

Examination of Patients Suspected as Having Hypersensitivity to Iodinated Contrast Media with Leukocyte Migration Test

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In vivo tests may be used for the diagnosis of allergy to iodinated contrast media (ICM); however, the tests do not provide definitive diagnosis and are associated with risks for patients. Diagnoses based on *in vitro* tests are limited, and there are almost no relevant studies. Herein, the authors examined involvement of allergic reaction from a multilateral standpoint in 39 patients suspected of having ICM allergies using leukocyte migration test (LMT). The positive rate of LMT was 44%. A comparison with the positive rate of LMT in drugs other than ICM (74%) indicated 30% difference, which was significantly low value, suggesting that there is poor involvement of these drugs in the allergic reaction. In LMT positives, 76% of hypersensitivity reactions were skin rash mainly erythema, and 18% was anaphylactic reactions. Cases considered as non-immediate hypersensitivity accounted for about 4 times as many as immediate-type hypersensitivity. In examination of relevancy between a history of drugs or food allergies, the incidence of ICM allergies was 35%. There is a high possibility that these adverse reactions were caused by pseudoallergy to drug. It was suggested that most hypersensitivity reactions were skin rash related to non-immediate hypersensitivity, and approximately 20% of the reaction was immediate anaphylactic reaction. Therefore attention should be paid not only to immediate-type hypersensitivity but also delayed reactions. Moreover, it was considered that patients with past history of drug or food allergies have a high potential for manifestation of the reactions.

Key words iodinated contrast media (ICM); leukocyte migration test (LMT); pseudoallergy; non-immediate hypersensitivity; allergic history

Since the late 1980s, a number of hypoosmotic, nonionic iodinated contrast media (ICM) have been used instead of ionic ICM. To meet this trend, development of serious adverse effects, such as pain, sensations of burning and anaphylactoid symptoms at the time of high-pressure injection, reduced significantly. On the other hand, allergic side effects caused by nonionic ICM became to attract attention, and not only immediate type hypersensitivity cases, which have a high severity, but also delayed hypersensitivity cases have been reported.^{1–5)} However, test methods used when ICM allergy is suspected are *in vivo* identification tests including patch test, intracutaneous reaction test, prick test and scratch test. As *in vitro* identification tests, radio-allergo-solvent test (RAST) using specific immunoglobulin E (IgE) antibody, histamine release test (HRT), basophil activation test (BAT) and drug-induced lymphocyte stimulation test (DLST) may be conducted. DLST are often used in clinical practice; however, clinical utility in the diagnosis of ICM allergy is not proved yet because of its low detection sensitivity. In other words, there is almost no research analysis that used *in vitro* identification methods for multiple cases to identify ICM allergy, and proved its utility.

Authors have reported that leukocyte migration test (LMT) is effective as a detection and identification method for hypersensitivity to drugs including drug eruption.^{6–10)} LMT is not a test method to identify cytokines or chemokines; however, we

have reported a possibility of involvement of a variety of cytokines to date.^{11–13)} Furthermore, LMT is more sensitive than DLST, and is also considered to have a high detection rate as an *in vitro* identification method.¹⁴⁾ Especially, a chemotaxis chamber, which was developed by the authors, has a higher sensitivity than existing agarose plate methods, and has been reported that this method has a high clinical utility.¹⁵⁾

Therefore, utility of LMT, involvement of allergic reaction, and relevancy to hypersensitivity reaction, latency period and past history of allergy were examined in patients suspected of being ICM hypersensitivity from a standpoint of *in vitro* identification methods by using LMT with high detection sensitivity.

MATERIALS AND METHODS

Subjects This study examined the possibility of hypersensitivity to ICM at Suibarago Hospital over a 20 year period (April 1991 to March 2010), after the matter was brought up by doctors. The pharmaceutical department was tasked with conducting LMT with a subject group of 49 patients. Of these patients, 10 patients, who indicated LMT positive to suspected drugs other than ICM, were eliminated, and 39 patients (18 males, 21 females) were included. The age was ranged from 25 to 79 years old (20's, 2 cases; 30s, 1 case; 40s, 7 cases, 50s, 10 cases; 60s, 10 cases; 70s, 9 cases), and the average age was 58.5±13.0 years old (mean±S.D.).

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Of the study patients, 9 patients (23%) had a past history of allergy, and the breakdown was 7 cases of drug allergy (30%) and 2 cases of food allergy (9%). In addition, there was no patient with allergic diseases such as asthma and atopic dermatitis.

The most common hypersensitivity reaction was skin rash (28 cases, 72%), and followed by anaphylaxis (including anaphylactoid symptoms, shock-like symptoms) (10 cases, 26%), and hepatic disorder (1 case, 3%). The LMT was conducted only with patients who had given consent, and after the significance of the test had been fully explained to the patients or their families. After doctors had requested LMT at the pharmaceutical department, patient information was gathered from various sources, including medical records held at the pharmaceutical department on hypersensitivity reactions, dosage periods and the like, as well as reports from attending physicians on patients suspected of susceptibility to side effects from drugs, and direct interviews with patients and their families. These steps involved in conducting an LMT are routinely systematized within the hospital. Informed consent satisfied "Ethical Guidelines for Epidemiology Research" of the Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare of Japan, and LMT was also obtained approval of the Ethical Committee of Suibaragou Hospital.

Suspected Drugs (Test Drugs) Suspected drugs used for 39 study patients were at least one drug and at most six drugs per one case, and 42 types of 85 drugs were used in total. As shown in Table 1, ICM (diagnostic products) include six types of 39 drugs, which consist of ionic ICM including ioxaglic acid (8 cases) and meglumine sodium amidotrizoate (1 case), and non-ionic ICM including iomeprol (8 cases), iopamidol (6 cases), ioversol (13 cases), and iotrolan (3 cases). Other drugs include 8 types of 13 drugs affecting metabolism, which are the highest number, and followed by 9 types of 11 drugs affecting the central nervous system, 6 types of 7 drugs affecting digestive organs, 6 types of 6 drugs of antibacterial drugs, and 7 types of 9 other drugs (suspected drugs are overlapped). Meanwhile, dose of these suspected drugs never exceeded the regular dose of each drug.

LMT Antigen Preparation Two types of drug antigen solutions were used for LMT. One antigen solution was prepared by dissolving bulk powder of each test drug into Hanks' balanced salt solution (HBSS; Gibco, Grand Island, NY, U.S.A.) and mixing 100 μ L of the drug solution with 100 μ L of culture medium (10% horse serum (Gibco), 10mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid (HEPES)

buffer added TC-199 medium (Gibco)) (antigen solution without patient serum; control solution was a mixed solution of 100 μ L of HBSS and 100 μ L of culture medium). Another antigen solution was prepared by mixing 100 μ L of the drug solution with 100 μ L of patient serum (antigen solution with patient serum; control solution was a mixed solution of 100 μ L of HBSS and 100 μ L of patient serum). Poorly soluble drugs were used by dissolving in ethanol or 0.1N sodium hydroxide solution, or dissolving in dimethyl sulfoxide (DMSO) and the diluted with HBSS so that the final concentration of DMSO becomes 1% or under. Phytohemagglutinin (PHA), which is a mitogen, was used at 1 μ g/mL of reaction concentration as a positive control to confirm the responsiveness of the test.

Maximum drug concentration ($C_{\max}$) of each test drug was used as the antigen concentration of the drug. However, if $C_{\max}$ of the drug was unknown, a concentration of 1/50000/mL and 1/10000/mL of a dosage were used for oral and injectable drugs, respectively. The drug antigens were prepared on the day of LMT. It was confirmed that concentrations of solvent and test drugs used do not exert immunological influence on the responsiveness of LMT induced by PHA stimulation.

Separation of Mononuclear Leukocytes and Reaction Culture LMT was conducted between 7d and 3 months after the discontinuation of test drug administration. In addition, this study was conducted such that drugs given during the test period or during treatment would not affect immunocompetence; the drugs used to treat the various hypersensitivity reactions and the drugs given subsequently did not have an effect on the LMT's reactivity. For the reaction between the patient mononuclear leukocytes and test drugs, mononuclear leukocytes (an intermediate layer) were collected from heparinized peripheral blood using specific gravity centrifugal method with Ficoll-Paque solution (Pharmacia Biotech, Uppsala, Sweden), and the separated mononuclear leukocytes after rinsing with HBSS were suspended into culture medium at a concentration of 1.25×10^6 cells/mL. Then, 800 μ L of this mononuclear leukocytes suspension was added to 200 μ L of antigen solution, and incubated in an incubator (BNA-111, ESPEC, Osaka, Japan) under 5% CO_2 at 37°C for 72–96h. Supernatant fluid was separated and stored at –20°C.

Migration Test Migration test was conducted using an agarose plate method (from April 1994 to March 2002) and chemotaxis chamber method (after April 2002). Heparinized peripheral blood from normal individuals was mixed with 1/4 volume of 5% dextran saline solution, incubated at 37°C for 40–60min, and then a leukocyte layer (supernatant liquid) was collected. Granulocytes (a precipitated layer) were collected from this supernatant liquid using specific gravity centrifugal method with Ficoll-Paque solution, contaminating red blood cells were hemolyzed with a lysing shock method, and then rinsed with saline solution to obtain leukocytes for migration test.

For an agarose plat method, leukocytes for migration test were suspended into previously separated reaction supernatant at a concentration of 2.5×10^8 cells/mL, and 7 μ L of the cell suspension was dispensed into each well (3mm in diameter) made of culture medium with 1% agarose on a plate. Then the plate was incubated in an incubator under 5% CO_2 at 37°C for 24h, and the area of leukocyte migration was measured with an immuno viewer (IMMUNO VIEWER-MU, Jokoh, Tokyo, Japan).

Table 1. The Suspected Drugs in Patients with Suspected Iodinated Contrast Media Allergies (Test Drugs for Leukocyte Migration Test)

Suspected drugs		Number of cases	Incidence (%)
Ionic type	Ioxaglic acid	8	20.5
	Meglumine sodium amidotrizoate	1	2.5
Non-ionic type	Iomeprol	8	20.5
	Iopamidol	6	15.4
	Ioversol	13	33.3
	Iotrolan	3	7.7
	Total	39	

For a chemotaxis chamber method, wells in a lower chamber of a chemotaxis chamber (96-well Chemotaxis Chamber AB96, Neuro Probe, Gaithersburg, U.S.A.) were filled with 30 μ L of culture medium, a membrane filter (Polycarbonate Filters PDF 5, Neuro Probe) was attached on the upper chamber, and the upper and lower chambers were coupled. Leukocytes for migration test were suspended into reaction supernatant at a concentration of 1×10^4 cells/ μ L. Then, 50 μ L of the suspension was dispensed into 4 wells of the upper chamber for each specimen, and incubated in an incubator under 5% CO₂ at 37°C for 90 min for migration. Leukocytes attached to the membrane between the upper and lower chambers were fixed with Diff-Quik fixing solution (Sysmex, Hyogo, Japan), stained with Diff-Quik staining solution (Sysmex); and then absorbance was measured at 490 nm using an immunoreader (Immuno Mini NJ-2300, Intermed Japan, Tokyo, Japan).¹⁵⁾

Determination Determination of agarose plate method was also conducted for normal individuals in a uniform manner. Migration index (MI)=(leukocyte migration area in leukocyte reaction supernatant with patient serum [antigen solution]/leukocyte migration area in leukocyte reaction supernatant with patient serum without patient serum [control solution]) $\times 100$ of the normal individuals was calculated, and a normal range (NR) was defined as the average MI ± 2 S.D. ($n=6$). MI value of a patient was 115 and above or 85 and less was considered as positive.

In LMT determination with chemotaxis chamber method, MI=(average absorbance of antigen solution with patient serum/average absorbance of antigen solution without patient serum) $\times 100$ was calculated, and positive was defined as when MI value was 60 and below or 150 and over, and significant difference of $p < 0.05$ was observed with Student's *t*-test for control group.¹⁵⁾

Analysis of LMT Results in Patients Suspected of Being Hypersensitivity to Drugs Other than ICM To examine involvement of allergic reaction and antigenicity of ICM, and relevance of a history of drug allergy and food allergy, investigation and analysis were conducted for drugs other than ICM based on LMT results implemented by authors during the past 20 years (April 1991 through March 2010). Especially, LMT positive rate of drugs, which are considered to induce drug allergy at high frequency^{6-8,10,14)} such as antimicrobial medias, non-steroidal anti-inflammatory drugs (NSAIDs), and enzyme preparations, were examined for each drug group. In addition,

a history of drug allergy and food allergy were also analyzed for each of the above 3 drug groups.

Statistical Analyses Statistical analyses of data were conducted with χ^2 -test, Fisher's exact probability test, and Student's *t*-test. Hazard ratio of below 5% ($p < 0.05$) was considered as a significant difference.

RESULTS

Results of LMT in Patients Suspected of Being ICM Hypersensitivity LMT was conducted with agarose plate method for 20 cases, and with chemotaxis chamber for 19 cases. Positive reaction was obtained 17 out of 39 cases in total (only ICM was positive for 12 cases, and ICM and other drugs were positive for 5 cases). A positive rate was 43.6%. As regard to the test methods, there were no major differences between both tests, although the value for the chemotaxis chamber (9/19 cases; 47.3%) was slightly higher than that for the agarose plate (8/20 cases; 40.0%). In addition, as shown in Table 2, the positive rate of LMT by drugs was 44.4% in ionic ICM: 3 out of 8 cases for ioxaglic acid, and 1 out of 1 case for meglumine sodium amidotrizoate indicated positive reaction. On the other hand, it was 43.3% in nonionic ICM: 4 out of 8 cases for iomeprol, 2 out of 6 cases for iopamidol, 6 out of 13 cases for ioversol and 1 out of 3 cases for iotrolan indicated positive reaction.

Positive Rates of LMT by Hypersensitivity Reactions

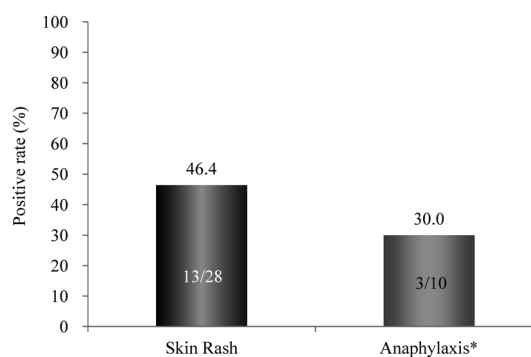


Fig. 1. The Proportions of Positive the Leukocyte Migration Test, Broken Down into Hypersensitivity Symptomatic Categories (Skin Rash and Anaphylaxis)

In addition, one case of hepatic disorder showed positive. *Anaphylaxis: In these cases, contain anaphylaxis-like or shock-like symptom.

Table 2. The Proportions of Positive the Leukocyte Migration Test, Broken Down into Suspected Drug Categories in Iodinated Contrast Media

Suspected drugs		Number of cases	LMT*		Incidence (%)
			Positive	Rate (%)	
Ionic type	Ioxaglic acid	8	3	37.5	44.4
	Meglumine sodium amidotrizoate	1	1	100	5.9
Non-ionic type	Iomeprol	8	4	50.0	43.3
	Iopamidol	6	2	33.3	11.8
	Ioversol	13	6	46.2	35.3
	Iotrolan	3	1	33.3	5.9
Total		39	17	43.6	

*LMT: The leukocyte migration test (16 cases by agarose plate method, 27 cases by chemotaxis chamber method). LMT-agarose plate method: the leukocyte migration test by agarose plate method, this test was regarded as positive if the migration indices of the subject patients or the control patients were above 115 or below 85. LMT-chemotaxis chamber method: the leukocyte migration test by chemotaxis chamber method, this test was regarded as positive if the migration indices (MI) of the subject patients or the control patients were above 150 or below 60 and had a significant difference from the MI of 4 negative controls ($p < 0.05$, Student's *t*-test).

with ICM As shown in Fig. 1, in the results of LMT by hypersensitivity reactions, 13 out of 28 cases of skin rash, and 3 out of 10 cases of anaphylaxis indicated a positive reaction. Furthermore, one case of hepatic disorder also indicated a positive reaction. Moreover, two of the thirteen subjects positive for a skin rash and two of the three subjects positive for anaphylaxis had been treated with the ionic type of ICM.

Comparison of the Positive Rates of LMT between ICM and Other Drugs The number of cases tested for suspected drugs other than ICM was 1330 cases as shown in Fig. 2. Of these cases, 980 cases indicated positive reaction, and the positive rate of LMT was 73.7%. On the other hand, the positive rate of LMT for ICM was 43.6%. These results showed that the positive rate of LMT of ICM was significantly low ($p=0.00003$) as compared with other drug groups. Furthermore, the positive rate of LMT by drug groups were 75.8% for antimicrobial medias, 65.6% for NSAIDs, and 67.2% for enzyme preparations. The positive rates of LMT of these drug groups indicated significantly high values ($p=0.02$ to 0.00002) as compared with the positive rate of LMT of ICM.

Latency Period in LMT Positive Cases Incidence of latency period (time or days from ICM administration to the onset of hypersensitivity reaction), positive drugs and hypersensitivity reaction are shown in Table 3. The latency period

was classified into acute (immediate) type, which is less than 1 h after initiation of ICM administration, and non-acute (non-immediate) type, which is 1 h or longer after initiation of ICM administration.¹⁶⁾ Incidence less than 1 h, which is considered as acute hypersensitivity, was 17.6% (3 cases), and these were all anaphylactic hypersensitivity reaction. On the other hand, incidences after 1 h or longer were 23.5% for ≥ 1 to <12 h, 35.3% for ≥ 12 to <24 h, 11.8% for ≥ 24 h to <5 d, and 5.9% for 5 d. All these hypersensitivity reactions were skin rash. For 1 case of hepatic disorder, detailed latency period was unknown.

Rate of Drugs and Past Food Allergies in LMT Positive Cases As shown in Fig. 3, the rate of patients who developed any allergy in the past in 17 of ICM-LMT positive cases was 5 cases (2 cases for NSAIDs, 1 case for antimicrobial medias, 1 case for affecting digestive organs, 1 case for anticancer drugs), and accounted for 29.4%. Similarly, furthermore, the incidence of patients who developed any allergy in the past was 13.1%, 17.3%, and 30.2% for antimicrobial medias, NSAIDs, and enzyme preparations, respectively.

On the other hand, the number of patients who developed any allergy in the past or at the present day in 17 of ICM-LMT positive cases was only 1 case (seafood allergy), and the rate was 5.9% as shown in Fig. 4. Similarly, the incidence

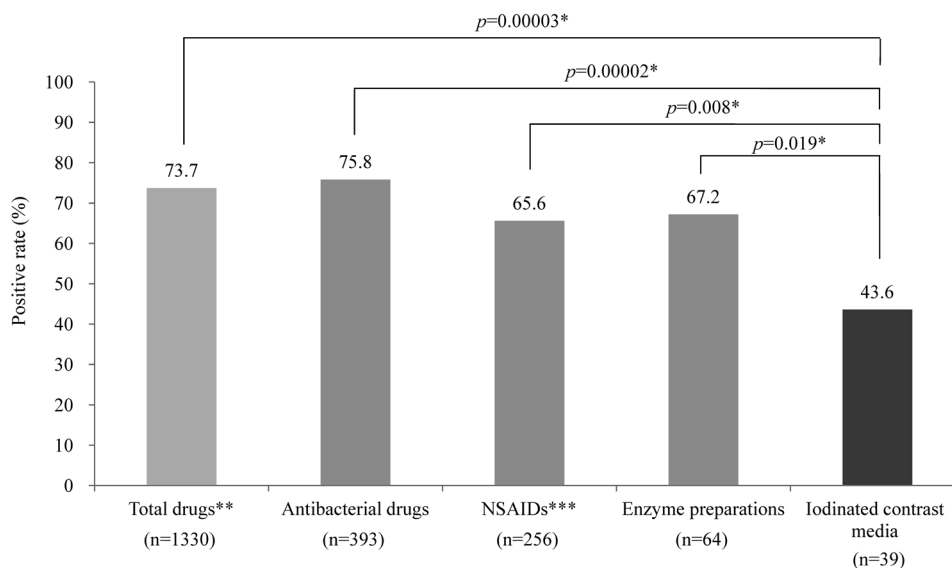


Fig. 2. The Proportions of Positive the Leukocyte Migration Test, Broken Down into Drug Categories

*Significantly different: χ^2 -test. **Total drugs: these tested agents were excluded iodinated contrast media. ***NSAIDs: non-steroidal anti-inflammatory drugs.

Table 3. The Latent Period in 17 LMT*-positive Patients Due to Iodinated Contrast Media

Latency period		Number of cases	Incidence (%)	Positive drugs	Hypersensitivity symptom
Acute type	Less than 1 h	3	17.6	Iomeprol (1), Ioversol (2)	Anaphylaxis (3)
	≥ 1 to <12 h	4	23.5	Ioversol (3), Ioxaglic acid (1)	Skin Rush (4)
Non-acute type	≥ 12 to <24 h	6	35.3	Iomeprol (2), Iotrolan (1)	Skin Rush (6)
				Ioversol (1), Ioxaglic acid (1)	
				Meglumine sodium amidotrizoate (1)	
	≥ 24 h to <5 d	2	11.8	Iopamidol (1), Ioxaglic acid (1)	Skin Rush (2)
	5 d	1	5.9	Iopamidol (1)	Skin Rush (1)
	Unknown	1	5.9	Iomeprol (1)	Hepatic disorder (1)
Total		17			

*LMT: The leukocyte migration test.

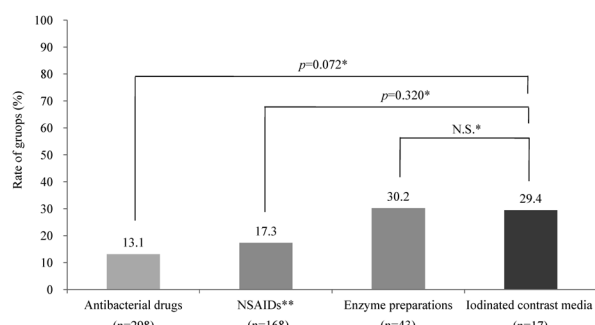


Fig. 3. The Proportions of Patients with Drug Allergic History, Broken Down into Drug Categories in Positive Cases by the Leukocyte Migration Test

*Significantly different: Fisher's exact probability test. **NSAIDs: non-steroidal anti-inflammatory drugs.

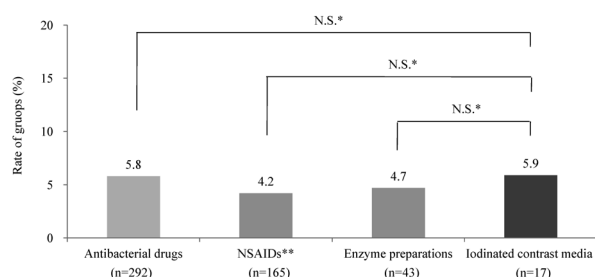


Fig. 4. The Proportions of Patients with Food Allergic History, Broken Down into Drug Categories in Positive Cases by the Leukocyte Migration Test

*Significantly different: Fisher's exact probability test. **NSAIDs: non-steroidal anti-inflammatory.

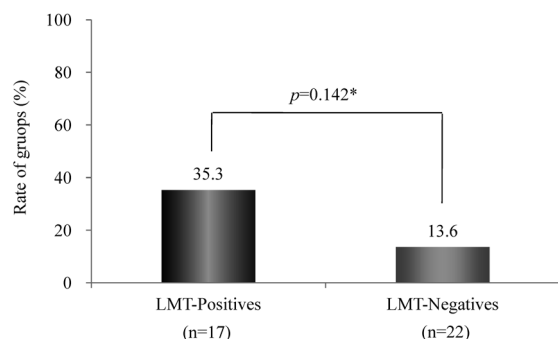


Fig. 5. The Proportions of Patients with Drug or Food Allergic History, Broken Down into Positive and Negative Cases by the Leukocyte Migration Test

*Significantly different: Fisher's exact probability test.

of patients who developed any allergy in the past was 6.0%, 4.2%, and 4.7% for antimicrobial medias, NSAIDs, and enzyme preparations, respectively.

In addition, when the incidence of patients who developed drugs or food allergies in the past was analyzed LMT positive group and negative group separately, the former was 35.3%, and the latter was 13.6% (Fig. 5). Moreover, two of the three subjects positive for anaphylaxis and four of the thirteen subjects positive for skin rash were found positive on the test.

DISCUSSION

Utility of LMT and Involvement of Allergic Reaction in ICM Hypersensitivity

Currently, nonionic ICM are

often used because safety of ICM is high in nonionic ICM as compared with ionic ICM.¹⁷⁾ When look at the number of test drugs used as suspected drugs in this study, 77% was non-ionic ICM and accounted for high percentage. It is considered that this result reflects that nonionic ICM are frequently used regardless of risk of hypersensitivity development.

Of side effects caused by ICM, anaphylactoid symptoms have a good chance that caused by a non-immunological mechanism. It is presumed that the non-immunological mechanism react as chemical toxicity caused by the contrast media itself, and is attributable to dosage (overdose), molecular toxicity, and physiological characteristics (osmotic pressure, viscosity, hydrophilicity, connectivity with protein, calcium binding capacity, sodium content *etc.*).^{18,19)} Furthermore, the drug itself has direct histamine release effect, and there are reports about complement activating action, and involvement in additive actions and mutual interactions of many transmitters (increase of acetylcholine, release of bradykinin, release of serotonin enhancer associated with activation of several biological cascades).^{20–22)} On the other hand, it has been reported that if the immunological mechanism is involved, it develops dose-independently, and is associated with a variety of chemical transmitters from mast cells and basophils through sensitized T cells.^{23–25)} However, it is indicated that there is a possibility that many cases are not caused by allergy reactions but non-immunological mechanism, as it is called pseudo allergy. The reason is because there are very poor evidences to support antibody occurred against the contrast media consistently.^{22,26)} Accordingly, the reliability of clinically necessary allergy testing is very low; *in vivo* testing methods such as intracutaneous testing can give negative results,^{27,28)} and most researchers reported a low detection rate, at around 10–20%. Even when this figure is relatively high it is still around 50%.^{1,29–32)} Thus, the standard *in vivo* tests have a low rate of detection.

In vitro identification tests are even worse, with no testing methods thought to be of clinical use.^{27,28)} For this reason, there is virtually no clinical research data on *in vitro* tests of contrast media, such as the DLST, which is most frequently used.

In such circumstances, the results of this study using LMT indicated 44% of positive rate. No significant difference was found between the two varieties of LMT, hence the variety was not considered to have affected the results of the study. There was no difference between ionic and nonionic ICM, and involvement of allergic reaction was confirmed in about a half of patients suspected of being hypersensitivity for both types of ICM. The finding that two of the three subjects with anaphylaxis had been treated with ionic ICM reflected the danger of the ionic variant as current knowledge suggests. LMT can detect both types, *i.e.*, delayed and immediate types of drug allergies. Authors indicated 73% of detection performance for patients suspected of being immediate type anaphylactic shock, and reported that hypersensitivity reaction (types of allergies) have no effect on the tests.⁶⁾ Accordingly, based on the results of this study, we believe that LMT is clinically useful in diagnosis of ICM hypersensitivity among existing *in vitro* identification methods, and is a valuable test method to prevent development of secondary side effects in the future. Accordingly, though the results of this study certainly do not indicate a high rate of detection of ICM hypersensitivity, the LMT has the highest detection rate among the existing *in vitro*

identification methods, and there is no burden on the patient. In addition, considering the current circumstance of low detection rate of *in vivo* identification methods such as skin test, it seems there is some meaning in using it, at least in clinical settings. In particular, in cases of serious hypersensitivity, it may be applicable when it is difficult to conduct a tolerance test or similar without the consent of the patient.

In addition, when examining the possibility that involvement of ICM induced allergy reaction based on the previous performances of LMT, the positive rate of 44% in LMT for ICM is a significantly lower value by about 30% as compared with hypersensitivity to any other drugs and antimicrobial media. If this positive rate is compared with the rates of NSAIDs and enzyme preparations, the rate is significantly lower by more than 20%; therefore, this value is not high. Based on these results, it is surmised that the detection rate of LMT in ICM itself is specifically low as compared with other drugs. In other words, it is also suggested in this study that there is a high possibility that there are many cases induced by the mechanism other than immune reaction as “pseudo-drug allergy” even if allergy-like symptoms are apparently observed. More specifically, it is considered that about half of patients who were diagnosed with suspected ICM allergy correspond to patients with “pseudo-drug allergy.” Especially, it is also suggested from a low positive rate of LMT (30%) in patients with suspected anaphylaxis. In any case, it is important to discriminate true allergy from false allergy from the standpoint of prevention of false diagnosis of allergic side effects, in the future. Especially, further studies to validate the safety of patients are required.

Therefore, we would like to advance our research further more to elucidate these issues in the future. In addition, although it cannot be identified with LMT what kind of cytokines and chemokines are produced; however, studies to prove involvement of allergic reaction through analyses at cytokine level will be required hereafter. In addition, although we did not examine it in this study, we believe that examination of IgE values and eosinophil count in the peripheral blood would be clinically important.

Hypersensitivity Reaction and Types of Allergy in ICM

In previous reports on adverse symptoms caused by ICM, what are especially seen as problems are immediate type allergy-like symptoms such as anaphylaxis. The frequency of these symptoms is extremely low, but it may result in clinical confusion. Anaphylaxis and skin rash accounted for about 97% of the entire symptoms of patients with suspected hypersensitivity in this study. Skin rash accounted for 72% of the total, and anaphylaxis was 26%; thus, ICM hypersensitivity cannot be disregarded after all.

In the analysis of latent period of LMT positive cases, the onset within 1 h, which is considered as immediate type hypersensitivity, indicated a low value (18%) (all of which were anaphylaxis). In other words, it is considered that there is a high possibility that most of cases were developed by the mechanism of non-immediate hypersensitivity. In our study, all 13 cases that presented skin rash and were positive in LMT were developed at 1 h or later, which are considered as non-acute type. Of these cases, 11 cases were not hives type caused by immediate type hypersensitivity but skin rash such as erythema type or similar rash; therefore, that means that 76% of positive 17 cases were detected hypersensitivity

reaction related to non-immediate hypersensitivity as the results. In other words, it is suggested that the non-immediate hypersensitivity may have a 4 times higher risk of the onset than immediate type hypersensitivity. In particular, in cases of non-immediate hypersensitivity, when a patient experiences a slight drug rash or similar problem, it was thought that fewer patients will report their symptoms and on many occasions relevant tests will not be performed. Hence, the true figures for the prevalence of hypersensitivity are probably higher than current estimates. The mechanisms in which the onset of non-immediate hypersensitivity to ICM, which is similar to that of delayed allergies to other drugs occurs when the patient was previously sensitized or sensitized for the first time and the symptoms occurred several days later, are considered and many reports have been issued.^{1,5,33–36} In a study that examined risk for onset of immediate type and non-immediate type hypersensitivities, it is reported that the non-immediate hypersensitivity developed at least two times and significantly high.³⁷ Furthermore, it is often reported that more erythema-type drug eruption are observed in non-immediate type hypersensitivity.³⁸ It is considered that these previous results are almost consistent with the results of this study.

In addition, we defined non-immediate type allergy as the cases that developed the reaction 1 h or longer after beginning ICM administration. The results of the examination suggested that identification of inducers other than IgE would be required for these non-immediate type allergy cases, and that the activation level of biological cascade substances would need to be determined. Especially, in terms of changes over time, involvement of cellular immunity is also suggested for four cases that developed the reaction 24 h or later in this study; therefore, it is necessary to understand the immunological risks of delayed type allergy occurrence.

Accordingly, it is considered based on the results of this study that it is required to pay attention to the onset of delayed-type allergic adverse reactions even after several hours and several days, although it is concerned that attention to anaphylaxis would be changed to immediate type anaphylaxis by the degree of seriousness. Immediate reactions such as anaphylaxis operate differently to non-immediate reactions such as most drug rashes, and should therefore be studied separately. However, as there were few subjects in this study, they were examined together. Future studies will need to examine the details of ICM allergies with a larger number of subjects.

Relevancy between ICM Allergy and a Past History of Allergy

In a relationship between the development of drug allergy and a past history of drug allergy, naturally patients with a past history of drug allergy had higher risk. According to our analysis using LMT, we reported that the incidence in patients without past history of drug allergy was about 0.3–0.4%; however, that was about 7.6–8.8% in patients with the past history.⁶ Thus, it is suggested that patients with a history of drug allergy have at least 20 times higher risk as compared with patients without history of drug allergy. The results of this study indicated especially high incidence for ICM, 29.4%, which was almost the same level as enzyme preparations, but this value was 2.2 times higher than antimicrobial medias and 1.7 times higher than NSAIDs, which indicated high incidence of drug allergy. In other words, it is suggested that there is a possibility that at least one in four patients with a history of drug allergy develop the ICM al-

lergy. Therefore, it is required that adequate attention would be required to use ICM. One case out of the 5 cases that was LMT positive and had a past history of drug allergy was when ICM was administered again. Accordingly, it is basically desired to diagnose drug allergy by *in vitro* test methods such as LMT even when administration is required for a patient with a previous ICM allergy history and is described as pseudo-drug allergy or when the case requires implementing challenge test.

In addition, for relationship between the history of drug allergy and food allergy, statistically significant difference was not obtained in this study. However, Katayama *et al.*³⁹⁾ suggested that patients with a history of food allergy have two times higher risk of ICM allergy as compared with patients without a history of the allergy. Therefore, we will examine further in this study using a large number of cases in the future.

Many researchers have been concerned that inducers of ICM allergy relate to allergy diseases and history of drugs and food allergies.^{27,39–42)} Furthermore, although there were no patients with allergy diseases in this study, when history of allergy to drugs and food were divided into LMT positive and negative groups, LMT positive group (35%) indicated about 3 times higher value as compared with LMT negative group. The fact that two out of the three subjects positive on the LMT for anaphylaxis also had a medical history of food or drug allergy suggested that this was also a risk factor.

Therefore, based on these results, it was suggested for patients with overall allergy that ICM has a high risk to develop allergy and the allergy may be developed one in three patients.

Today, diagnostic imaging has an important role in diagnosis. Especially, the range of diseases that conduct examinations with contrast media is expanding, and of course its value is also widely recognized. However, on the other hand, side effects caused by contrast media are unavoidable. Especially in the case of allergy, which is very difficult to predict, there is a possibility that the patient results in death when the condition was serious and proper treatment was not conducted. Based on the results of our study, it is required to confirm a past history (search of a possibility of sensitization) and the presence and absence of allergic predisposition thoroughly before the administration from the standpoint of proper use with consideration of safety, after all. In addition, it is desired to administer ICM at a concentration as low as possible as is conventionally done in light of induction based on non-allergic mechanism. Considering the fact that the number of patients with allergic tendency and affected patients is increasing in these years, we believe that more detailed studies are required.

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