Original Article

**In vitro analysis of mediators of contact urticaria caused by cefotiam hydrochloride**

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**ABSTRACT**

Contact urticaria syndrome is a rare but potentially serious problem for hospital staff that handle antibiotics and other agents. While the cause is immunologic, little is known about the mediators involved. To investigate these mediators, three nurses with contact urticaria syndrome caused by exposure to cefotiam hydrochloride (CTM), and five normal controls were evaluated. IgE antibodies specific for CTM were detected by the radioallergosorbent technique (RAST). In response to CTM, cytokines were released from peripheral blood mononuclear cells (PBMC), and sulfidoleukotrienes were released from peripheral blood leukocytes. BAST counts in the three nurses with this syndrome exceeded those in the normal controls. The stimulation index of the leukotrienes released from the peripheral blood leukocytes in response to CTM also exceeded those in the normal controls. Higher levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) were also detected in the supernatant of the patients’ PBMC. The results suggest that contact urticaria syndrome caused by exposure to CTM was induced by an immunologic mechanism via IgE antibodies, and that GM-CSF and leukotrienes may be involved in this reaction.

**Key words:** antibiotics, CAST, cephalosporin, contact urticaria, cytokine, leukotriene, specific IgE

**INTRODUCTION**

Contact urticaria was first reported in 1972 by Fisher. It is classified as non-immunologic, immunologic or undefined origin. While the incidence was initially thought to be low, reports appear to be increasing, with numerous cases associated with handling foods, latex, disinfectants and antibiotics. Cefotiam hydrochloride (CTM), a cephalosporin, is one of the most commonly used parenteral antibiotics in Japan. We previously reported two cases of contact urticaria caused by CTM.

Although the mechanism of contact urticaria caused by antibiotics is generally considered to be immunologic, there has not been any precise examination. We aimed to clarify the mechanisms of contact urticaria related to CTM by measuring specific IgE against CTM using the radioallergosorbent technique (RAST) and evaluating the response of leukotrienes from blood leukocytes and of cytokines from mononuclear cells in peripheral blood to in vitro exposure to CTM.

**METHODS**

We evaluated three nurses aged 24, 26, and 27 years who had come to our outpatient department of dermatology at Kyushu University Hospital with contact urticaria caused by contact with CTM. We previously reported about these patients in 1993. They had developed generalized urticaria, abdominal pain, or a shock reaction soon after touching a solution of CTM. Each of the nurses had a history of hand eczema characterized by scaly erythema and fissura and showed a strongly positive flare-and-weal reaction in open or closed patch testing for CTM for 20 min. The lymphocyte stimulation test (LST) for CTM was positive in all three patients. After we had obtained informed consent, we collected peripheral blood by venepuncture for study. At the same time, peripheral blood was obtained from five healthy adults as controls. The CTM preparation used was Halospore (Ciba-Geigy Co., Basel, Switzerland).

**IgE antibodies specific for CTM**

CTM was conjugated with human serum albumin (HSA) by carbodiimide. Briefly, 46.0 mg CTM was mixed with 32.6 mg HSA in 13 mL ion exchanged water for 60 min at pH 6. The mixture was dialysed against 0.02 mol/L phosphate-buffered saline (PBS). Conjugation of CTM with HSA was confirmed photo-metrically. The CTM-HSA conjugates were then coupled to cyanogen bromide (CNBr)-activated filter paper dishes. Serum samples of 50 μL were applied to paper dishes for 3 h. RAST was then performed using RAST kits (Shionogi Pharmaceutical Co. Ltd., Osaka, Japan). Results of RAST were expressed as % RAST count, calculated as follows:
% RAST count = \( \frac{125\text{I}-\text{anti IgE bound (cpm)}}{125\text{I}-\text{anti IgE bound (cpm) x 100}} \)

In the RAST inhibition assay, varying concentrations of CTM (1.5, 7.5, and 15 mg/mL) were added to the patient’s serum in equal volume, and RAST was then done.

**CAST-ELISA**

The cellular allergen stimulation test (CAST) was performed. Dextran solution 0.5 mL was added to 2 mL of the patients’ blood, and the mixture was incubated for 90 min at 20–30°C. After centrifugation, sedimented leukocytes were resuspended in 2 mL of stimulation buffer containing interleukin (IL)-3. Then 50 µL of varying concentrations of CTM (10, 50, and 100 µg/mL) was added to 200 µL of the cell suspension, and incubated for 40 min at 37°C. After centrifugation, the cell-free supernatant was collected. The sulfidoleukotrienes (sLT), leukotriene (LT)C4 and its metabolites LTD4 and LTE4, that were released into the medium were measured simultaneously in an enzyme-linked immunosorbent assay (ELISA) using ELISA kits (Buhlmann Laboratory AG, Allschwil, Switzerland). Results were expressed as the stimulation index (SI):

\[ SI = \frac{\text{value of released sLT in response to CTM stimulation}}{\text{control value of released sLT with buffer stimulation}} \]

**Cytokines**

Peripheral blood mononuclear cells (PBMC) were obtained by densimetric centrifugation and suspended in RPMI medium. The PBMC suspension (10⁶/1.5 mL) was cultured with or without 0.1 mL of varying concentrations of CTM (10, 50, and 100 µg/mL) for 72 h at 37°C in a humidified atmosphere containing 10% CO₂. After centrifugation, the cell-free supernatant was supplied for the following assay. Interferon-γ (IFN-γ), IL-2, GM-CSF, and IL-4 activities were determined in a solid-phase ELISA using ELISA kits (Otsuka Co. Ltd, Tokyo, Japan). Results are shown as the ratio of the values with and without the antigen.

**Statistical evaluation**

Student’s t-test was used to test for differences among the groups.

| Table 1. Serum IgE antibodies specific for cefotiam hydrochloride (CTM) and test for released leukotrienes and granulocyte-macrophage colony-stimulating factor (GM-CSF) in response to CTM in vitro. |
|-----------------|-----------------|-----------------|-----------------|
| Patient no.    | CTM-specific IgE (R.AST) | CAST (S.I.) | GM-CSF release (Ratio) |
| 1              | 10.0            | 1.65           | 4.71           |
| 2              | 6.8             | 1.99           | 4.00           |
| 3              | 2.0             | 1.21           | 3.40           |
| Patient (mean ± s.d.) | 6.27 ± 4.03* | 1.62 ± 0.39*  | 4.04 ± 0.65** |
| Control (mean ± s.d.) | 0.88 ± 0.08 (n=5) | 0.90 ± 0.39 (n=5) | 1.51 ± 0.40 (n=4) |

R.AST, Radioallergosorbent technique; CAST, Cellular allergen stimulation test.

* P<0.05 vs control; ** P<0.01 vs control;

**RESULTS**

The % RAST counts of the serum from the three patients were 10.0, 6.8 and 2.0% (6.27±4.03; mean ± s.d.), while those of the five normal controls were 0.9, 1.0, 0.8, 0.8 and 0.9% (0.88±0.08; mean ± s.d.) (Table 1). There was a significant difference between the two groups (P<0.05). In RAST inhibition assay, pre-incubation with CTM reduced the % RAST counts in the three patients to the level seen in the normal controls at the concentrations [data not shown].

We took the highest SI in the three concentrations of CTM solution as the SI value of the CAST-ELIZA. The SI values in the three patients were 1.65, 1.99 and 1.21 (1.62±0.39; mean ± s.d.), while those of the controls were 0.95, 0.69, 0.52, 1.00 and 1.35 (0.90±0.32; mean ± s.d.). There was a significant difference between the two groups (P<0.05).

High levels of GM-CSF were detected in the patients’ PBMC in response to CTM stimulation. The values were 4.71, 4.00 and 3.40 (4.04±0.66; mean ± s.d.), while the values in the controls were 1.35, 1.67, 1.97 and 1.04 (1.51±0.40; mean ± s.d.), and the difference was significant (P<0.01; Table 1). IL-2 and IL-4 were not detected, and there were no changes in the levels of IFN-γ either with or without CTM stimulation (data not shown).

**DISCUSSION**

Contact urticaria syndrome is an immediate type of contact reaction that usually accompanies a systemic reaction such as asthma, abdominal pain, and even shock, upon exposure to the offending substance. In Japan, there were only a few reports of contact urticaria induced by penicillin or streptomycin until 10 years ago but about 20 cases of contact urticaria syndrome caused by antibiotics, including CTM, have been reported since. All of the cases were nurses; nearly all had a history of hand eczema, and the symptoms were relatively severe. Although in some cases two or more antibiotics were the causative agents CTM was the only agent common in all cases.

Tadokoro et al. reported contact anaphylaxis in two nurses caused by CTM, and showed the presence of CTM-specific IgE in their serum. We also demonstrated specific IgE against CTM in the serum of three patients, suggesting that the reaction might be mediated, to a certain extent, by IgE antibodies.
CAST is a new diagnostic method that is based on the production of sulfidoleukotrienes by peripheral blood leukocytes upon challenge with allergens.\textsuperscript{12,13} Results are considered to reflect the intensity of the hypersensitivity. The sLT are produced by mast cells, basophils, and stimulated eosinophils, monocytes and endothelial cells.\textsuperscript{13} In our study, sLT were released from sedimented leukocytes in response to CTM, including the basophils and eosinophils, which are the main sources of sLT. Basophils have IgE antibodies attached to their surface. Eosinophils express the high-affinity IgE receptor, FccRI.\textsuperscript{14,15} Considering that all three patients showed serum levels of CTM-specific IgE antibodies, it appears that sLT may be involved in the reaction to CTM.

Cytokines are involved in the IgE-mediated immediate type of hypersensitivity reaction. IL-4 accelerates the production of IgE antibodies from B cells.\textsuperscript{17} IL-5 is related through the eosinophils to the late phase reaction of immediate hypersensitivity.\textsuperscript{18} We detected only GM-CSF in the supernatant of the patients' PBMC in response to CTM. GM-CSF, which is mainly secreted by the helper T cells and monocytes, exerts haematopoietic effects on the granulocytes including the eosinophils and basophils, and accelerates the production of LTC\textsubscript{4} in the eosinophils.\textsuperscript{19} It is therefore suggested that sLT are released from the basophils and eosinophils which were activated by GM-CSF, and that these mediators are involved in contact urticaria syndrome.

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REFERENCES