

A comparison of cutaneous testing and ELISA testing for assessing reactivity to ethylene oxide-human serum albumin in hemodialysis patients with anaphylactic reactions

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In five patients who have experienced anaphylaxis and in 29 patients who have not had such episodes during hemodialysis, we have performed two immunologic studies: cutaneous testing with ethylene oxide-human serum albumin (ETO-HSA) and ELISA for IgE against ETO-HSA. Four of five patients with reactions had positive cutaneous tests, whereas only one nonreactor had a positive skin test ($p < 0.0002$). The same four of five patients with reactions also had positive ELISA results, whereas three nonreactors had positive ELISA results ($p < 0.003$). In this group of patients, the positive predictive value of cutaneous testing (80%) is somewhat higher than that of ELISA testing (57%). However, the sensitivity, specificity, and negative predictive values are similar. We conclude that cutaneous testing with ETO-HSA probably offers a small advantage over IgE against ETO-HSA as determined by ELISA. (J ALLERGY CLIN IMMUNOL 1991;87:674-6.)

Anaphylaxis, which is a symptom complex that may include urticaria, bronchospasm, angioedema, hypotension, or cardiopulmonary arrest, has been reported as a reaction to hemodialysis in some patients.^{1,2} The significance of the problem of anaphylaxis to hemodialysis is underscored by the fact that life-threatening reactions and even death have been reported.³ The original hypothesis that the sterilizing agent, ETO, conjugated to HSA could act as an allergen was proposed by Dolovich and Bell.¹ Subsequently, we have reported a strong association between anaphylactic reactions to hemodialysis and IgE against ETO-HSA.⁴ In spite of the strong association, a significant proportion, approximately one third, of patients with dialysis anaphylaxis do not have detectable IgE against ETO-HSA.

Abbreviations used

ETO-HSA: Ethylene oxide-human serum albumin
PBS-T: Phosphate-buffered saline-Tween

Cutaneous tests, in particular, intradermal tests, tend to be more sensitive than in vitro tests for detection of specific IgE. Therefore, we performed this study to determine whether cutaneous testing might be more useful than ELISA testing in the evaluation of patients who have had hemodialysis anaphylaxis and in predicting which patients receiving dialysis might develop anaphylaxis.

MATERIAL AND METHODS

Patients

For two consecutive years, we clinically and immunologically evaluated each hemodialysis patient in our medical center who would consent to participate in this study. All patients were dialyzed with devices that were sterilized with ETO. Reactors were defined as those patients who had dialysis reactions of the immediate type that included one or more of the following: urticaria, angioedema, bronchospasm, or hypotension. Serum was obtained from five reactors, 29 nonreactors, and three nonatopic laboratory personnel with no known ETO exposure. Sera were stored at -20°C until assays were performed.

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ETO-HSA

Human albumin (25% solution) approved for human use was from the New York Blood Center. The albumin solution was exposed to ETO for 4 hours and aerated for 12 hours. This is the usual gas sterilization procedure. To remove any free ETO, the ETO-HSA solution was dialyzed in buffered saline containing 0.4% phenol. The ETO-HSA solution was then passed through 0.20 μ m filters (Nalgene Labware, Rochester, N.Y.) and sterility tested. HSA solution without ETO was dialyzed and filtered in the same manner to serve as a control.

Patients were tested by the prick test method with 1 mg/ml of ETO-HSA, 1 mg/ml of HSA, and 1 mg/ml of histamine base. The ETO-HSA and HSA solutions contained equivalent amounts of HSA. If the prick test was negative, incremental intradermal testing was performed with 0.02 ml of ETO-HSA at 10 μ g/ml, 100 μ g/ml, and 1 mg/ml and HSA at 10 μ g/ml. If the histamine resulted in a wheal that was not at least 2 mm larger than the HSA wheal, the skin tests were considered uninterpretable. A positive skin test was defined as a wheal that was at least 2 mm larger than the HSA control wheal.

ELISA

We estimated IgE against ETO-HSA by a modification of a previously described ELISA method.^{5,6} Immulon flat-bottom 96-well micro-ELISA plates (Greiner & Sons, Nürtingen, Germany) were coated with 200 μ l of either ETO-HSA or HSA at 100 μ g/ml in carbonate buffer, pH 9.6, for 1 hour at 37° C. The plates were then washed three times with PBS-T (Sigma Chemical Co., St. Louis, Mo.); 200 μ l of serum diluted in PBS-T was placed in individual wells and incubated for 30 minutes at 37° C. Plates were again washed three times, and 200 μ l of rabbit antihuman IgE (Calbiochem-Behring, La Jolla, Calif.) diluted 1:1000 in PBS-T was added to each well and incubated for 30 minutes at 37° C.

Plates were again washed, and 200 μ l of goat antirabbit gamma globulin conjugated to alkaline phosphatase was diluted 1:1000 in PBS-T, added to each well, and incubated for 1 hour at 37° C. After a final wash, 200 μ l of enzyme substrate, p-nitrophenyl phosphate (Sigma Chemical Co.), 1 mg/ml in 10% diethanolamine buffer, pH 9.8, was added and incubated at 37° C until color was developed. The optical density of each well was read at 405 nm in an Artek model 210 reader (Artek Systems Corp., Farmingdale, N.Y.). ELISA titers were the lowest dilution of patient serum that had an optical density in the ETO-HSA-coated wells of at least twice that of the HSA-coated wells.

Sensitivity, specificity, and predictive value

Sensitivity is all patients with a positive test divided by all reactors, whereas specificity is all patients with a negative test divided by all nonreactors. Positive predictive value is the true positive tests divided by all positive tests, whereas negative predictive value is true negative tests divided by all negative tests.⁷

TABLE I. ETO-HSA reactivity by cutaneous test and ELISA in dialysis reactors and nonreactors

	Reactors	Nonreactors
ELISA		
Positive	4	3
Negative	1	26
	χ^2 , 8.7540; p < 0.003	
Skin test		
Positive	4	1
Negative	1	28
	χ^2 , 14.2889; p < 0.0002	

RESULTS

Cutaneous tests with ETO-HSA, HSA, and histamine

Four of the five patients who had dialysis reactions had positive cutaneous tests to ETO-HSA. One patient had a positive prick test, two had a positive intradermal test with 10 μ g/ml, and one patient had a positive intradermal test with 100 μ g/ml. One of the 29 patients who had never had a reaction had a positive intradermal test to 1 mg/ml of ETO-HSA (Table I). By chi-square analysis, there is a significant association between a positive cutaneous test and anaphylactic reactions to dialysis (χ^2 , 14.2889; p < 0.0002). The positive predictive value, the negative predictive value, sensitivity, and specificity of cutaneous testing in this population are 80%, 96%, 80%, and 96%, respectively. In all patients, the histamine test resulted in a wheal that was at least 2 mm larger than the HSA test wheal.

ELISA for IgE against ETO-HSA

Four of the five patients who had reactions had detectable IgE against ETO-HSA, whereas three of 29 patients who never had a reaction had a positive IgE against ETO-HSA (Table I). By chi-square analysis, there is a significant association between a positive IgE by ELISA against ETO-HSA and hemodialysis anaphylaxis (χ^2 , 8.7540; p < 0.003). The positive predictive value, the negative predictive value, sensitivity, and specificity of IgE against ETO-HSA by ELISA in this population are 57%, 95%, 80%, and 96%, respectively.

DISCUSSION

Anaphylactic reactions are an uncommon but potentially life-threatening complication of hemodialysis.⁸ Consequently, it would be desirable if all the

antigens responsible for such reactions could be identified and if tests could be developed to evaluate and predict those patients at risk. Patients at risk for anaphylaxis to a given agent could be dialyzed via techniques that would avoid exposure to that agent. In this comparative study of cutaneous testing and ELISA testing, positive results of either test were significantly associated with anaphylactic reactions to hemodialysis in the population at our medical center.

We have previously reported in vitro results of 24 patients with anaphylactic reactions to hemodialysis. Most of those patients were at other medical centers, not available for cutaneous testing, and therefore not included in this study. In our medical center patient population, the positive predictive value of cutaneous testing (80%) is somewhat better than that of ELISA testing (57%). However, the negative predictive value, sensitivity, and specificity are very similar.

In embarking on this study, it was our conjecture that we might find some patients who had reactions, a negative ELISA test, but a positive cutaneous test. We did not find such patients to explain the absence of positive in vitro tests in approximately one third of reactors.⁴ Most probably, as has been hypothesized, there are other, as yet unidentified, antigens that are responsible for the anaphylaxis or the mechanism may be anaphylactoid in some patients.⁸

In summary, both positive cutaneous testing results

and positive ELISA testing results are associated with anaphylaxis during hemodialysis in the patient population studied at our medical center. There appears to be a small advantage to performing cutaneous tests rather than an ELISA for IgE against ETO-HSA.

REFERENCES

1. Dolovich J, Bell B. Allergy to product(s) of ethylene oxide gas. *J ALLERGY CLIN IMMUNOL* 1978;62:30-2.
2. Nicholls AJ, Platts MY. Anaphylactoid reactions due to haemodialysis, haemofiltration, or membrane plasma separation. *Br Med J* 1982;285:1607-9.
3. Popli S, Ing TS, Daugirdas JT, et al. Severe reactions to cuprophane capillary dialyzers. *Artif Organs* 1982;6:312-5.
4. Grammer LC, Paterson BF, Rixe D, et al. IgE against ethylene oxide-altered human serum albumin in patients with anaphylactic reactions to dialysis. *J ALLERGY CLIN IMMUNOL* 1985;76:511-4.
5. Voller A, Bidwell DE, Bartlett A. Enzyme immunoassays in diagnostic medicine: therapy and practice. *Bull WHO* 1976;53:55-6.
6. Sepulveda R, Longbottom JL, Pepys J. Enzyme-linked immunosorbent assay (ELISA) for IgG and IgE antibodies to protein and polysaccharide antigens to *Aspergillus fumigatus*. *Clin Allergy* 1979;9:359-71.
7. Galen RS. Use of predictive value theory in clinical immunology. In: Rose NR, Friedman H, Fahey JL, eds. *Manual of clinical laboratory immunology*. 3rd. ed. Washington, D.C.: American Society for Microbiology, 1986:966-70.
8. Miller GB Jr, Sikes RK. Acute allergic reactions associated with reprocessed hemodialyzers—Virginia. 1989. *MMWR* 1989;38:873-4.