

Latex allergen elimination in natural latex sap and latex gloves by treatment with alkaline potassium hydroxide solution

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Antigenic proteins in latex products can cause type I allergy. We investigated the effects of potassium hydroxide (KOH) solution on the allergenicity of proteins in natural latex sap and latex gloves by using an EAST competitive inhibition immunoassay and skin prick test. Latex sap was mixed with KOH solution at different concentrations and incubated over various periods of time at room temperature. Latex gloves were washed in KOH solution for different times at 30°C and 90°C. Our immunoassay results for the subsequently extracted latex proteins demonstrated a KOH concentration-, temperature-, and time-dependent decrease in allergenicity, finally resulting in complete loss of IgE-binding activity. In the skin prick test, we found only four weakly positive reactions to proteins extracted from KOH-washed gloves in 30 latex-sensitized patients. In addition, up to 97% of the aqueous extractable protein content could be removed from latex gloves by washing in KOH solution under certain conditions. These results suggest that antigenic proteins in natural latex sap and latex gloves can be changed by treatment with KOH solution, which is followed by a loss of their capability to bind specific IgE antibodies from most latex-sensitized patients.

Key words: allergen elimination; latex allergy; potassium hydroxide; specific IgE.

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Immediate-type hypersensitivity reactions to latex products, particularly to medical gloves, have become a serious problem in recent years. They now affect approximately 0.1% of the general population (1), up to 10% of health-care workers (2–5), and up to 38% of children undergoing multiple operations (predominantly spina bifida patients) (6, 7).

The main cause of latex sensitivity is the presence of soluble proteins in natural rubber products (8–13). More than 240 different polypeptides from natural latex sap could be detected by two-dimensional electrophoresis, and, according to results from two-dimensional immunoblotting, nearly 60 of them showed reactivity to IgE antibodies from latex-sensitized patients (13).

As the presence of the proteins partially adsorbed to the surface of the rubber particles in natural latex sap is important in maintaining latex stability (14, 15), it does not seem to be possible to produce rubber articles completely free from latex proteins. Several methods to reduce the extractable

protein content in dipped natural rubber latex articles have been developed so far: latex sap treatments, such as recentrifugation (14, 16, 17) or enzyme addition (14, 16, 18), and manufacturing process treatments, such as leaching (16–20), enzyme treatment (16), chlorination (16, 19, 21), or coating of latex product surfaces (16, 21).

In this paper, we introduce an alternative procedure which effects pH-induced changes of latex protein structures, so that the antigenic proteins lose their capability to bind specific IgE antibodies of patients with latex allergy. This is achieved either by adding potassium hydroxide (KOH) solution to natural latex sap or by washing finished latex products in KOH solution. We describe the treatment of latex sap and latex gloves with potassium hydroxide solution under different conditions (variation of KOH concentration, temperature, and/or time) and show the change or even the complete loss of allergenicity of the subsequently extracted proteins in serologic and skin prick tests.

Material and methods

Ammoniated (0.7%) latex sap from Malaysia provided by the company MAPA (Zeven, Germany) was used in this study.

Surgical gloves (Sempermed® classic, sterile, and powdered) from Semperit (Vienna, Austria) served as test material for the treatment of finished latex products.

Treatment with KOH solution

For the treatment of latex sap, KOH solutions at five different concentrations (1–5 M) were mixed with latex sap (1:9 v/v) so that the final KOH concentrations in the latex samples ranged from 0.1 to 0.5 M. These latex samples were incubated at room temperature over various periods of time (up to 5 weeks) on a horizontal "rotomixer" that ensured permanent blending. Latex samples incubated with distilled water under the same conditions served as controls.

By KOH treatment of latex gloves, each glove was first cut at the side to ensure that the KOH solution also reached the glove interior during treatment. The gloves were then washed in agitated 0.5 M KOH solution at temperatures of 30 or 90°C at different times. At each temperature, four gloves were immersed in 800 ml (1:16 w/v) 0.5 M KOH solution, and this mixture was stirred with a heatable magnetic stirrer which was also used to control the temperature in the solution. After 1, 5, 15, and 30 min, one glove was removed from the KOH solution and used for the subsequent protein extraction. Gloves treated with distilled water under the same conditions served as controls.

Extraction of latex proteins

For protein extraction, samples of latex sap were diluted with PBS buffer (pH 7.5; 1:1 v/v) and then centrifuged at 40 600 g for 1 h to separate the rubber particles. The liquid fraction was dialyzed against distilled water with a dialysis tube (SERVA, Heidelberg, Germany) with a cutoff size of 3.5 kDa at 4°C for 48 h. The dialysate was then lyophilized.

For protein extraction from treated gloves, each glove was cut into small pieces (1–2 cm²) and extracted 1:8 (w/v) in PBS buffer (pH 7.5) for 2 h at 37°C. After removal of the rubber pieces, the resulting extracts were centrifuged (2400 g for 15 min), dialyzed against distilled water (at 4°C for 72 h, membrane tube from SERVA with a cutoff size of 3.5 kDa), and lyophilized.

The lyophilisates were dissolved in PBS buffer (pH 7.5) and stored at –70°C until used.

Protein determination

The protein content of the latex sap extracts was determined according to Bradford's method with an analysis kit from Bio-Rad (Munich, Germany), with bovine serum albumin (BSA) as reference protein.

To avoid interference in the protein assay by chemical additives used in the manufacture of latex gloves, the protein content of the glove extracts was determined by the modified Lowry method according to the recommendation of the American Society for Testing and Materials (22). Ovalbumin was used as the assay standard.

Allergen determination by inhibition immunoassay

The binding capacity of extracted latex proteins to specific IgE antibodies was determined by EAST competitive inhibition immunoassay. All protein extracts were diluted with PBS buffer to obtain a uniform protein concentration of 0.1 mg/ml. A serum pool from six latex-allergic patients served as IgE antibody source. The latex IgE concentration in the serum pool was 11.45 kU/l, as determined by EAST Phadezym (Pharmacia Diagnostics, Uppsala, Sweden).

In the first step, proteins extracted from untreated latex sap (as described above) were bound to CNBr-activated cellulose disks and used as target allergen. Several dilutions (1:1, 1:20, 1:40) of each latex protein extract were pre-incubated in volumes of 10 µl with 50 µl of the IgE serum pool overnight at 4°C. This mixture was then incubated with the latex target allergen for 3 h at room temperature. After the cellulose disk was washed, the amount of latex-specific antibodies bound to the target allergen was determined by EAST Phadezym.

A reference allergen extract from untreated latex sap (extracted as described above) with a protein concentration of 0.1 mg/ml was used in several dilutions of 1:1 to 1:200 to obtain a standard inhibition curve. The allergen content of each latex protein extract was determined by comparison with this standard curve. The allergenicity of the extracted proteins was expressed as the ratio of the determined allergen amount to the protein amount used in the assay. The standard proteins were assigned an allergenicity of 100% (ratio=1).

Skin prick test (SPT)

Latex proteins extracted from gloves which had been washed in 1.0 M KOH solution at 90°C for 30 min as well as from untreated gloves were used to prepare the SPT solutions. The extraction was

performed as described above. The lyophilisates were dissolved in skin test solvent (0.4% phenol in 0.9% NaCl) to obtain a protein concentration of 0.01 mg/ml.

Both solutions were used in SPT with 30 latex-sensitized (SPT- and RAST-positive) health-care workers. All patients were informed about the study and gave their consent to participate. Histamine (1%) served as positive control and solvent as negative control. A wheal diameter of at least 3 mm surrounded by erythema was regarded as a positive reaction.

Results

Latex sap treatment with KOH

The protein content in latex sap was not significantly changed by the treatment with KOH solution and varied in all samples by 15% at most.

The allergenicity of latex proteins extracted from latex sap which was treated with KOH solution at different concentrations and by different incubating times is shown in Fig. 1. The proteins from the control samples in which latex sap was incubated only with distilled water inhibited IgE binding totally and did not exhibit any change of their allergenicity over time.

At a KOH concentration of 0.1 M in latex sap, a time-dependent decrease in IgE antibody binding occurred. After an incubation period of 5 weeks, the extracted proteins showed an allergenicity of 18% of the initial value only.

A 0.2-M KOH concentration in latex sap resulted in a decrease of protein antigenicity to about 20% of the initial value after 1 day. After 3 days, the proteins showed an IgE binding activity of only about 4%. The complete allergenicity loss

of the latex sap proteins was achieved after an incubation period of 3 weeks.

At a KOH concentration of 0.3 M in latex sap, the proteins showed only 2% of their initial antibody-binding capacity after 1 day and did not bind any IgE antibody after 5 days.

Proteins extracted from latex sap with 0.4 and 0.5 M KOH exhibited an IgE antibody binding activity of less than 1% after an incubation period of 1 day. A complete allergenicity loss by these concentrations was already achieved after 2 days.

Latex glove treatment with KOH

The residual extractable protein contents of the latex gloves after washing at 30°C and 90°C are shown in Figs. 2 and 3.

By washing the gloves, extractable proteins could be greatly reduced in the first minute, followed by a much slower, further reduction. At 30°C, washing in 0.5 M KOH solution for 30 min effected a decrease of extractable protein content to 94 µg/g (about 9% of the initial value), while there were still 287 µg (about 28%) of extractable proteins in 1 g of glove material detectable after washing in distilled water under the same conditions. At 90°C, the effect of leaching was even more marked. An extractable protein content of 32 µg/g (about 3%) was achieved by washing in KOH solution for 30 min at this temperature. After washing in distilled water under the same conditions, a reduction in protein content to 103 µg/g (about 10%) could be measured.

The allergenicity values of the extracted proteins after washing are shown in Figs. 4 and 5.

Proteins extracted from untreated gloves had an allergenicity of only 84%. Washing in distilled water at 30°C did not influence the IgE-binding

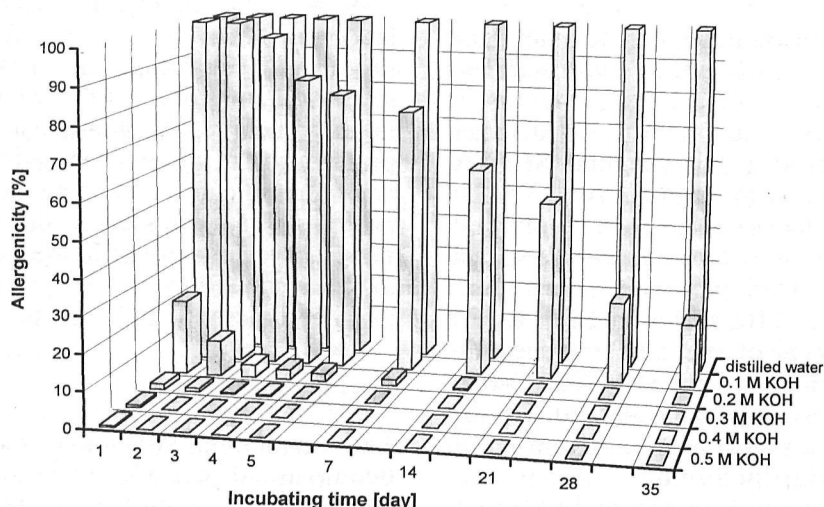


Fig. 1. Allergenicity of proteins extracted from latex sap after treatment with different KOH concentrations at room temperature.

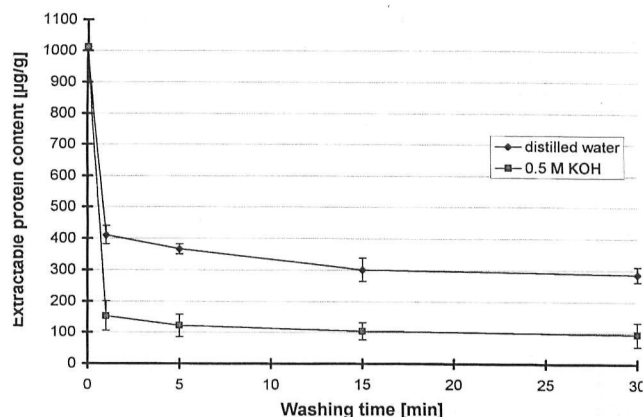


Fig. 2. Amount of extractable proteins from latex gloves washed in 0.5 M KOH or distilled water at 30°C.

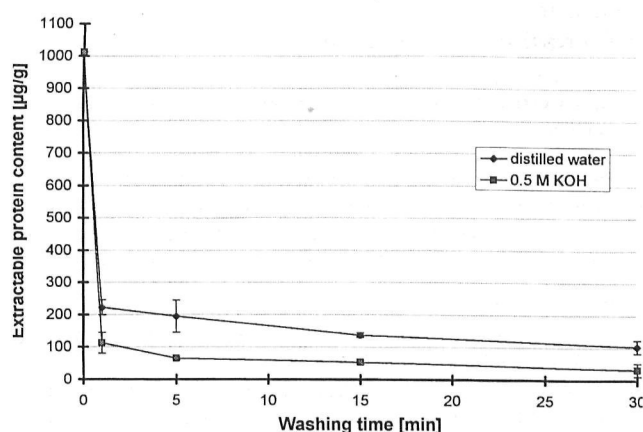


Fig. 3. Amount of extractable proteins from latex gloves washed in 0.5 M KOH or distilled water at 90°C.

activity of glove proteins. Treatment with 0.5 M KOH resulted in a loss of more than 50% of the IgE-binding activity of extracted proteins within the first minute. After 30 min, decrease to an allergenicity of about 10% occurred. At 90°C, treatment by distilled water was associated with a reduction of nearly 30% of the IgE-binding capacity within the first minute. After 30 min, the allergenicity dropped to about 10%. However, washing in 0.5 M KOH at 90°C resulted in a decrease of allergenicity to less than 0.5% within 1 min and caused complete loss of allergenicity after 15 min.

Skin prick test (SPT)

In the SPT, all 30 latex-sensitized subjects demonstrated moderate to strong wheal and flare reactions to proteins extracted from untreated gloves. The SPT solution containing an identical concentration of proteins from gloves washed in 1.0 M KOH solution at 90°C for 30 min elicited weakly positive reactions (wheal diameter ≤ 4 mm) in only four patients (RAST classes 3–6) (Fig. 6).

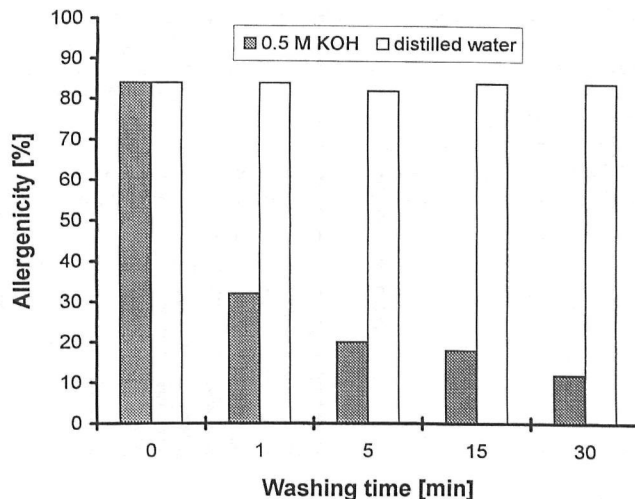


Fig. 4. Allergenicity of proteins from latex gloves washed in 0.5 M KOH or distilled water at 30°C.

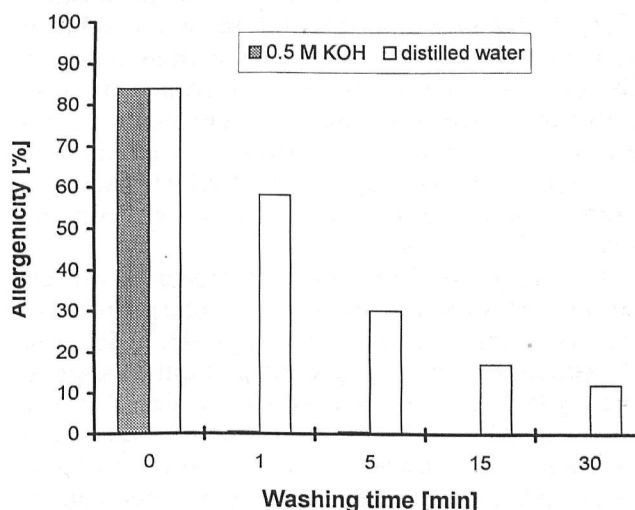


Fig. 5. Allergenicity of proteins from latex gloves washed in 0.5 M KOH or distilled water at 90°C.

Discussion

Our experiments demonstrate that antigenic proteins in natural latex sap and latex gloves can be changed by treatment with KOH solution, with subsequent loss of their capability to bind specific IgE antibodies from latex-sensitized patients. In this change of protein structure, the denaturing effects of alkaline pH and temperature are closely connected.

By treatment with KOH solution, antigenic proteins from latex sap and latex gloves showed a KOH concentration-, temperature-, and time-dependent decrease in allergenicity, finally resulting in a complete loss of IgE-binding activity in the inhibition immunoassay.

However, according to the SPT results, proteins from gloves treated by 1.0 M KOH at 90°C

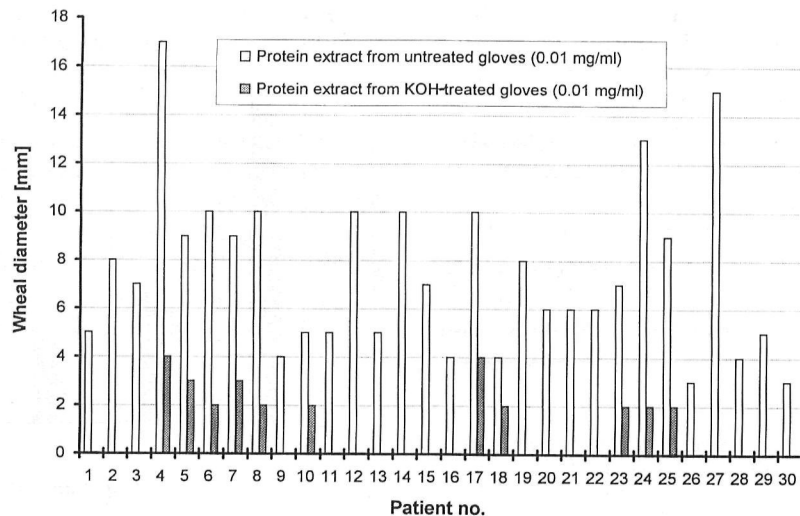


Fig. 6. Skin prick test responses obtained by extracts from untreated and KOH-treated latex gloves.

for 30 min still seem to have a small residual allergenicity, since four out of 30 sensitized subjects showed weak wheal and flare reactions to them. The glove treatment results demonstrate that proteins can be leached from latex gloves in a time- and temperature-dependent manner. Compared to distilled water, washing in 0.5 M KOH leads to a stronger reduction of extractable protein content, both at 30°C and 90°C.

Regarding the reduction of extractable protein content as well as the loss of allergenicity of residual extractable proteins by KOH, a decrease of extractable antigenic protein content of approximately 99% can be achieved by washing gloves in 0.5 M KOH solution at 30°C for 30 min. After washing at 90°C under the same conditions, there are no extractable antigenic proteins detectable by inhibition immunoassay. In contrast to KOH solution, washing in distilled water under the same conditions effects a reduction of the extractable antigenic protein content of about 70% at 30°C and about 98% at 90°C. The influence of the treatment on the structure and properties of natural latex sap and latex gloves should be further investigated.

The industry certainly has several means to apply this new procedure to eliminate allergens in latex sap as well as in finished latex products. The optimal conditions of treatment, e.g., KOH concentrations or concentrations of other OH⁻ sources, duration, and temperature, will depend on the production methods and on the products themselves.

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