

Study on Patients Who Underwent Suspected Diagnosis of Allergy to Amide-Type Local Anesthetic Agents by the Leukocyte Migration Test

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ABSTRACT

Background: There are problems in diagnosis of allergy to amide-type local anesthetic agents (ALAs), because definitive diagnosis is not obtained by *in vivo* tests, which are used for the diagnosis. Consequently, patients may be exposed to risk. There are few diagnoses based on *in vitro* tests, and there are almost no relevant studies.

Methods: Authors examined involvement of allergic reaction using the leukocyte migration test (LMT) through multiple standpoints in 43 patients who underwent suspected diagnosis of allergy to ALAs.

Results: Rate of LMT-positives was 54%, and especially the positive rate of lidocaine hydrochloride preparations was significantly high. In 15 positives to lidocaine hydrochloride preparations, all cases were indicated as positive in a test with drugs containing antiseptic agent, but only 3 cases were indicated as positive in a test with lidocaine hydrochloride alone. In addition, test with paraben was conducted in 4 cases; 2 cases were confirmed as positive. In relevance of histories of drug or food allergies, development rates of ALAs-allergies were the highest in both allergies, and were 35% and 13%, respectively.

Conclusions: There is a high possibility that these adverse reactions were caused by pseudoallergy to drug. Even by allergic reactions, it was assumed that 80% of them might be caused by antiseptic agents such as paraben. In addition, it was suggested that ALAs, especially lidocaine hydrochloride preparations have high antigenicity (sensitizing property). Furthermore, it was considered that patients with past history of drug or food allergies have a high potential for manifestation of the reactions.

KEY WORDS

amide-type local anesthetic agents, paraben, past history of allergy, pseudoallergy to drug, the leukocyte migration test

INTRODUCTION

Local anesthetic agents fall into the general classification of ester types and amide types according to their chemical structures. Regarding adverse reactions caused by local anesthetic agents, it has been pointed out that local anesthetic agents may have a risk of al-

lergic responses, since 1920 when contact dermatitis caused by an ester-type local anesthetic agents was reported.¹ However, reports of adverse reactions decreased notably from 1943 when ALAs became used more frequently at the bedside. Because these types of drugs have different mechanism of allergy manifestation due to different metabolic pathways,^{2,3} it is rec-

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ognized that the ALAs have low antigenicity as compared with ester-type agents^{4,5} and are relatively safe local anesthetic agents. Nowadays the ALAs are used widely for various procedures in clinical practice including dental treatments, general surgery, and obstetric service. Even though the ALAs became widely used, however, reports of adverse events were not eliminated. Various adverse reactions including mild rash and nausea, and even severe anaphylactic response have been reported.⁶⁻¹⁷

On the other hand, it is known that adverse reactions due to ALAs, which often tend to consider that apparently it was induced by allergic reactions, may include reactions caused by patient's fear of surgical procedure and administration of anesthetic agent involved in medical practice, manifestation of minor reactions mediated by various pharmacological mechanisms, and development of accidental symptoms.¹⁸⁻²⁰ Therefore, cases, which are clinically, generally called "local anesthetic (mainly amide-type) agent allergy" often include cases that were diagnosed as allergy induced by local anesthetic agents without confirming involvement of allergic reaction and without immunological evidence. Such indeterminate diagnosis results in various therapeutic limitations. It actually creates confusion when administration of local anesthetic agents becomes required. More specifically, it is important to conduct examinations to clarify the involvement of allergic reactions in consideration of the fact that hypersensitivity induced by local anesthetic agents may be caused by not only simple drug allergy but also minor reaction and accidental symptoms.

Examination methods used for local anesthetic agent allergy are mainly challenge test, intradermal test and patch test, and most of reported cases indicated positive reaction were determined by *in vivo* identification tests. The specific IgE-antibody radioallergosorbent test (RAST), histamine release test (HRT), basophil activation test (BAT), drug-induced lymphocyte stimulation test (DLST), and other methods may be performed in *in vitro* identification tests. DLST has been widely used in clinical practice; however, evidence of clinical efficacy in diagnosis of allergy to local anesthetic agents has not been obtained yet because of its low detection sensitivity. In other words, there are almost no valuable studies, which included multiple cases analyzed with *in vitro* identification methods in allergy to ALAs.

Authors previously reported that LMT is valuable as a method to detect and identify responsible agents for drug hypersensitivity including drug rash.²¹⁻²⁵ Potential involvement of a variety of cytokines has also been reported to date although LMT is not a testing methodology to identify cytokines and chemokines.²⁶⁻²⁸ In addition, because LMT has a higher sensitivity than DLST, it is considered that LMT may have higher detection rate than DLST as *in vitro* identifica-

tion method.²⁹ Specifically, we recently reported that a chemotaxis chamber method developed by authors has an even higher sensitivity than existing agarose plate method and has a high clinical value.³⁰

Therefore, we examined relationships of the *in vitro* identification method with hypersensitivity reaction, antigenicity, mechanisms underlying, and past history of allergy in patients who underwent suspected diagnosis of allergy to ALAs using LMT, a highly sensitive detection method.

METHODS

SUBJECTS

Patients underwent LMT were 60 suspected patients of hypersensitivity to ALAs at Suibaragou Hospital, Japan during the past twenty years (April 1991 through March 2010). Of 60 cases, 17 cases that were indicated as LMT positive to test drugs other than ALAs were eliminated. The remaining 43 patients (14 males, 29 females) were included in this study. The age of patients were ranged from 4 to 94 years old (under 10 years old, 6 cases; 10's, 1 case; 20's, 11 cases; 30's, 6 cases; 40's, 4 cases; 50's, 6 cases; 60's, 3 cases; 70's, 5 cases; 80's, 0 case; and 90's, 1 case), and the average age 39.8 ± 23.4 years old (mean $\pm$ S.D.).

The purpose of drug administration to the objectives were dental and oral surgical procedure in 32 cases (74%), surgical procedure in 9 cases (21%), and endoscopic procedure in 2 cases (5%).

Of the subjects, patients with a history of allergy were 15 cases (29%), including 13 cases (30%) of drug allergy and 4 cases (9%) of food allergy. In addition, 2 patients (5%) with atopic dermatitis, and 1 patient (2%) with chronic bronchial asthma were also included as allergic diseases. (The numbers of allergic histories are cumulative.)

The majority of hypersensitivity reactions observed in the subjects was anaphylaxis including anaphylactoid symptoms and shock-like symptoms (25 cases, 66%), followed by 11 cases (29%) of rash, and then 2 cases (5%) of hepatic disorders. Eight of 11 rash cases were urticaria-type drug eruption. In addition, there were five suspected cases of drug allergy although these were not clearly diagnosed as hypersensitivity reaction, including nausea due to administration of anesthesia, in each previous history. Patient information, such as hypersensitivity reaction and a period of medication, was obtained from reports about patients who underwent suspected diagnosis of drug adverse effect submitted by attending physicians, medical records, and direct interviews with patients. All LMT were conducted with sufficient explanation about the significance of the study, and consent of the patients. The informed consent was obtained according to the "Ethical Guidelines for Epidemiological Research" of the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare. The Ethical

Table 1 The suspected drugs in patients with suspected local analgesic agents allergies (Test drugs for leukocyte migration test)

Suspected drugs [†]	Number of cases	Incidence (%)	Classification by administered purpose (Number of cases)
Lidocaine hydrochloride	39	90.7	Dental and oral operator (32) Surgical manipulation (4) Endoscopy (3)
Propitocaine hydrochloride	18	41.9	Dental and oral operator (18)
Mepivacaine hydrochloride	14	32.6	Dental and oral operator (11) Surgical manipulation (3)
Bupivacaine hydrochloride hydrate	4	9.3	Surgical manipulation (4)
Total	75		

[†] In this table, the same patients are counted in two or more categories in case of suspected drugs.

Committee of Suibaragou Hospital also approved the implementation of LMT.

TEST (SUSPECT) DRUG

The number of test drugs of 43 subjects was at least 1 drug to at most 10 drugs per case, and 55 types and 171 drugs in all cases, of which drugs for peripheral nerves was 8 types and 75 drugs. In addition, for ALAs, lidocaine hydrochloride preparations were 39 cases, propitocaine hydrochloride preparations were 18 cases, mepivacaine hydrochloride preparations were 14 cases, and bupivacaine hydrochloride hydrate preparations were 4 cases as shown in Table 1. All ALAs used were ethical drugs, and no over the counter drug (OTC) containing amide-type local anesthetic agent was used. The majority of other drugs were drugs for central nerve, which were 14 types and 46 drugs, followed by antimicrobial agents, which were 14 types and 25 drugs, agents affecting metabolism, which were 5 types and 5 drugs, drugs for the digestive system, which were 4 types and 5 drugs, and others were 10 types and 15 drugs. (The numbers of test drugs are cumulative.)

In addition, for 30 of 39 cases tested for lidocaine hydrochloride preparations, both (two lines) commercialized antiseptic-containing lidocaine hydrochloride preparations themselves and lidocaine hydrochloride alone, which is a main component of the preparation, were tested separately. Moreover, methyl parahydroxybenzoate, which is one of inactive ingredients (antiseptic), was also tested in 4 of 43 cases. No cases, which received a dose in excess of usual dosage of each drug, were observed.

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, USA) and mixing 100 μ L of the drug solution with

100 μ L of culture medium (10% horse serum [Gibco], 10 mM 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 0.1 N 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 1/50,000/mL and 1/10,000/mL of a dosage were used for oral and injectable drugs, respectively. The final antigen concentration of all test drugs was determined based on control studies conducted at several concentration values to confirm that there was no influence of the drug *per se* on immune activity. 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 7 days and 3 months after the discontinuation of test drug administration. The drugs used for treatment of hypersensitivity reaction and other drugs used after the treatment included drugs that exert influence on the responsiveness of LMT, and the test was conducted under conditions without influence on immunological capacity

in this study. 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% CO₂ at 37°C for 72-96 hours. 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 40-60 min, 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 plate 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 (3 mm in diameter) made of culture medium with 1% agarose on a plate. Then the plate was incubated in an incubator under 5% CO₂ at 37°C for 24 hours, and the area of leukocyte migration was measured with an immuno viewer (IMMUNO VIEWER-MU, Jokoh, Tokyo, Japan).

For a chemotaxis chamber method, wells in a lower chamber of a chemotaxis chamber (96-well Chemotaxis Chamber AB96, Neuro Probe, Gaithersburg, USA) 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, Ja-

pan).³⁰

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 $\pm$ 2 SD ($n = 6$). 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.³⁰

ANALYSIS OF LMT RESULTS IN PATIENTS WHO UNDERWENT SUSPECTED DIAGNOSIS OF DRUG HYPERSENSITIVITY TO DRUGS OTHER THAN ALAs

To examine involvement of allergic reaction and antigenicity of ALAs, and relevance of a history of drug allergy and food allergy, investigation and analysis were conducted for drugs other than ALAs 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^{21-23,29,30} such as antimicrobial agents, 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

LMT RESULTS IN PATIENTS WHO UNDERWENT SUSPECTED DIAGNOSIS OF DRUG HYPERSENSITIVITY TO ALAs

LMT was implemented with an agarose plate method in 16 cases, and with a chemotaxis chamber method in 27 cases. As the results, 23 of 43 cases (15 cases were positive to only ALAs, 8 cases were positive to amide-type and other local anesthetic agents) indicated positive reactions and a positive rate was 53%.

Furthermore, in the LMT results by drugs, positive

Table 2 The proportions of positive the leukocyte migration test, broken down into suspected drug categories in amide-type local anesthetic agents

Suspected drugs [†]	Number of cases	LMT [‡]			Incidence (%)
		Positive	Rate (%)	χ^2 -test	
Lidocaine hydrochloride	39	20	51.3	$p = 0.0002$ $p = 0.0160$	87.0
Propitocaine hydrochloride	18	0	0		0
Mepivacaine hydrochloride	14	2	14.3		8.7
Bupivacaine hydrochloride hydrate	4	1	25.0		4.3
Total	75	23	30.7		

[†] In this table, the same patients are counted in two or more categories in case of suspected drugs.

[‡] 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 (M.I.) of the subject patients or the control patients were above 150 or below 60 and had a significant difference from the M.I. of 4 negative controls ($p < 0.05$, Student's t-test).

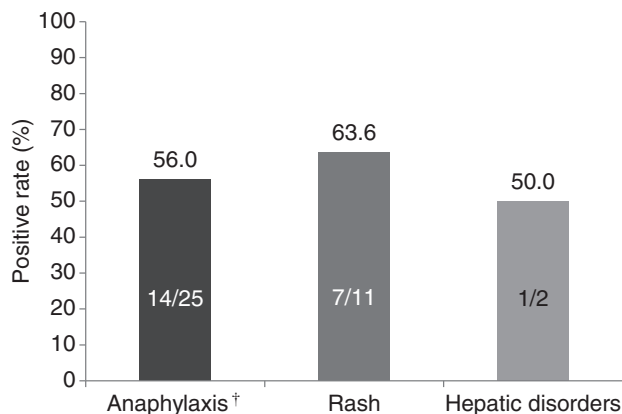


Fig. 1 The proportions of positive the leukocyte migration test, broken down into hypersensitivity symptomatic categories. [†] Anaphylaxis, In these cases, contain anaphylaxis-like or shock-like symptom.

response was indicated in 20 of 39 cases for lidocaine hydrochloride preparations, 0 of 18 cases for propitocaine hydrochloride preparations, 2 of 14 cases for mepivacaine hydrochloride preparations, and 1 of 4 cases for bupivacaine hydrochloride hydrate preparations as shown in Table 2. The lidocaine hydrochloride preparations showed significantly high positive rate as compared with propitocaine hydrochloride preparations and mepivacaine hydrochloride preparations ($p < 0.02$).

In addition, only 1 amide-type local anesthetic agent was identified as positive for each case, and no cases has 2 or more positive drugs in the entire 23 positive cases.

LMT POSITIVE RATE BY DIFFERENT HYPERSENSITIVITY REACTIONS TO ALAs

In the LMT results by hypersensitivity reactions 14 of 25 cases for anaphylaxis, 7 of 11 cases for rash, and 1

of 2 cases for hepatic disorders indicated positive reaction as shown in Figure 1. In addition, 1 of 5 cases for no clear hypersensitivity symptoms indicated positive reaction. In addition, 4 of 7 positive cases of rash were urticaria-type drug eruption.

LMT POSITIVE RATE OF DRUGS WITH OR WITHOUT ADDITIVE IN LMT POSITIVE CASES CAUSED BY LIDOCAINE HYDROCHLORIDE PREPARATIONS

For 15 of 20 LMT positive cases caused by lidocaine hydrochloride preparations, test was conducted with two drugs, a lidocaine hydrochloride preparation containing antiseptic and lidocaine hydrochloride alone, which is a major ingredient. As shown in Table 3, all 15 cases indicated positive reaction to the lidocaine hydrochloride preparation containing antiseptic. On the other hand, only 3 cases indicated positive reaction to the lidocaine hydrochloride alone, and 12 cases were negative.

Hypersensitivity reactions caused by the lidocaine hydrochloride preparation containing antiseptic included 9 cases of anaphylaxis and 3 cases of rash. On the other hand, the 3 cases of hypersensitivity reactions caused by lidocaine hydrochloride alone included 2 cases of rash and 1 case of shock.

RESULTS OF LMT WITH AN ANTISEPTIC AGENT (METHYL PARAHYDROXYBENZOATE)

Test was conducted using methyl parahydroxybenzoate itself. As the results, positive reaction was observed in 2 of 4 cases. The results of positive 2 cases are shown in Table 4, one case was a lidocaine hydrochloride preparation, and another case was mepivacaine hydrochloride preparation.

COMPARISON OF LMT POSITIVE RATES BETWEEN ALAs AND OTHER DRUGS

The number of cases tested for in test drugs other

Table 3 The incidence rate of positive the leukocyte migration test, broken down into suspected drugs with or without preservatives in the 15 patients developed lidcaine hydrochlorise formulations

Suspected drugs [†]	LMT [‡]		
	Number of positive cases	Rate (%)	Hypersensitivity symptom (cases)
Lidocaine hydrochloride + Preservatives	15	100	Anaphylaxis [§] (9)
			Rash (3)
Lidocaine hydrochloride only	3	20	Anaphylaxis [§] (1)
			Rash (2)

[†] In this table, the same patients are counted in two or more categories in case of suspected drugs.

[‡] LMT, The leukocyte migration test. [§] Anaphylaxis, In these cases, contain anaphylaxis-like or shock-like symptom.

Table 4 The incidence rate of positive the leukocyte migration test, broken down into suspected drugs with or without preservatives in the patients developed lidcaine hydrochlorise formulations

Cases				LMT [†]			
Age	Sex	Hypersensitivity symptom	Tested drugs	Antigen concentration (μg/mL)	M.I. [‡]	P value, Student's t-test	Judgment
25	Fe-male	Rash	Mepivacaine hydrochloride	2 μg	119.6	0.08686	Negative
			Methyl p-hydroxybenzoate	50 ng	237.0	0.00035	Positive
			PHA [§]	1 μg	316.3	0.00002	Positive
38	Fe-male	Anaphylaxis	Lidocaine hydrochloride	1 μg	105.4	0.82602	Negative
			Methyl p-hydroxybenzoate	50 ng	184.2	0.00239	Positive
			PHA [§]	1 μg	384.2	0.00039	Positive

[†] LMT, The leukocyte migration test by chemotaxis chamber method. [‡] M.I., The migration index.

[§] PHA, Phytohemagglutinin (mitogen as a positive control in the LMT).

than ALAs was 1330 as shown in Figure 2, and 980 cases of them indicated positive reaction, and LMT positive rate was 74%. On the other hand, LMT positive rate of the ALAs was 54%. In these results, LMT positive rate of the ALAs indicated significantly low value as compared with other drug groups ($p = 0.003$). In addition, LMT positive rates of different drug groups were 76% for antimicrobial agents, 66% for NSAIDs, and 67% for enzyme preparations. These LMT positive rates of antimicrobial agents and NSAIDs were significantly high as compared with LMT positive rate of the ALAs ($p < 0.01$).

RATES OF DRUG ALLERGY AND FOOD ALLERGY IN LMT POSITIVE CASES

Of 23 cases of LMT positive cases induced with ALAs, patients who had a past history of drug allergy to any kind of drugs were 8 cases (2 cases to ALAs, 3 cases to antimicrobial agents, 1 case to NSAIDs, and 2 cases to unknown drugs), which accounted for 35%, as shown in Figure 3. Also, the percentage of patients with a history of drug allergy in LMT positive patients groups for antimicrobial agents, NSAIDs and enzyme preparations were 13%, 17.3% and 30%, respectively. Antimicrobial agents indicated significant low value as compared with the ALAs ($p = 0.010$).

On the other hand, of 23 cases of LMT positive cases induced with ALAs, patients with a past or current history of food allergy were 3 cases (13%) as shown in Figure 4. Also, the percentage of patients with a history of food allergy in LMT positive patients groups for antimicrobial agents, NSAIDs and enzyme preparations were 6%, 4% and 5%, respectively.

DISCUSSION

ANTIGENICITY OF ALAs

In local anesthetic agents, it has been known that amide-type drugs have lower antigenicity and are safer than ester-type drugs; therefore, many amide-type preparations are used widely. Especially, use frequency of lidocaine hydrochloride preparations is currently the highest. There are several types of amide-type preparations and risks causing toxic reaction has been actively studied,³¹⁻³⁴ and lidocaine hydrochloride are used widely because there are relatively safer as compared with other drugs. However, there are almost no investigations about risks inducing allergy reactions in terms of difference of antigenicity.

In this study results, LMT positive rate of lidocaine hydrochloride preparations indicated significantly high value, which was more than 2 times higher, as

Drug Allergies in Local Anesthetics

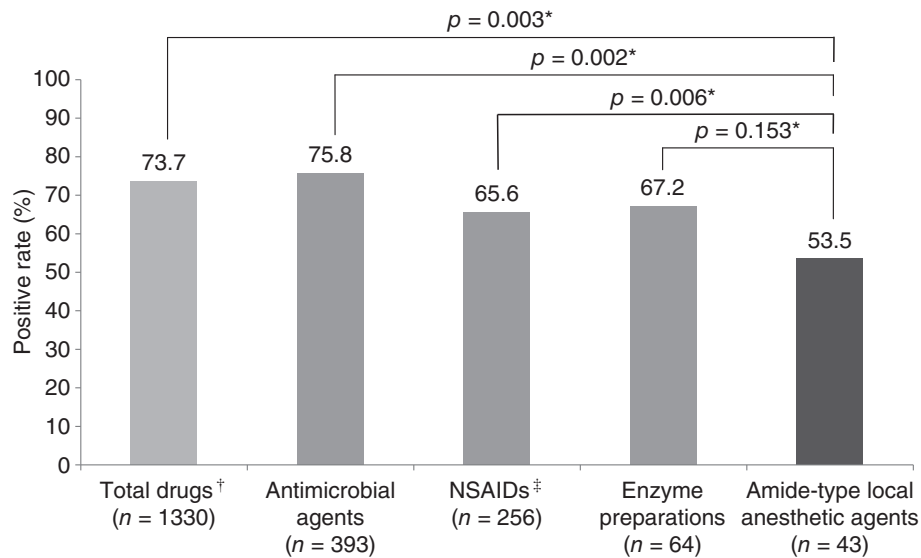


Fig. 2 The proportions of positive the leukocyte migration test, broken down into drug categories. *Significantly different, χ^2 -test. †Total drugs, These tested agents were excluded amide-type local anesthetics. ‡NSAIDs, Non-steroidal anti-inflammatory drugs.

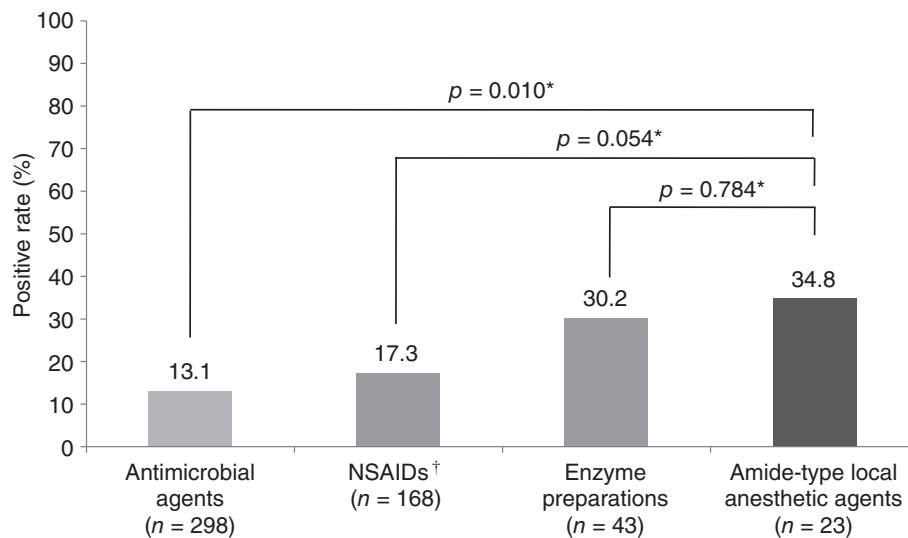


Fig. 3 The proportions of patients with drug allergic history, broken down into drug categories in positive cases by the leukocyte migration test. *Significantly different, Fisher's exact probability test. †NSAIDs, Non-steroidal anti-inflammatory drugs.

compared with other propitocaine hydrochloride preparations and mepivacaine hydrochloride preparations. Therefore, it is suggested that lidocaine hydrochloride preparations may have the highest antigenicity or sensitizing property, and thus have a high risk to induce allergy symptoms in ALAs. For lidocaine hydrochloride preparations, there are reports about contact dermatitis^{35,36}; however, it is considered that such reports are reflect not its high antigenicity but a high risk to induce allergy symptoms, because and use frequency of lidocaine hydrochloride prepara-

tions is currently the highest, for example lidocaine hydrochloride preparations are contained in some OTC drugs. Therefore, detailed studies including difference of antigenicity of each drug in terms of the chemical structure would be required to prevent secondary hypersensitivity reactions caused by cross-over reaction, etc.

INVOLVEMENT OF HYPERSENSITIVITY REACTIONS AND ALLERGIC REACTIONS IN ALAs

Immediate allergy-like symptoms, such as anaphy-

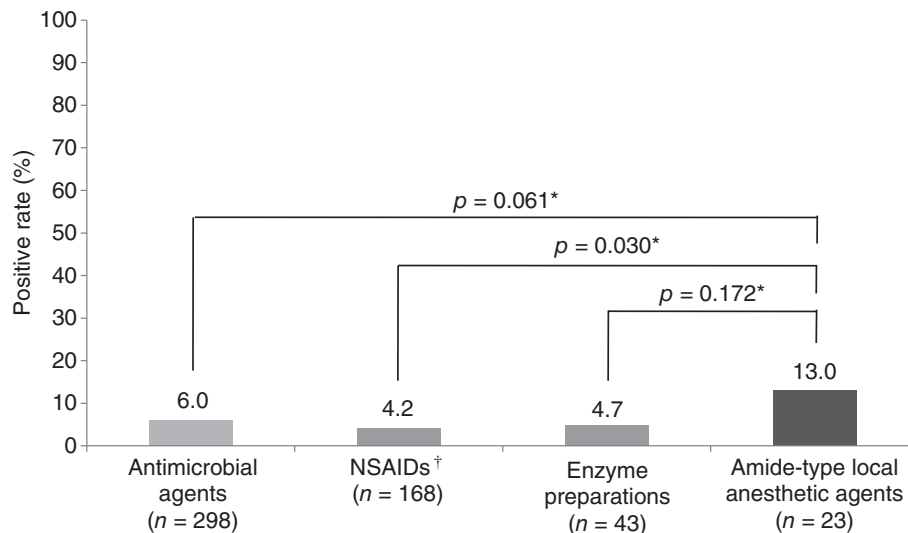


Fig. 4 The proportions of patients with food allergic history, broken down into drug categories in positive cases by the leukocyte migration test. *Significantly different, Fisher's exact probability test. [†]NSAIDs, Non-steroidal anti-inflammatory drugs.

laxis, are especially seen as a problem among previous reports on harmful symptoms caused by ALAs. Occurrence of immediate allergy-like symptoms is extremely low, but these symptoms may cause clinical confusion. In symptoms occurred in patients who underwent suspected diagnosis of hypersensitivity in this study, anaphylaxis and rash accounted for 95% of the entire symptoms except unknown cases. Especially, anaphylaxis accounted for 66% of the entire symptoms. In addition, 8 of 11 patients with rash developed rash classified into urticaria-type drug eruption. More specifically, it is considered that there is a high likelihood that symptoms of most cases were developed by a mechanism of type I allergy or immediate allergy, in which Th2 is strongly involved in helper T cells (Th). Considering that 4 of 7 LMT positive cases of rash were urticaria-type drug eruption, hypersensitivity reactions, including anaphylaxis, involved with immediate allergy were detected in 78% of 23 positive cases as a result. In other words, it is also suggested that the risk of developing delayed allergy may be about 20%. Only ethical drugs were examined in this study; however, it is expected that occurrence of delayed allergy would become increased in view of contact dermatitis cases due to OTC drugs containing ALAs.

When considering about a possibility of induction due to involvement of allergic reactions, LMT positive rate of ALAs is only 54%, which is about 20% lower value as compared with the entire groups of other drugs, and this is by no means high value. LMT can detect both types of drug allergies, immediate allergy and delayed allergy. Previously, authors reported that LMT indicated 73% of detection performance in patients who underwent suspected diagnosis of immedi-

ate anaphylactic shock, and that the test was not influenced by (allergy type) hypersensitivity reaction.³⁰ Therefore, it is suggested that detection with LMT indicates specifically low detection rate for ALAs regardless of the development mechanism of allergy. More specifically, it is also suggested in this study that it is highly likely that there are many cases induced by mechanisms other than immune response as "pseudoallergy to drug" even if the patient presented allergy-like symptoms on the surface.

For allergic symptoms caused by ALAs, there were many reports that suggested potential pseudoallergy to drugs. However, most of these diagnoses were based on *in vivo* examinations, such as challenge test and intracutaneous test, and examples of *in vitro* validation are extremely poor. Although there is a report that positive results were obtained with DLST,³⁷ Suzuki *et al.*³⁸ conducted DLST in 9 cases and reported that all cases were indicated as negative, and concluded that diagnosis with DLST was invalidity for ALAs. DLST has a problem of false-positive result and many questions, but is a useful test for delayed allergy; therefore this is not suitable for elucidation of the mechanism of local anesthetic agent allergy.^{29,39} Moreover, there is no report verified using histamine release test. Therefore, it is considered that our research results using LMT is highly useful, and there are many hypersensitivity reactions that are not caused by allergic reactions. Although LMT is not able to identify the produced cytokines and chemokines; however, further research would be required to show involvement of immune reaction through analysis at a cytokine level. In addition, it was considered that it is clinically important to verify IgE values and eosinophil counts in the peripheral blood at the same

time although it was not included in this study.

Development of hypersensitivity reactions based on non-allergic mechanism may include psychogenic response and vasovagal reflex. Especially, there is a report that about 80% of patients who underwent suspected diagnosis of allergy to local anesthetic agents at dental treatment may be caused by vasovagal reflex.¹⁹ In either case, further detailed studies would be required in the future, and especially studies to conduct *in vitro* examination are also sought in terms of patient safety.

INVOLVEMENT OF ANTISEPTIC ADDITIVES IN ALAs

Allergy to parabens and sodium pyrosulfite that are contained as antiseptics of ALAs has already been reported.⁴⁰⁻⁴² Based on these reports and so forth, it is becoming popular to use products containing no antiseptics. In our study results, in examination of 15 of 20 cases that indicated LMT positive with lidocaine hydrochloride preparations, antiseptics containing tests indicated 100% of positive reaction. However, test with lidocaine hydrochloride alone, which contained no antiseptics, indicated only 20% of positive reaction. In other words, it is suggested that 80% of actual amide-type local anesthetic agent allergy, in which involvement of not pseudoallergy to drug but allergic reaction was confirmed from LMT results, was induced by not lidocaine hydrochloride itself but antiseptic. Therefore, in amide-type local anesthetic agent allergy, it is considered that the percentage of involvement of antiseptic is high except for pseudoallergy to drug, and the amide-type local anesthetic agent itself, which is a main ingredient, has much lower antigenicity than that of antiseptic, about 1/5 of that of antiseptic.

In addition, although these were data from only 2 cases, it is obvious that antiseptic involves in the amide-type local anesthetic agent allergy when considering the cases that LMT strong positive was actually indicated to methyl parahydroxybenzoate directly.

Because most of allergy reports caused by paraben additives are contact dermatitis, it is more likely that establishment of sensitization is caused by use of daily commodities such as cosmetics and hairdressings. In other words, it is suggested that there are risks that allergy is suddenly induced even if the patient use the ALAs for the first time because of establishment of sensitization due to use of daily commodities, even if a patient has no previous use experience of ALAs. Based on these results, therefore, it is considered that administration of preparation containing no antiseptics is desired regardless of previous history of allergy if at all possible.

RELATIONSHIPS BETWEEN THE AMIDE-TYPE LOCAL ANESTHETIC AGENT ALLERGY AND PAST HISTORY OF ALLERGY

For relationships between the amide-type local anesthetic agent allergy and past history of allergy, the risk of developing drug allergy is higher in patients with a past history of drug allergy than in patients without the history, as was expected. In our analysis using LMT, it was reported that development rate of drug allergy was about 0.3-0.4% in patients without a past history of drug allergy; meanwhile, development rate of drug allergy was about 7.6-8.8% in patients with a past history.²¹ Hence, about 20 times higher development risk is suggested for patients with a past history of drug allergy as compared with patients without the history. According to results from this study, especially ALAs indicated high development rate of 34.8% among them, which was about 3 times higher than antimicrobial agents, which have high development rate of drug allergy, and about 2 times higher than NSAIDs. More specifically, it is suggested that there is a possibility that drug allergy could be induced in 1 of 3 patients with a past history of drug allergy. Therefore, it is required to exercise adequate caution for use of ALAs. Of 8 LMT positive cases with a past history of drug allergy, lidocaine hydrochloride preparations were re-administered in 2 cases. Hence, traditional diagnosis with *in vitro* examinations such as LMT would be desired even when administration in cases, in which a past history of allergy caused by amide-type local anesthetic agent, was diagnosed as pseudoallergy to drug, or challenge test are required.

In relationship between past history of drug allergy and food allergy, ALAs indicated more than 2 times higher values as compared with antimicrobial agents and NSAIDs, and it was indicated that there is a tendency development rate caused by the amide-type local anesthetic agent is high as compared with other drugs. Moreover, of 3 patients with allergic diseases, both of 2 patients with atopic dermatitis presented LMT positive. Based on these results, it is suggested that drugs, especially ALAs, have high risks of allergy development in patients with overall allergic tendency to any of drugs, foods, and diseases.

It is said that amide-type local anesthetic agent allergy is very rare. However, it is considered that further studies are required in the future, because recently a number of not only ethical drugs but also OTC drugs with ALAs have been commercialized, there is possibility that use of daily commodities may cause sensitization because antiseptics also contribute to the sensitization due to its antigenicity at high rates, and the number of patients with allergy or allergic tendency is increasing.

Diagnosis of hypersensitivity to ALAs is difficult in many cases; however, it is required to give the highest priority to use formulations containing no antiseptics.

tics and to pay special attention to administration to patients with a history of allergy, in terms of proper usage of ALAs with consideration for safety aspect. Moreover, it is desirable as in the past to administer ALAs at concentration as low as possible in light of induction of the hypersensitivity based on the non-allergic mechanism.

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