
Comparative studies of latex extracts used in skin testing

Jordan N Fink, MD; Kevin J Kelly, MD; Nancy Elms; and Viswanath P Kurup, PhD

Background: There has been considerable variability reported in latex extracts used for skin testing and the results of those skin tests in the evaluation of latex allergy. Some test extracts have been associated with the induction of anaphylactic reactions in sensitive patients, while others have been reported as without adverse reactions.

Objective: The present study was designed to evaluate latex extracts by comparing antigens from latex gloves.

Methods: Latex gloves were extracted for from 15 minutes to 24 hours for their protein contents and in vitro reactivities with IgE antibodies using sera of latex allergic patients and controls by ELISA.

Results: A higher yield of proteins was obtained after prolonged extraction, although reactivity of the sera by ELISA showed no major differences among the extracts. Extracts of 15 minutes elicited stronger reaction with health care workers and patients with spina bifida than with 24-hour extracts. The 15-minute extract demonstrated comparatively more reactive antigenic components in the fractions representing 3 to 10 and 10 to 30-kDa compared with fractions having less than 3 and over 30-kDa antigens.

Conclusion: The study provided no evidence regarding the safety of one latex extract over the other related to time of extraction. The results indicated that a 15-minute extract can be used in ELISA for in vitro and, as in the literature, safe in vivo demonstration of latex specific IgE antibody.

Ann Allergy Asthma Immunol 1996;76:149-52.

INTRODUCTION

Latex hypersensitivity is commonly manifested as contact dermatitis due to chemical accelerators used in latex products. In recent years, however, immediate reactions ranging from contact urticaria to anaphylaxis have been increasingly recognized.¹⁻⁴ The latex reactions have been shown to be latex protein antigen-IgE-mast cell mediated; but the responsible specific antigen(s) has not yet been identified. As no standardized antigen is available, it

has become practice to use extracts of finished latex products, usually gloves, in the performance of prick tests to identify specific IgE in individuals such as health care workers (HW) or patients with spina bifida (SB) suspected of having latex allergy.

While skin testing is the most reliable technique for the detection of clinically relevant specific IgE, adverse reactions to the test may occur. We have previously reported a series of adverse reactions induced by prick skin testing with minute amounts of latex antigen and have thus avoided the procedure.⁵ Investigators in Finland^{6,7} and elsewhere in the US⁸ have reported no serious adverse effects from prick tests with latex extracts prepared in a different manner. These findings suggest that the variances noted may be due to differences in techniques, antigen preparations, or perhaps population sensitivities. This study was therefore

undertaken to determine whether significant differences in the antigen preparation played a role in the reactions noted.

MATERIALS AND METHODS

Patients

Sera were obtained from five patients with spina bifida and five health care workers. All had histories compatible with latex allergy including urticaria, asthma and/or anaphylaxis and had immediate wheal and flare skin reactions as well as systemic reactions to prick testing with latex extracts prepared in our laboratory.⁵ All patients had elevated latex-specific IgE levels as determined by ELISA.⁹ Five normal subjects without any clinical evidence of latex allergy were also included as controls.

Antigens

Three sources of latex gloves were used. Baxter Triflex, Smith and Nephew (Perry X-am 100), and Ansell. Some of these gloves have also been used in skin prick testing in Finland.⁶

One set of gloves was extracted with a short period of extraction (15 minutes) according to the method described by Turjanmaa.^{6,7} The other five sets were prepared according to the method previously reported by our group (30 minutes to 24 hours), but with some modification.⁹ Gloves were cut into pieces of 0.5 × 0.5 cm size and about 400 mg of the gloves (about 20 pieces) were extracted with 5 mL of sterile physiologic saline at room temperature. The extractions were carried out for 15 minutes, 30 minutes, 1 hour, 2 hour, 8 hours, and 24 hours and aliquots removed at each time period for study. The liquid was separated, centrifuged at 3,000 rpm for 15 minutes and the supernatant collected. These extracts were studied for protein

From the Allergy-Immunology Division, Departments of Medicine and Pediatrics, The Medical College of Wisconsin, and the Research Service, Veterans Affairs Medical Center, Milwaukee, Wisconsin.

Supported in part by a grant from Ansell International and the Medical Research Service of the Department of US Veterans Affairs.

Received for publication March 27, 1995.

Accepted for publication in revised form July 3, 1995.

content and were used for antibody assays. All extractions and other studies were carried out in duplicate.

Characterization of the Extracts

The extracts were studied for protein content using the Lowry¹⁰ and the Biscinchoninic acid methods (Pierce Chemicals, Rockford, IL) using bovine serum albumin as standard and following the procedure of the manufacturer. All antigens were also subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis after reduction as previously described.⁹ The gels were stained with Coomassie blue and the proteins bands resolved on the gels were compared.

Enzyme-Linked Immunosorbent Assay (ELISA)

All sera from each group of subjects were used to evaluate the 15-minute and 24-hour extracts. Two of these sera from each group were used in evaluating the extracts of intermediate timed washings.

Wells of polystyrene microplates (Immulon 2, Dynatech Laboratories,

Chantilly, VA) were coated with the glove extracts after making dilutions in PBS.^{9,11,12} The plates were incubated at room temperature for three hours followed by overnight incubation at 4°C. After washing and blocking the wells, serum samples were added and incubated for two hours at room temperature. This was followed by the addition of goat anti-human IgE, biotinylated rabbit anti-goat IgG, and streptavidin peroxidase. The plates were incubated at room temperature for one hour each with different reagents and washed with PBS-Tween in between the addition of reagents. Orthophenylenediamine substrate was added, and the color developed was measured in an automatic ELISA reader with a 490-nm filter (Dynatech Laboratories, Chantilly, VA). The optical density values were compared with known positive and negative samples run with each plate. Similarly processed crude antigen-coated plates (5 µg/mL) were also compared. All reagents were standardized by checker-

board titration with previously determined positive and negative sera.

Comparison of Different Preparations Based on Molecular Size of Peptides

Two extracts of the gloves obtained at 15 minutes and at 24 hours were compared for the distribution of antigens with diverse molecular weights. The antigen extracts were further fractionated by molecular sieving using Centriprep (Amicon, Inc, Beverly, MA) of 3,000 Da, 10,000 Da, and 30,000 Da. The different fractions were then studied for their reactivity by ELISA as described above.

RESULTS

Characterization of Latex Proteins

The protein extracted from the three gloves at various extraction times varied considerably (Fig 1). There was also no agreement between the two protein assays used in the study; however, a progressive increase was noted in the protein content of the extractions carried out from 15 minutes to 24 hours. The longer extraction antigens showed an increase from 60% to 90% more protein (depending on the assay method used). Interestingly, there were still residual proteins present in the rubber gloves as evidenced by further extractions (data not shown). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, when stained with Coomassie brilliant blue, showed several faint bands representing 5 to 60 kDa in all extracts.

Enzyme-Linked Immunosorbent Assay (ELISA)

No major differences in antibody reactivity could be detected with the different antigens obtained by extraction of gloves at the 15-minute to 24-hour periods of time (Fig 2). All patients showed significantly elevated IgE titers against the various extracts used in the study. Baxter glove extracts invariably showed more reactivity with sera of all three groups compared with Ansell and Perry gloves. The reactivity against the patients by the different extracts were also not comparable. An-

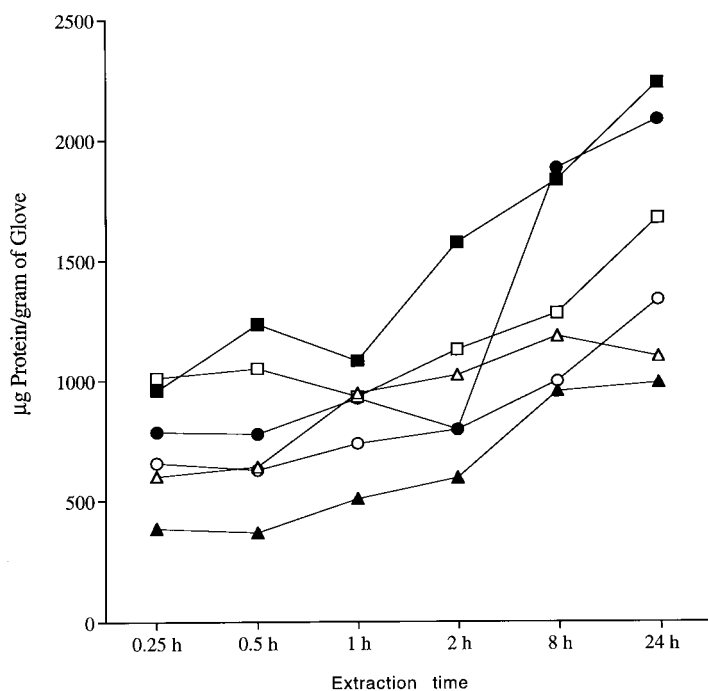


Figure 1 Protein content in the various extracts of gloves for 15 minutes to 24 hours estimated by the Lowry and biscinchoninic acid methods. Protein estimated by the Lowry method from Ansell ▲, Perry ■, and Baxter ●, and biscinchoninic acid method from Ansell △, Perry □, and Baxter ○ gloves.

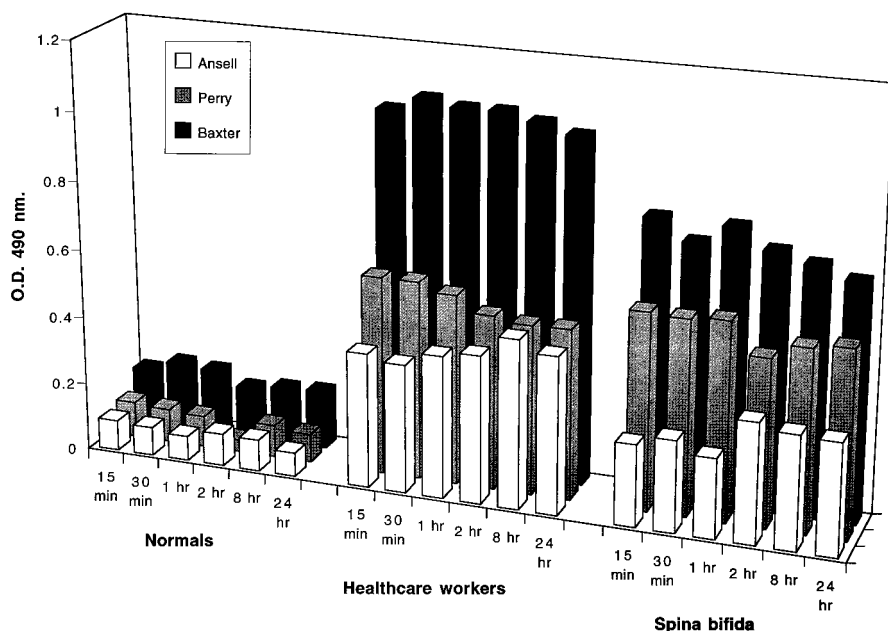


Figure. 2 The reactivity of various extracts 15 minutes to 24 hours from three brands of gloves studied for IgE binding activity in the sera of normal subjects, health care workers (HW), and patients with spina bifida (SB).

tigen extracted from Ansell gloves by longer extraction usually contained more reactive components compared with 15-minute extracts. The reverse was true with Perry glove extracts, which showed more antibody binding with a 15-minute extract than with a 24-hour extract. Baxter gloves extracted at different periods showed no major differences. Normal sera showed only low levels of antibody activity with all the extracts, although Baxter glove extracts showed more reactivity than with the other two glove extracts.

Reactivity of Antibody with Different Molecular Weight Fractions of Latex Peptides

The IgE reactivity of sera from the different groups to different molecular weight fractions of the 15-minute and 24-hour glove preparations are shown in Figure 3. Both health care workers and spina bifida patients reacted strongly with all fractions of the 15-minute extract. Spina bifida patients showed higher levels of IgE to proteins having 10 to 30 and over 30-kDa molecular weights. Health care workers

showed almost similar reactivity with all four fractions of antigens with highest reactivity to 10 to 30 kDa fraction. Normal sera reacted less than health care workers or spina bifida sera. There were no major differences between the 15-minute and 24-hour reactivities of these sera, except against the 10 to 30-kDa fraction, which showed lesser reactivity, particularly with the 15-minute extract.

DISCUSSION

The protein content of the three glove extracts were variable; differences can be attributed to the manufacturing process and/or the source of the natural rubber latex.^{9,13,14} It also appeared that the extraction time had a direct bearing on the extractable quantity of protein, as a marked increase in protein was detected after two hours of extraction (Fig 1). There was also a wide variation in the protein content noted between the two methods of protein detection. All extracts produced a precipitate on standing, whether at 4°C or at room temperature. Further, the precipitation was accentuated in anti-

gen preparations extracted from gloves for more than two hours.

Although 15-minute extracts have been used in skin tests, antigens prepared at extraction periods of longer times have not been compared by this technique.^{6,7} Because of the precipitation of antigens observed in extracts prepared by prolonged extraction methods (over two hours), the demonstrable skin and systemic reactions seen in our previous study⁵ may be the result of residual soluble antigens retained after the removal of this precipitate, although the Multi-Test technique used in that study may have played an important role in the reactions observed. The ELISA results, showing no major differences in extraction solutions, suggests that such precipitation may not be significant.

In previous report, the stronger reactivity of the 15-minute extracts used in skin testing has been attributed to low molecular weight peptides^{13,14}. The present study, however, suggests that the enhanced reactivity of 15-minute extracts over other extracts is probably due to the presence of polypeptide allergens of 10 to 30- and >30 kDa.

Latex proteins undergo rapid degradation with time (Lu, Liangjun et al submitted for publication). It is interesting to note that the 15-minute extracts showed components of all molecular sizes, particularly the presence of more 10 to 30-kDa allergen components than did the 24-hour extracts. This 15-minute extract also reacted strongly with both HW and SB patient sera compared with the 24-hour extract (Fig 3). Allergens of >30 kDa fractions from both 15-minute and 24-hour extracts elicited almost identical reactivity with both groups of patients. Other molecular fractions, ie, sizes less than 10 kDa, failed to show any major differences in their reactivity with IgE (Fig 3). It can be postulated that antigens having 10 to 30-kDa size from 15-minute extract may react strongly with sera from patients as antigens having >30 kDa components from 24-hour extraction.

We could not identify the specific antigenic proteins in these extracts due

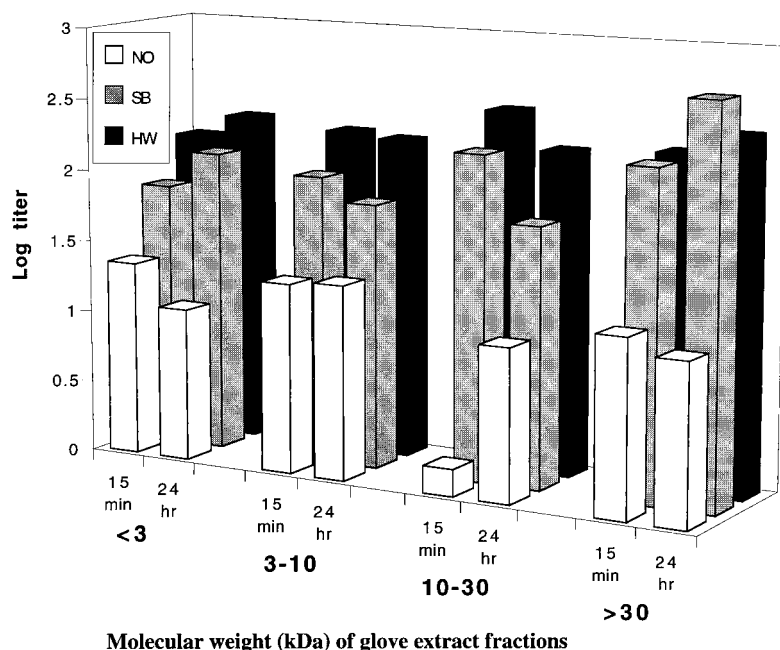


Figure 3 The reactivity of normal subjects' (NO), patients' with spina bifida (SB), and health care workers' (HW) sera to different molecular weight fractions of 15 minutes and 24 hours extracts of gloves studied for IgE antibody by ELISA.

to the low concentration of proteins available for Western blot analysis. Concentration methods such as freeze drying and molecular concentrations resulted in degradation and denaturation of the proteins; thus, a meaningful comparison could not be made of the different antigens extracted from the various gloves. Further, the brands of gloves also showed differences in protein contents, perhaps a corollary to differences in antigen content as demonstrated by others.¹⁵ Interestingly, several latex proteins bound strongly to the gloves and were not completely extractable by any of the extraction methods. Increasing the number of wash cycles to more than 15 did not eliminate latex proteins, which could still be demonstrated in the glove residuals. In addition, direct ELISA using such washed gloves also demonstrated latex allergens.

Gloves extracted for 15 minutes have been reported as useful sources of latex skin test antigen for the diagnosis of latex allergy; few adverse reactions

have been reported with this preparation.^{6,7} Antigens obtained after 24 hours of extraction have caused adverse reactions in patients with latex allergy following skin testing.⁵ The 24-hour extract, with increased quantities of protein, may contain potential allergens associated with anaphylaxis. In summary, it appears that, at this time, the 15-minute extract of latex is as immunoreactive as a 24-hour extract and hence, may be used to evaluate sera for specific IgE antibody by ELISA and as a skin test reagent, and may also be useful in screening latex products and to prepare standardized extracts for diagnosis.

REFERENCES

1. Carrillo T, Cuevas M, Hinojosa M, et al. Contact urticaria and rhinitis from latex surgical gloves. *Contact Dermatitis* 1986;15:69-72.
2. Fuchs T, Wahl R. Immediate reactions to rubber products. *Allergy Proc* 1992; 2:61-6.
3. Slater JE. Rubber anaphylaxis. *N Engl J Med* 1989;320:1126-30.

4. Kelly KJ, Setlock M, Davis JP. Anaphylactic reactions during general anesthesia among pediatric patients. *MMWR* 1991;40:437-43.
5. Kelly KJ, Kurup VP, Zacharisen M, et al. Skin and serologic testing in the diagnosis of latex allergy. *J Allergy Clin Immunol* 1993;91:1140-5.
6. Turjanmaa K. Incidence of immediate allergy to latex gloves in hospital personnel. *Contact Dermatitis* 1987;17: 270-5.
7. Turjanmaa K, Reunala T, Alenius H, et al. Allergens in latex surgical gloves and glove powder. *Lancet* 1990;336: 1588.
8. Mathew S, Melon A, Wagner W. Latex hypersensitivity: a case study. *Ann Allergy* 1993;70:483-6.
9. Kurup VP, Kelly KJ, Resnick A, et al. Characterization of latex antigen and demonstration of latex specific antibodies by enzyme linked immunosorbent assay in patients with latex hypersensitivity. *Allergy Proc* 1992;13: 329-37.
10. Lowry OH, Rosebrongh NJ, Farr AL, et al. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.
11. Kurup V, Kumar A, Kelly K, et al. Characterization of a monoclonal antibody against latex protein associated with latex allergy. *J Allergy Clin Immunol* 1993;92:638-43.
12. Kurup VP, Kelly KJ, Turjanmaa K, et al. Immunoglobulin E reactivity to latex antigens in the sera of patients from Finland and the United States. *J Allergy Clin Immunol* 1993;91: 1128-34.
13. Slater JE, Chhabra S. Latex antigens. *J Allergy Clin Immunol* 1992;89:673-8.
14. Chambeyron C, Dry J, Leynadier F, et al. Study of the allergenic fractions of latex. *Allergy* 1992;47:92-7.
15. Yunginger JW, Jones RT, Fransway AF, et al. Extractable latex allergens and proteins in disposable medical gloves and other rubber products. *J Allergy Clin Immunol* 1994;93: 836-44.

Request for reprints should be addressed to:
Viswanath P Kurup, PhD
VA Medical Center
Research Service 151-I
5000 West National Ave
Milwaukee, WI 53295