Radioallergosorbent Test (RAST) for Specific IgE Antibody to Lidocaine, Procaine and Methylparaben

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Although anaphylactoid reactions to local anesthetics are well known, a radioallergosorbent test (RAST) to detect specific drug reagin (IgE) anti-body has not been developed. We established RAST for local anesthetics by using carboxylic acid derivatives of lidocaine, procaine and methylparaben. Serum samples were taken from 100 volunteers who were regarded to be nonallergic to the drugs used. Negative RAST values obtained from these volunteers were 1653 ± 254(SD) cpm (lidocaine), 2750 ± 264 cpm (procaine), and 2805 ± 336 cpm (methyl paraben). (Key words: RAST, local anesthetics, lidocaine, procaine, methylparaben)

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Anaphylactoid, hypersensitive side effects of local anesthetics and methylparaben are rare, but well-known[1–5]. Some of the untoward responses following the use of local anesthetics tend to be thought of as a "sensitivity response". The exact incidence has not been ascertained, because there are no reliable diagnostic methods to determine allergy to local anesthetics. Neither skin test nor clinical examinations are satisfactory to determe allergy to local anesthetics. Although the indirect skin test (P-K reaction) is effective, but it carries the danger of infection.

Recently, we developed the radioallergosorvent test (RAST)[6–10] for lidocaine, procaine and methylparaben, in which the synthesized allergen were chemically coupled to unsolved carrier. Present study was designed to determine the RAST values of nonallergic subjects to local anesthetics, and to evaluate the diagnostic usefulness of this method to find out the allergic subjects to local anesthetics.

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Material and Methods

1) Synthesis of carboxylic acids derivatives

Only proteins or amino polipeptides can bind with a CNBr-activated cellulose disk. The preparation of the antigen began with the synthesis of carboxylic acid derivatives of lidocaine, procaine and methylparaben.

Lidocaine-COOH was prepared from 2,6-xylidine and N-methyliminodiacetic acid (fig. 1), and purified by column chromatography. Procaine-COOH was prepared from procaine and succinic anhydride (fig. 2), and purified by column chromatography.

Ethyl ρ-hydroxy O-benzoglycolate prepared from ρ-hydroxy benzoic acid and ethylbromacetics was hydrolyzed to give methylparaben-COOH (fig. 3).

2) Conjugate with BSA

These carboxylic acid derivatives were conjugated with bovine serum albu-

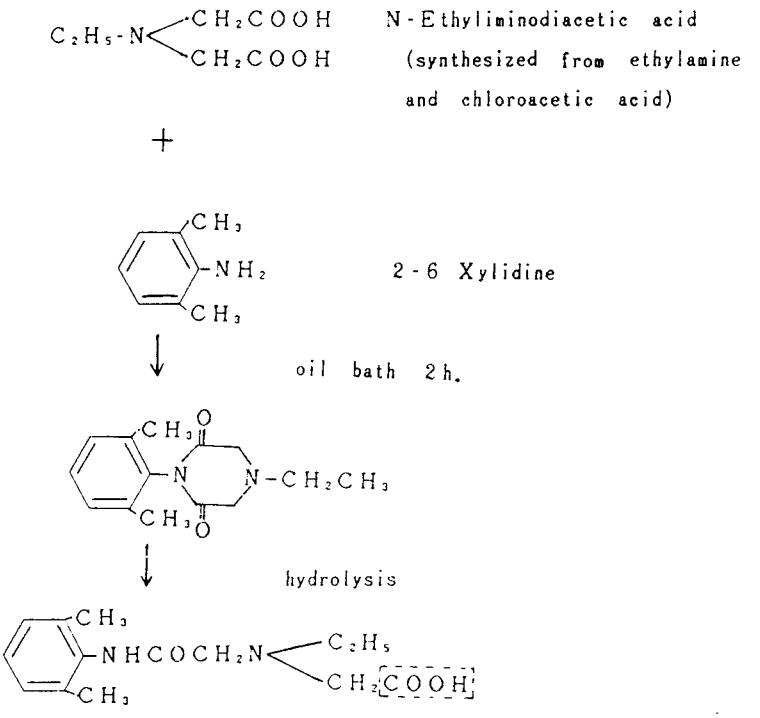


Fig. 1. Synthesis of lidocaine carboxylic acid derivative.

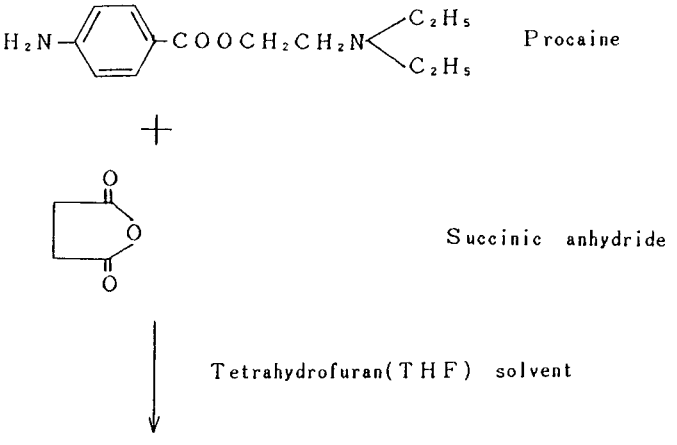
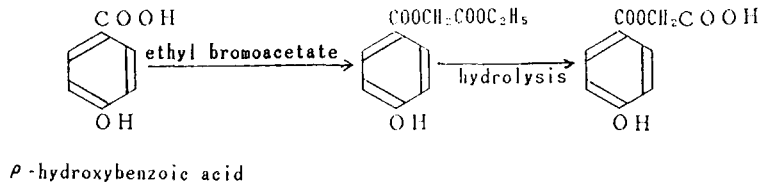


Fig. 2. Synthesis of procaine carboxylic acid derivative.

min (BSA) using tri-N-buthylamine and isobuthyl chloroformate by mixed anhydride method.

The molar ratio of lidocaine-COOH to BSA conjugate was determined spectrophotometrically (fig. 4). Spectrophotometric analysis was carried out by comparing the absorbance at 263 nm of the conjugate with



ρ-hydroxybenzoic acid

Fig. 3. Synthesis of methylparaben carboxylic acid derivative.

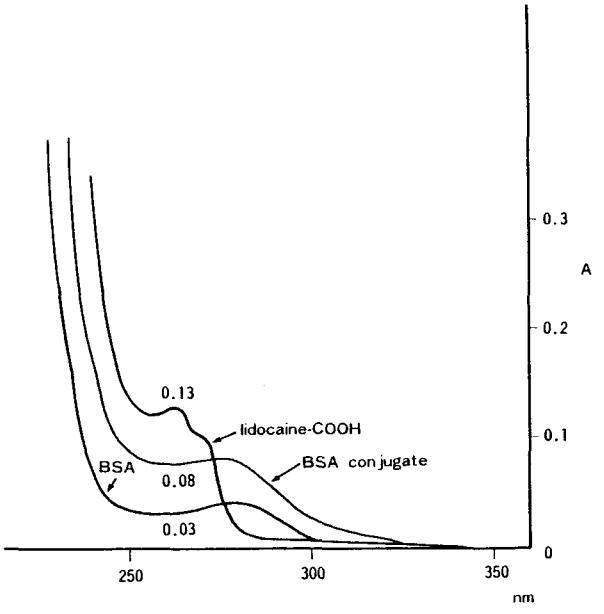


Fig. 4. Spectrophotometric absorbance of lidocaine-COOH, BSA and lidocaine-COOH conjugated with BSA.

At 263 nm, absorbance of lidocaine-COOH is 0.13, that of BSA is 0.03, and that of lidocaine-COOH conjugated with BSA is 0.08.

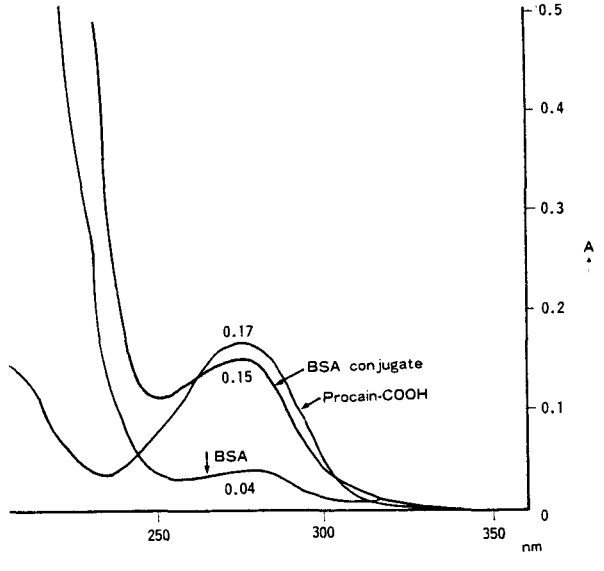


Fig. 5. Spectrophotometric absorbance of procaine-COOH, BSA and procaine-COOH conjugated with BSA.

At 273 nm, absorbance of lidocaine-COOH is 0.17, that of BSA is 0.04 and that of lidocaine-COOH conjugated with BSA is 0.15.

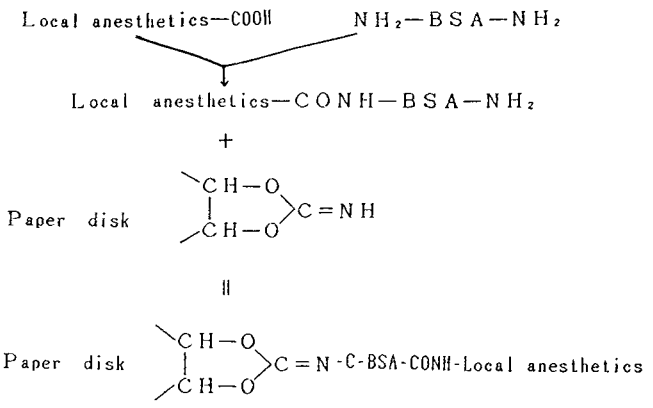


Fig. 6. Coupling of antigen to CNBr activated disk.

Table 1. RAST values (c.p.m.) obtained from 100 volunteers who were regarded to be nonallergic to the drugs-lidocaine, procaine and methylparaben

	mean	SD	Normal RAST values
Lidocaine	1.653	254	~2,161
Procaine	2.750	264	~3,278
Methylparaben	2,805	336	~3,477

RAST values less than mean +2SD have been regarded as a negative reaction to drugs.

Table 2. The c.p.m. value obtained from the patients who showed allergic positive reaction to lidocaine or procaine by skin test
All values are in the normal range.

Patient	The sera from positive skin test for lidocaine	The sera from positive skin test for procaine
1	1,294	2,159
2	1,363	2,001
3	1,965	
4	2,133	
5	2,117	
6	1,991	
mean±SD	1,810±346	2,080±79

that of BSA and lidocaine-COOH by using the following constants: molecular weight of BSA, 65000; ε value for lidocaine-COOH, 0.313×10^4. The number of lidocaine-COOH molecules linked to a BSA molecule was determined to be 10 by the method of Bernard and his colleagues[11].

In a similar way, the number of procaine-COOH molecules linked to BSA molecule was determined to be 16 (fig. 5).

3) Coupling of antigen to CNBr activated disk

CNBr activated paper disks were incubated with 30 ml of 10% allergen solution (pH7~8) over night at 4°C. The BSA conjugated allergens were then fixed on the paper disk (fig. 6).

4) Radioimmunoassay

The RAST was performed in the following way.

Fifty microliters of serum was incubated for 3 hours at room temperature with the allergen coated paper disk. After the incubation, every disk was washed with 3 ml of saline three times, and incubated with 50 microliters of ^{125}I-anti human IgE, corresponding to approximately 40,000 c.p.m., over night at room temperature and washed as above. The amount of radioactivity was measured in a gammascintilation counter. The RAST values were expressed as the c.p.m.

Results

Table 1 shows the results for sera obtained from 100 individuals, who were regarded as nonallergic with lidocaine, procaine and methylparaben by skin test. The c.p.m. values obtained are 1653 ± 254 (mean ± SD) (lidocaine), 2750 ± 264 (procaine), and 2805 ± 336 (methyl paraben). The subjects whose RAST values less than 2161

c.p.m. (mean + 2SD) have been regarded as nonallergic to lidocaine. Similarly, the subjects whose values less than 3278 c.p.m. (procaine), and 3477 c.p.m. (methylparaben) have been regarded as nonallergic to procaine and methylparaben.

Table 2 shows the responses of sera from 6 patients who had allergic history to local anesthetics.

Positive RAST value was not obtained from any of these patients. The mean RAST value of sera from the six patients who had a positive allergic reaction to lidocaine is 1810 ± 346 c.p.m. The RAST values of sera from two patients who had positive skin test to procaine were also in the normal range.

Discussion

Anaphylactic and hypersensitive reactions of procaine are well-known as procaine shock[12]. It has been assumed that the ester-link group of local anesthetics and methylparaben possess antigenic properties whereas the amide-link group of local anesthetics have not possess such antigenic properties.

Allergic reactions such as hypotension, skin rashes and respiratory depression can not be used as an absolute diagnosis of type 1 anaphylatic reaction. Neurogical response caused by pain due to the injection may be mistaken for anaphylactic shock. Accidental intravenous injection of local anesthetics may cause the loss of conciousness particulary in children. A large number of patients thought to be allergic to local anesthetics are found negative with skin test. Too often a patient is described as allergic to local anesthetics without adequate evaluation.

In order to clarify whether the patient is indeed allergic to the drugs, more precise immunological screening is necessary. Preliminary studies have indicated that RAST method may be valuable as screening test, and that it is useful for the invitro diagnosis of specific allergies. Stenius and Wide[13] found an 83 percent correlation between RAST and the results of skin prick test. RAST has the obvious advantage of not risking the occurrence of potentially serious reactions.

Since Wide and his collegue[6] first performed the RAST by using penicillin coupled to BSA as the hapten, RAST has been used as a diagnostics test for several different antigens[7-10]. Only amino polypeptides or proteins can bind chemically to CNBr activated cellulose and sephadex. Therefore, to perform the RAST for local anesthetics, it is necessary to prepare the antigen which can bind to the CNBr activated cellulose disk.

In this study, lidocaine-COOH, procaine-COOH and ethyl ρ-hydroxy O-benzoglycolate were synthesized and conjugated with BSA. These products were able to bind the activated cellulose disk. It was thought that allergenic action of local anesthetics was maintained in those products because the intermediate chain i.e ester-link and amide-link of local anesthetic remain unchanged. The RAST procedure can readily be used for the detection of IgE antibody specific for a given anesthetic in sera. In the present study, RAST values were not high in six patients who had reported allergic to local anesthetics. This may be associated with the fact that the half-life of IgE antibody is very short (2.4 day). These sera were taken from the patients more than six months after the anaphylactic reaction occurred. The value of the RAST may gradually decrease with time[9]. In allergic disease, however, the other classes of immunogloblins may also have an effect.

The allergic reaction of these patients may be due to the other classes of immunogloblin such as IgG, and not to IgE antibody.

If these allergic reactions are not a type I, RAST values from these patients should be normal. The RAST method is simple and useful procedure to detect the drug specific IgE anti-body in vitro. A limitation of the present study is that it measures allergy associated only with IgE anti-body. It is extremely important to establish the RAST for the other classes of immunogloblin in future studies.

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References

1. Fregert S, Tegner E, Thelin I: Contact allergy to lidocaine. Contact Dermatitis 5:185–188, 1979
2. Arora S, Aldrete JA: Investigation of possible allergy to local anesthetics drug. Anesthesiology review 13–16, 1976
3. Aldrete JA, Johnson DA: Allergy to local anesthetics. JAMA 207:356–357, 1969
4. Timbury MC: Allergy to local anesthetics. Med J Australia 19:386, 1980
5. Falace DA, Hill JS: Allergy to lidocaine and mepivacaine: Report of a Case. Compend Contin Educ Denti 6:280–284, 1985
6. Wide L, Bennich H, Johansson SG: Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet 2:1105–1107, 1967
7. Ceska M, Eriksson R, Varga JM: Radioimmunosorvent assay of allergens. J Allergy Clin Immunol 49:1–9, 1972
8. Baldo BA, Fisher M McD: Detection of serum IgE antibodies that react with alcuronium and tubocuraine after lifethreatening reactions to muscle-relaxant drugs. Anesth Intensive Care 11:194–197, 1983
9. Yasuno H: A study on penicillin and cephalosporin allergy using radioallergosorbent test (Eng Abst). West Jap Derma 43:416–422, 1981
10. Aras K, Johansson SGO: The radioallergosorbent test in the in vitro diagnosis of multiple reaginic allergy. J Allergy Clin Immunol 48:134–143, 1971
11. Bernard FE, Felix B, Sam MB, Seymour L: Steroid-Protein conjugates. J Biol Chem 228:713–727, 1957
12. Waldbott, GL: Allergic shock from local and general anesthetics, Anesth Analg 14:199–204, 1935
13. Stenius B, Wide L: Reaginic antibody (IgE), skin and provocation tests to Dermatophagoides culinae and house dust in respiratory allergy. Lancet 2:455–457, 1969