LEUCOCYTE MIGRATION INHIBITION TEST AND ELISA IN PATIENTS WITH SUSPECTED BETA-LACTAM ANTIBIOTIC ALLERGY

Naoki Nagakura[1], Tadayori Shimizu[1], Yasutake Yanagihara[1], and Katsuji Uno[2]

1) : Department of Microbiology, School of Pharmaceutical Sciences, University of Shizuoka, 395 Yada, Shizuoka 422, Japan

2) : Pharmacy, Suibarago Hospital

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We studied the role of immediate and delayed type hypersensitivity (DTH) in beta lactam-induced hypersensitivity by performing a leucocyte migration inhibition test (LMIT), which proves the development of DTH, and by measuring serum antibodies by enzyme linked immunosorbent assay (ELISA).

1. An LMIT was performed in 89 patients with suspected beta lactam antibiotic allergy (50 with drug rash, 29 with drug fever, 24 with hepatopathy, 12 with eosinophilia and 2 with anaphylaxis, some patients having two or more concomitant symptoms). The LMIT was positive at a high rate of 74 % (66 cases).

2. In contrast, ELISA was positive in only 8 % (7 cases), with IgG in 2 and IgM in 5 cases.

3. The LMIT results showed high correlation for the 35 drugs tested, except for latamoxef, which was positive in only one of four cases. In contrast, the ELISA was positive at a high rate for ceftizoxime (3/5 cases) and cephalexin (2/4 cases).

4. The LMIT was positive in 56−100 % in patients with symptoms other than urticaria, while ELISA was positive in only 0−20 % of the symptoms.

5. The seven patients who showed a positive response in the ELISA were also positive in the LMIT, suggesting that cellular and humoral immunity play a role in some patients with beta-lactam antibiotic allergy.

Key words : Beta-lactams, Hypersensitivity, LMIT, ELISA

INTRODUCTION

Allergic reactions are a common side effects of beta-lactam antibiotics. The pathogenic mechanisms of allergic reactions are generally divided into the two categories of immediate-type hypersensitivity, associated with humoral antibody-mediated immunity, and delayed-type hypersensitivity (DTH), associated with cell-mediated immunity, although Coombs and Gell[1] proposed a classification into four categories. At present, all four categories are thought to play a role in beta-lactam induced allergic reactions[2]. However, the relation between cellular and humoral immunity in patients with beta-lactam induced hypersensitivity is still obscure.

We performed a leucocyte migration inhibition test (LMIT), which shows the developement of DTH, and an enzyme-linked immunosorbent assay (ELISA), which detects the presence of serum antibodies, in 89 patients with suspected beta-lactam hypersensitivity in order to investigate the relation between cellular and humoral immunity. We also compared the positive results of an LMIT and ELISA according to the 35 drugs used and the different symptoms in the patients.

PATIENTS AND METHODS

Subjects. The subjects comprised 89 patients (46 males and 43 females) with allergic manifestations thought to have been caused by beta-lactam antibiotics in the Suibarago Hospital from 1985 to 1989. The group consisted of (i) those with suspected drug rash manifested by eruptions between days 1−25 after administration of the drug began, (ii) those with suspected drug-induced hepatopathy manifested by elevated transaminase (GOT and GPT) levels on days 10−40 after drug treatment was initiated, and (iii) those with suspected drug fever manifested by temperature elevations on days 7−14 after the commencement of drug administration, which subsided within 48 h after the drug was discontinued. From clinical aspect, the patients suspected of hypersensitivity induced by drugs other than beta-lactams were excluded. The patients who were given anti inflamatory drugs or steroids were also excluded. The control group consisted of 28 patients who had been given beta-lactams between 5 and 19 days but nevertheless displayed allergic reactions. The immediate-type skin reaction determined by intradermal injection of beta-lactams was negative in all these patients.

LMIT An LMIT was performed by an improved version of the agarose plate method of Clausen[3]. The lymphocytes were collected from the patients' heparinized peripheral blood samples with Ficoll-Conray solution (Pharmacia Fine Chemicals, Uppsala, Sweden), washed with Hanks' solution, suspended and adjusted to a cell count of 1×10^6 cells/ml in TC-199 medium (GIBCO, Grand Island Biological Co., USA) supplemented with 10 % horse serum and 10 mM HEPES buffer. The antigen solution (a mixture of 100 μl heat-inactivated serum from the patient and 100μl of a solution of the tested drug) was added to 800 μl of the lymphocyte suspension and incubated at 37 ℃ for 24−48 h in a rotary incubator. The supernatant was separated, frozen, and stored at −20 ℃ until used. The optimum concentration of beta-lactam antibitotic used in this fluid as antigen was 250 μg/ml, as determined by previous reports[4,5]. Next, leucocyte-rich plasma was separated from heparinized normal human peripheral blood with physiological saline containing 5 % dextran and washed with Hanks' solution, thus obtaining the leucocytes for a migration test. These leucocytes were suspended and adjusted to a density of $2.5 \cdot 10^8$ cells/ml in the supernatant which had been frozen and stored as described above; 7 μl samples of this suspension were placed in each well of an agarose plate prepared from a culture medium containing 1 % agarose, and after incubating at 37℃ in a moist chamber, the area of the field of migration of the leucocytes was measured with an immunoviewer.

The testing criteria were as follows. Migration tests were performed in the above described manner with leucocytes from normal individuals suspended in culture fluid aliquots with 250 μg/ml concentrations of the various beta-lactam antibiotics under consideration. For each antibiotic, the migration index (MI) of the normal individuals was calculated from formula (A) below, and the mean ± 2 SD (n= 6) of these values was regarded as the normal range (NR). The MI values of the patients were calculated from formula (B). If the MI of the patients was below the normal range, this was interpreted as indicating the presence of leucocyte migration inhibiting factor, and the test was then regarded as positive. Also, if the patient's MI was above the NR, this was attributed to the presence of leucocyte migration activating factor, and interpreted as indicating the development of DTH with a weak degree of sensitivity; in this case, the result was classified as weakly positive[6~8]

(A) The MI of leucocytes from normal humans (%) = migration area in medium with drug/migration area in medium without drug × 100

(B) The MI of leucocytes from patients (%) = migration area in medium with drug/migration area in medium without drug × 100

Preparation of beta-lactam-protein conjugates Beta-lactam-carrier conjugates (alkaline-conjugates) were prepared according to the method of Iwata et al. [9]. In brief, human serum albumin (HSA: Pasel, West Germany) as carrier and 35 drugs (listed in Table 1) as hapten were used. Beta-lactams were added at the ratio of 5 to 1 (w/w) protein and the pH was kept at 10−11 with 5N NaOH for 24 h at 37 ℃. The free beta-lactam derivatives were removed

from the conjugates by dialysis against phosphate buffered saline (PBS) for 4 days and distilled water for 24 h, and then lyophilized.

ELISA The ELISA was performed by the following method. Microplates of 96-wells (Falcon, No. 3915) were coated with 50 μl of beta lactam carrier conjugates solution (10 μg/ml) overnight at 4 ℃. Each well was washed with 0.05 % Tween 20-Dulbecco's PBS and blocked with Block Ace® (Dainippon Pharmaceutical, Co., Osaka) at room temperature for 1 h. The wells were washed and serial dilutions of the patients' serum were added to each well for 2 h at 37 ℃. The plates were washed with 0.05 % Tween 20-PBS, and 50 μl of peroxidase conjugated anti-mouse IgE, IgM, or IgG (Capple, Organon Teknika Corp., USA) was added. After incubation at 37 ℃ for 2 h, the plates were washed and a substrate solution (0.56 mg of 2,2'-azino-bis(3-ethyl-benzthiazolin-sulfonate per ml, 0.07 μg of H_2O_2 per ml) was added. After incubation at 37 ℃ for 15 min, the optical density (OD) was measured at 415 nm with an EIA reader (Corona Electric Co., Tokyo).

By using the standard deviation ratio method[10], antibody titer was determined as follows:
The OD of the reaction between patient's serum and beta-lactams = (OD of the reaction between patient's serum and beta-lactam-HSA conjugate) − (OD of the reaction between patient's serum and HSA).

RESULTS

Positive rates of LMIT and ELISA according to the difference in drugs The results of LMIT and ELISA according to the difference in drugs are shown in Table 1. In six penams, the positive rates of LMIT and ELISA in 14 cases were 71 % (10 cases) and 7 % (1 case), respectively. On the other hand, in 26 cephems, the positive rates of LMIT and ELISA in 69 cases were 72 % (50 cases) and 9 % (6 cases). Furthermore, two monobactams caused positive reactions in the LMIT in all 4 cases, not in the ELISA, while imipenem/cilastatin also showed positive in 2 cases in the LMIT but in the ELISA were negative.

According to drug, the positive rates of LMIT in 34 drugs, except latamoxef which showed positive in one case out of 4 cases, were almost the same. By contrast, each positive rate of mezlocillin (1/1) cephaprin (1/1), ceftizoxime (3/5) and cephalexin (2/4) in ELISA was high, suggesting that the positive rate of ELISA is affected by the kind of drug.

Positive rates of LMIT and ELISA according to symptoms The positive rates of LMIT and ELISA according to symptoms are shown in Table 2. In the 50 patients with suspected beta lactam hypersensitivity, the positive rate of the LMIT in cases with skin eruption was 72 % (36 cases), and 12 % (6 cases) in ELISA. In the 32 patients with eruption only, the positive rate of the LMIT was 56 % (18 cases) and of the ELISA was 9 % (3 cases), and all 18 cases with concomitants were positive in the LMIT but in the ELISA only 17 % (3 cases) were positive.

The above results indicate that the patients with concomitant symptoms showed a higher positive rate than the patients with skin eruption only, and the positive rate of the ELISA showed the same tendency. The LMIT in the patients with erythematous and fixed eruptions exhibited a high positive rate of 88 % (23/26) and 100 % (5/5), whereas the LMIT of the cases with urticaria showed a low positive rate of 42 % (8/19). On the other hand, 2 cases of erythema, 2 of urticaria, and one of fixed eruptions showed positive in ELISA.

In pyrexia, the positive rate of the LMIT was 93 % (27/29), but only one case with concomitant symtoms was positive in ELISA. The number of positive cases of pyrexia alone was 5 of 7 in the LMIT, but 22 cases with concomitant symptoms were all positive. Twenty out of 24 cases with hepatopathy were positive (83 %) in the LMIT, but only one case was positive in the ELISA. The LMIT was positive in 11 of 12 cases (92 %) with eosinophilia while ELISA was positive in only one case. Of the patients with anaphylactic shock, 50 % were positive (1/2) in the LMIT, but negative in the ELISA.

Relation between symptoms and antibody titer in ELISA-positive patients Of seven ELISA-positive patients, 5 cases were female and 6 displayed leucocyte migration activating factor in the LMIT (Table 3). The immunoglobulin class of antibody detected was IgM (5 cases) and IgG (2 cases), but IgE

Table 1 1. Positive rate of LMIT and ELISA by difference of drugs in 89 patients with suspected of beta lactam antibiotic allergy

Beta-lactam preparation	Drug	Case	Number (%) of LMIT positive cases	Number (%) of ELISA positive cases
Penams		14	10 (71)	1 (7)
	Ampicillin[a]	8	4	0
	Amoxicillin	1	1	0
	Carbenicillin	1	1	0
	Flucloxacillin	1	1	0
	Mezlocillin	1	1	1
	Piperacillin	2	2	0
Cephems		69	50 (72)	6 (9)
	E 1040	4	2	0
	FK 482	1	1	0
	Cefodizime	6	6	0
	Cephapirin	1	1	1
	Cefsulodin	1	0	0
	Cefazolin	2	1	0
	Cefoxitin	2	1	0
	Cefminox	2	2	0
	Cefmenoxime	1	1	0
	Cefmetazole	1	1	0
	Cefpimizole	1	1	0
	Cefpiramide	3	3	0
	Cefoperazone	7	5	0
	Cefotiam	3	1	0
	Cefotetan	2	2	0
	Cefotaxime	2	2	0
	Ceftazidime	3	2	0
	Cefuzonam	4	3	0
	Ceftizoxime	5	4	3
	Latamoxef	4	1	0
	FMOX	2	1	0
	CPZ, SBT	1	0	0
	Cephalexin	4	4	2
	Cefaclor	3	2	0
	Cefatrizine	3	2	0
	CPDX-PR	1	1	0

[a] Including talampicillin

FMOX: flomoxef sodium, CPZ: cefoperazone, SBT: sulbactam, CPDX-PR: cefpodoxime proxetil

Table 1-2. Positive rate of LMIT and ELISA by difference of drugs in 89 patients with suspected of beta lactam antibiotic allergy

Beta lactam preparation	Drug	Case	Number (%) of LMIT positive cases	Number (%) of ELISA positive cases
Monobactams		4	4 (100)	0
	Aztreonam	2	2	0
	Carumonam	2	2	0
	IPM/CS	2	2 (100)	0
Total		89	66 (74)	7 (8)

IPM/CS, imipenem/cilastatin sodium

Table 2. Positive rate of LMIT and ELISA according to allergic symptoms in patients suspected of beta lactam antibiotic allergy

Allergic symptom	Number of tests	Number (%) of LMIT positive cases	Number (%) of ELISA positive cases
Skin eruptions	50	36 (72)	6 (12)
Eruptions only	32	18 (56)	3 (9)
With concomitant symptoms	18	18 (100)	3 (17)
Erythematous	26	23 (88)	2 (8)
Urticarial	19	8 (42)	2 (11)
Fixed	5	5 (100)	1 (20)
Fever	29	27 (93)	1 (3)
Fever only	7	5 (71)	0
With concomitant symptoms[a)]	22	22 (100)	1 (5)
Hepatopathy	24	20 (83)	1 (4)
Hepatopathy only	15	12 (80)	1 (7)
With concomitant symptoms	9	8 (89)	0
Eosinophilia	12	11 (92)	1 (8)
Anaphylaxis	2	1 (50)	0
Total	89	66 (74)	7 (8)

a) The same patients are counted in two or more categories in the case of concomitant symptoms of skin eruption, fever or hepatopathy.

Table 3. Clinical data of ELISA positive patients

Patient	Age	Sex	Administered antibiotics	Allergic symptom	LMIT	ELISA (titer)
P-5	8	F	Cephalexin	Urticaria	I	IgM (320)
P-40	20	F	Cephalexin	Hepatopathy	A	IgG (640)
P-48	7	F	Ceftizoxime	Erythema	A	IgM (640)
P-51	12	F	Ceftizoxime	Erythema	A	IgM (640)
P-63	41	M	Ceftizoxime	Fever, Thrombopenia	A	IgM (640)
P-74	69	F	Mezlocillin	Urticaria	A	IgG (80)
P-84	46	M	Cephapirin	Fixed, Fever	A	IgM (320)

F: female, M: male, I: migration inhibition, A: migration activation

was not detected. Serum showed a relatively high titer of 80-fold (1 case), 320-fold (2 cases) and 640-fold (4 cases).

DISCUSSION

In this experiment, the positive rates of LMIT and ELISA in 89 patients with suspected beta-lactam antibiotic allergy were 74 % and 8 %, suggesting that DTH plays an important role in beta-lactam antibiotic allergy. The above results agree with those quoted in our previous report[11] regarding the presence of detectable antibodies by haemagglutination test in patients with suspected beta-lactam-induced hypersensitivity, which was rare in the ELISA but the positive rates of the LMIT showed a very high incidence.

From the results of the LMIT according to drug, the positive rate of patients given latamoxef was lower than those of other beta-lactams, for which no differences in positive rates was observed. In contrast, ceftizoxime and cephalexin showed high positive rates in the ELISA. The reason for the difference may be connected with the chemical structures of the beta-lactams. But this is unclear, and further research is needed.

According to the difference in symptoms, 23 of 26 patients (88 %) with erythematous skin eruptions were positive in the LMIT and 5 patients with fixed skin eruptions, indicating that DTH is also important in these symptoms. Further, in the LMIT the positive rates in patients with hepatopathy were 83 % (20/24), but they were very low in patients with urticaria. The DTH seems to be responsible for the expression of the drug-induced hepatopathy, and there are several papers showing an enhancement of the positive rate of the lymphocyte stimulation test[12,13]. The positive rates of the LMIT also exhibited a high incidence of the drug-induced hepatopathy[14]

When we carried out an ELISA using normal human serum as control, anti-beta-lactam-antibodies were detected in 6 of 12 patients with urticaria induced by beta-lactams (unpublished data). In contrast, anti-penicillin antibodies have been detected in patients with no symptoms of beta-lactam induced hypersensitivity[15]. Our ELISA detected antibodies in only 2 of 19 patients with urticaria, using the sera of patients given beta-lactams as control. Since an immediate-type skin reaction test provide negative in all these patients, a method of identifing sensitizing drugs should be established for patients with urticaria.

Seven patients in whom antibodies were detected in the ELISA, showed positive reactions in the LMIT. Thus in some patients, there appears to be an association between cellular and humoral immunity and the expression of hypersensitivity. The immunoglobulin class of antibodies detected was IgG and IgM, no IgE having been found. Since these patients were all negative in the immediated-type skin reaction, the role of IgE in the symptoms is likely to be small. Five of 7 ELISA-positive patients were female and the migration-activating factor

was detected in 6 patients in the LMIT. Our results indicate the necessary of further investigations on the relation between the positive rates of LMIT and ELISA in patients with suspected beta lactam antibiotic allergy.

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β-ラクタム剤過敏症疑疹患者における酵素抗体法による血中特異抗体の検出と白血球遊走阻止試験の比較

永倉 直樹[1]・清水 忠順[1]・柳原 保武[1]・宇野 勝次[2]

[1]：静岡県立大学薬学部微生物学教室*

[2]：水原郷病院薬剤科

Enzyme linked immunosorbent assay (ELISA) による血中抗体測定と delayed-type hypersensitivity (DTH) の成立を証明する leucocyte migration inhibition test (LMIT) を併用することにより，β-ラクタム剤過敏症における即時型過敏症反応と DTH 反応の関与を検討した。

1．即時型皮内反応が陰性を示したβ-ラクタム剤過敏症疑疹患者89例（皮疹50例，発熱29例，肝機能障害24例，好酸球増多12例，ショック2例，各症状は重複している場合がある）を対象として調べたとき，LMIT では74％（66例）と高い陽性率を示した。

2　同じ患者血清での ELISA による陽性率は8％(7例)と低いものであり，2例が IgG 抗体を，5例に IgM 抗体が検出された。

3．試験を行った35薬剤中，LMIT では latamoxef が，4例中1例と陽性率が低かったが，他の34剤は，ほぼ等しい陽性率を示した。一方，ELISA では，ceftizoxime が5例中3例，cephalexin が4例中2例と高い陽性率を示した。

4　LMIT における蕁麻疹以外の症状を示す患者の陽性率は56から100％であったが，ELISA では症状にかかわりなく0から20％であった。

5．ELISA 陽性の7例は，全例，LMIT 陽性を示し，これら一部の患者では細胞性免疫と体液性免疫の両方が過敏症発現に関与することが示唆された。

*静岡市谷田395