serum samples serially diluted in 1% BSA/PBS were applied to the blocked wells and incubated overnight at 4°C. Then the plates were washed and incubated with horseradish peroxidase (HRP)-conjugated rat anti-human IgE monoclonal antibodies (Pharmingen, San Diego, CA, USA) diluted in 50% goat serum (Gibco, Grand Island, NY, USA) in PBS for 2 h at 37°C. After washing, wells were developed using OPDA solution (0.3% o-phenylenediamine dihydrochloride, 0.02% H_2O_2, 0.15 mol/L citrate buffer, pH 4.9) and the absorption at 492–620 nm was determined using a microplate autoreader.

To confirm the immobilization of antibiotics, immune serum was applied instead of human serum in the above method. Anti-cephalosporin C rabbit serum (Biogenesis Ltd, Poole, UK), anti-cefpirome (CPR), or anti-cefmetazole (CMZ) rat serum were used as immune sera. The latter two sera were made in our laboratory according to a method described previously,[5] with some modifications. Briefly, CPR or CMZ was conjugated with ovalbumin (grade V; Sigma) and used with complete Freund's adjuvant for immunization of rats (8-week-old male Brown Norway rat) twice at an interval of 2 weeks. Blood was collected at 10 days after the second immunization. As secondary antibodies, HRP-conjugated goat anti-rabbit IgG (Rockland, Gilbertsville, PA, USA) or goat anti-rat IgG (Chemicon International Inc., Temecula, CA, USA) were used.

For pre-incubation study, the serum was pre-incubated with various concentrations of the compounds in wells blocked with 1% BSA/PBS. After 1 h incubation at room temperature, serum was added to the plates as a sample.

RESULTS

The patient confirmed that cefotiam (CTM) was one of the causal drugs. Therefore, CTM was tested first in the ELISA system. We successfully detected IgE antibody reacting equally with wells coated with this compound diluted in both saline and 0.1 mol/L $NaHCO_3$ (pH 8.3) (data not shown). Since the structure of CTM contains aminothiazole and tetrazole side chains, we selected CPR and CMZ, each of which has one common side chain with

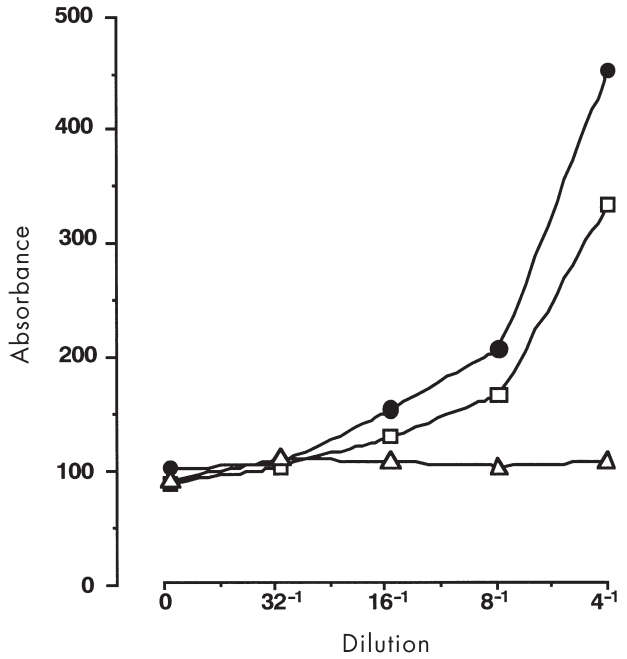

Fig. 1 Detection of IgE antibody to cefotiam (●) and cefpirome (□) but not cefmetazole (△) in the patient's serum by enzyme-linked immunosorbent assay.

CTM, to test the specificity. We found that the patient's serum IgE reacted with wells coated with CTM or CPR but not with CMZ. A representative result is shown in Fig. 1. The structures of the three compounds are shown in Fig. 2. As shown in Fig. 3, anti-CTM IgE antibody was detected in the serum of the patient described above, but not in the two atopic asthmatic patients nor in the healthy volunteer. None of these sera reacted with wells coated by CMZ. Further reactivity was tested and it was found that the patient's serum contained IgE reacting with wells coated by ceftazidime (CAZ).

To confirm the immobilization of antibiotics on the plate, we applied immune sera in this ELISA system (Table 1). Anti-cephalosporin C antibody reacted with wells coated with CTM but not CPR, CAZ or CMZ, while anti-CPR immune serum reacted with wells coated with CPR or CAZ, but not CTM or CMZ. Anti-CMZ immune serum did not react with wells coated with either CTM, CPR, CAZ or CMZ. Therefore, we concluded that CTM, CPR and CAZ, but not CMZ, could be immobilized directly to the surface of the plates.

The structure of the ligand for the IgE antibody was

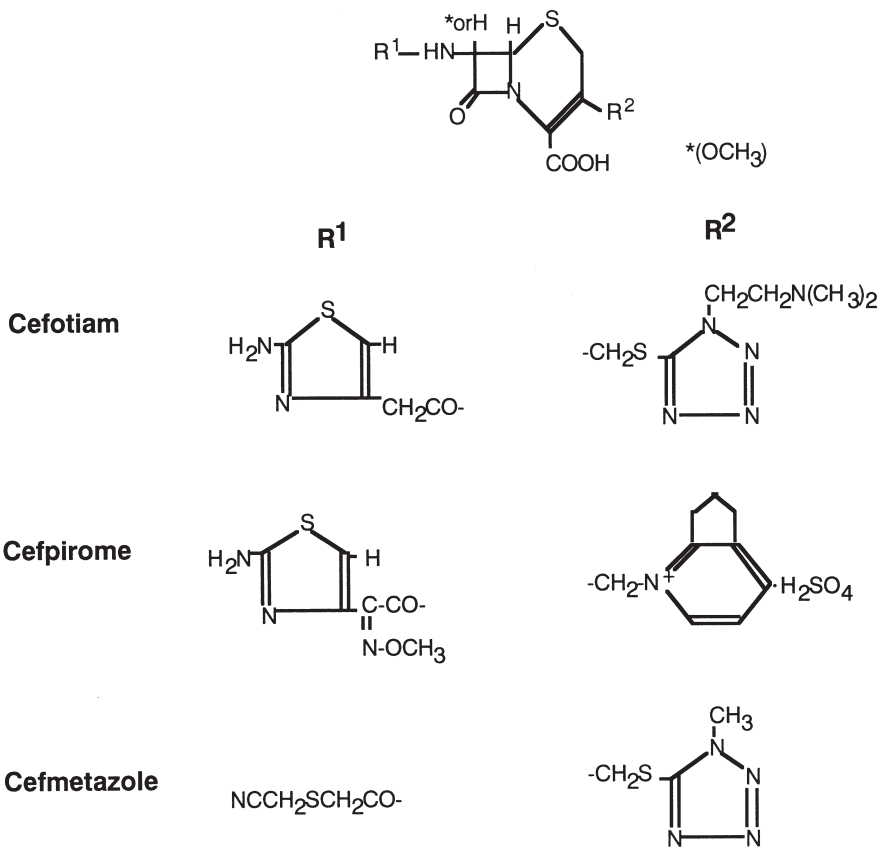

Fig. 2 Molecular structures of cefotiam, cefpirome and cefmetazole.

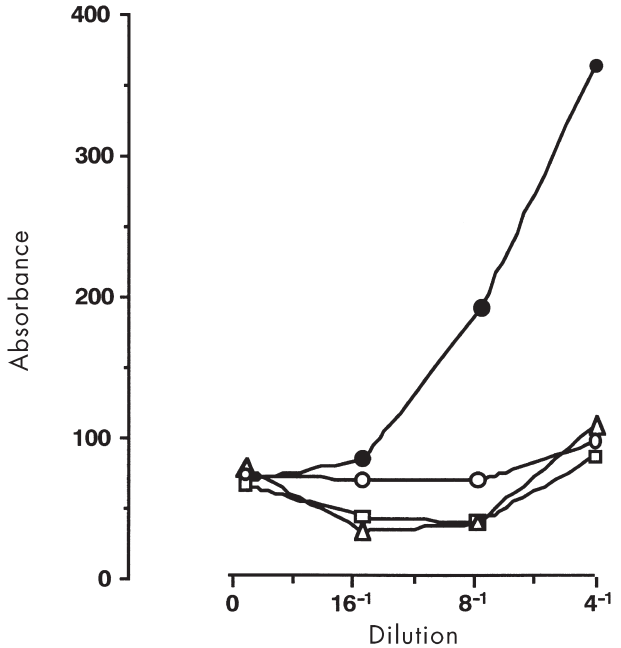

Fig. 3 The IgE antibody to cefotiam was detected in the present patient (●) but not patients with elevated levels of IgE or normal control. Patient 1 (□): bronchial asthma, 37-year-old male, serum IgE of 1383 IU/L. Patient 2 (△): bronchial asthma and atopic dermatitis, 41-year-old female, serum IgE of 4370 IU/L. Normal (○): no allergic diseases, 26-year-old male.

Table 1 Reactivity of various immune sera to immobilized antibiotics

Immune serum	CTM	CPR	CMZ	CAZ
Anti-cephalosporin C	O	X	X	X
Anti-CPR	X	O	X	O
Anti-CMZ	X	X	X	X

X, negative; O, positive; CTM, cefotiam; CPR, cefpirome; CMZ, cefmetazole; CAZ, ceftazidime.

confirmed by the absorption studies. Pre-incubation with CTM completely absorbed the reactivity to CTM and CPR. Similarly, pre-incubation with CPR, aminothiazole and amino-thiazoleacetic acid, but not tetrazole, markedly diminished the reactivity to CTM and CPR (Fig. 4). The inhibition of aminothiazole was dose dependent as shown in Fig. 5. There were no differences in the reactivity of the anti-cephalosporin C antibody to CTM-coated wells that had been pre-incubated with AT, CTM, or saline, indicating no possibility that the addition of free AT or CTM can remove immobilized CTM. The structures

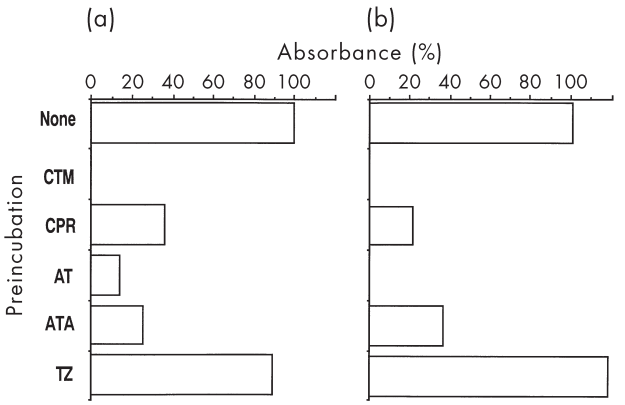

Fig. 4 Pre-incubation studies. The patient's serum was tested for IgE antibody to (a) cefotiam (CTM) and (b) cefpirome (CPR), following pre-incubation with CTM, CPR, aminothiazole (AT), amino-thiazoleacetic acid (ATA) and tetrazole (TZ) at concentrations of 300 μg/mL. The value of IgE in serum just left for the pre-incubation time was used as positive control (100%).

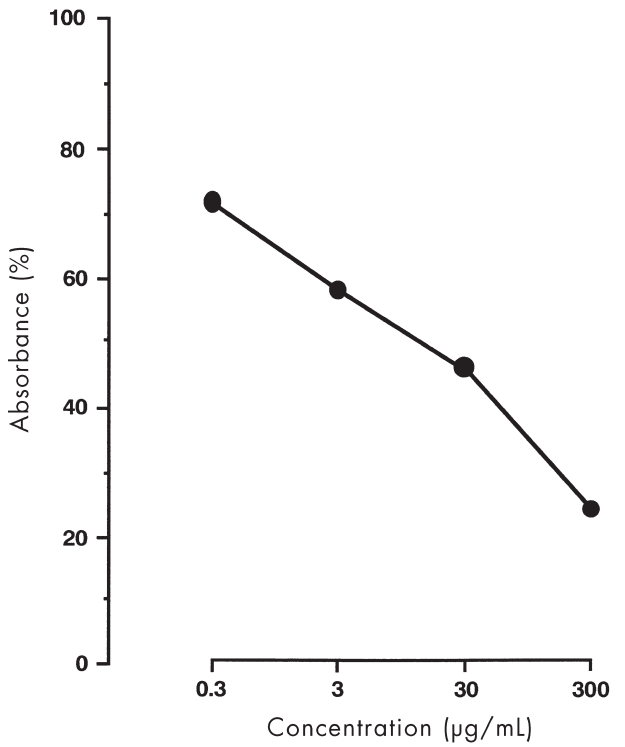

Fig. 5 Pre-incubation of serum with aminothiazole at various concentrations. The patient's serum was tested for IgE antibody to cefotiam (CTM). Aminothiazole showed a dose-dependent inhibition for the reactivity to CTM. The value of IgE in serum just left for the pre-incubation time was used as positive control (100%).

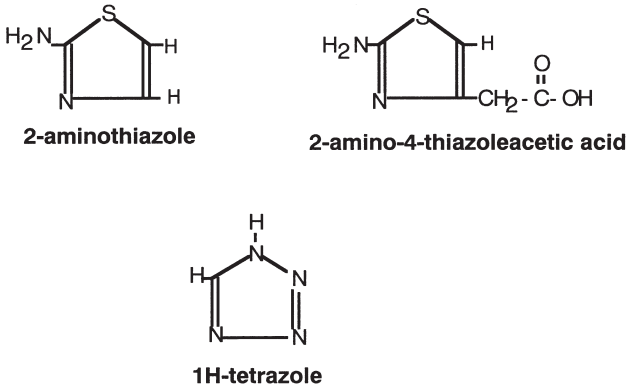

Fig. 6 Molecular structures of aminothiazole, aminothiazoleacetic acid and tetrazole.

of the compounds used in this pre-incubation study are shown in Fig. 6.

Discussion

In the present study, we tried to immobilize antibiotics alone (i.e., without conjugation with protein) for an ELISA system to test whether specific IgE could be detected in this system. Direct detection using serum from a patient with drug-induced contact urticaria demonstrated IgE antibody reacting with the aminothiazole side chain of the cephalosporins. Because the coating of the solid phase was done by simple adsorption, it was essential to ensure sufficient absorption by a plastic surface. By using anti-cephalosporin C and anti-CPR immune serum, we confirmed the immobilization of CTM, CPR and CAZ. Thus, conjugation with protein, such as human serum albumin, may not be needed for these antibiotics at least. From the results of direct detection, there is a possibility that the serum of our patient had reactivities other than the aminothiazole side chain. However, inhibition studies revealed that the aminothiazole side chain would be a major antigenic determinant for the reactivity to CTM or CPR.

The present ELISA system has some obvious limitations. First, immobilization could not be achieved for all antibiotics. Our results indicated that certain antibiotics such as CMZ cannot be immobilized. Second, this method cannot be applied to new determinants formed by interaction between compounds and carrier protein, such as albumin. Mizutani *et al*. have reported that the IgE fraction of their patient's serum contained IgE that recognized CTM linked to human serum albumin but not to CTM or albumin alone.[6] In spite of these limitations, omission of protein conjugation would be convenient for *in vitro* search for the responsible compounds in patients with IgE-mediated allergy to cephalosporin.

Several reports concerning antigenic site on cephalosporins have been published. Foti *et al*. have reported the results of a patch test with different cephalosporins in 18 occupationally exposed nurses with contact dermatitis.[7] Positive results were obtained in seven patients. The common structure was the tetrazolic group in six of them, and the aminothiazolyl group in one patient.[7] Similarly, in patients with drug-induced fever or skin eruption, the tetrazolyl group was reported to have a major responsibility in type IV allergy determined by the leukocyte migration inhibition test.[8] Therefore, a tetrazolyl group may be a major allergen in these patients.

Contact urticaria has been described for more than 20 years.[9,10] It can be primarily divided into immunologic and non-immunologic types. Because immunologic-contact urticaria may cause not only local skin lesion but also non-cutaneous involvement such as asthma and anaphylaxis, it is important for physicians to bear it in mind. Many agents are reported to cause immunologic-contact urticaria, including food, animals, plants, industrial chemicals and medicaments. Among the medical agents, antibiotics are unique because medical staff can be occupationally exposed to them. In Japan, there are 14 case reports of contact urticaria caused by occupational exposure to antibiotics, as reviewed by Shimizu *et al*.[11] All were nurses and, interestingly, a common causal drug was CTM. Because CTM at least can apply in the present ELISA system, this ELISA method would be useful for such patients, although simple immobilization cannot be achieved for all antibiotics. Among reported cases, IgE directed to CTM conjugated with HSA was demonstrated in only three cases.[6,12] All of these patients had IgE directing to tetrazolic ring on CTM. Using the prick test, suspected cross-reactivity to tetrazole was demonstrated in nine of nine cases in previous studies.[11,13–15] Therefore, the usual structure of cephalosporins reacting with IgE was tetrazolic ring. Our patient seems to be the first case demonstrating aminothiazole as an allergen for contact urticaria. However, it should be noted that the results may be influenced by the detection system, for which the present case was different from previous studies.

In conclusion, an ELISA system could be constructed with some particular antibiotics immobilized in solid phase without carrier protein conjugation. Using this system, we detected IgE antibody reacting with aminothiazole on

cephalosporin molecule; accordingly, it may be useful for detection of antibodies to drugs in patients with an allergic reaction to cephalosporins.

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