

Reduction of latex-allergen content in Swedish medical catheter balloons – a survey of 3 years' production

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Anaphylactic reactions after intravascular exposure to natural rubber latex (NRL) have been reported. Thus, there is an urgent need to produce medical devices with the lowest possible latex-allergen content. The latex-allergen concentration in extracts prepared from 92 lots of medical catheter (MC) balloons, manufactured by Nolato Polymer AB, Torekov, Sweden, from April 1993 to March 1996, was measured with an EAI (IgE antibody inhibition) assay. Inhibitory capacity was expressed in arbitrary units/ml (U/ml) in relation to reference NRL sap, given an arbitrary value of 1000 U. Extracts from randomly selected lots were measured for protein by the modified Lowry method. Water leaching, chlorination, and treatment with savinase were used experimentally to study reduction of the latex-allergen content. The latex-allergen content in extract from the regular MC balloons varied from 0.1 to 2.9 U/ml. All the methods used to reduce the allergen content were effective, and increased leaching stabilized the allergen content at a low level. The protein concentration of the extracts varied between 9 and 100 mg/l. No correlation was found between protein and allergen content. As a result of this study, the manufacturer has extended the stage of water leaching in the production process. This study shows that cooperation between immunologists and manufacturers may result in product development and improvement.

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Many medical devices include elements made of natural rubber latex (NRL), often containing allergenic proteins; i.e., latex allergens (1).

Since the 1980s, severe allergic reactions due to latex allergy have been reported in many medical situations (2, 3), including intravenous exposure resulting in anaphylactic reactions (4). Medical catheter (MC) balloons of NRL represent a potential risk (5, 6) of severe reactions, but since the elastic properties are essential, NRL is difficult to replace with synthetic materials. Thus, there is an urgent need to produce MC balloons with the lowest possible content of latex allergens, and different ways to reduce allergenic latex proteins have been reported (7).

For study of the latex-allergen content in MC balloons, cooperation was established between

a Swedish manufacturer (Nolato Polymer AB, Torekov, Sweden) and the departments of Clinical Immunology and Occupational Dermatology of Karolinska Hospital, Sweden. The study aimed to monitor the latex-allergen content in MC balloons from one production line and to evaluate the efficiency of different techniques for latex-allergen reduction that could be used by the manufacturer.

Material and methods

Raw latex

Raw ammoniated latex from rubber trees (*Hevea brasiliensis*) grown in Malaysia and used by the manufacturer to produce MC balloons was

examined. As a reference, a latex preparation, E8-Latex Reference Preparation, was obtained from the Food and Drug Administration (FDA), Rockville, MD, USA.

Regular MC balloons

MC balloons 2 mm in diameter, intended for intravascular applications, i.e., dilation of arteriosclerotic constrictions of coronary vessels, were examined. From the same production line, samples were taken by the manufacturer at intervals of 1 week to 1 month. Altogether, 92 samples collected between April 1993 and March 1996 were examined.

The regular manufacturing process includes dipping the catheter mandrels in NRL, followed by 2-min drying, after which they are leached in water for 1 h. As a last step, the balloons are powdered with dry cornstarch. At the beginning of 1995, the leaching time was increased from 10 to 20 min and the temperature was raised from 40°C to 50°C.

Specially treated MC balloons

Different procedures aimed at reducing the latex-allergen content of the MC balloons were studied. One batch was manufactured in the regular way with a standard water leaching bath. Unpowdered samples from this batch were then chosen for experimental allergen-reducing procedures.

To reduce the allergen content in the MC balloons, the manufacturer used the following four alternative procedures:

- 1) additional leaching of the balloons in deionized water at 15°C for 1, 10 and 30 min after vulcanization (flow rate of water 2 l/min)
- 2) treatment of the balloons with 0.0025% solution of chlorine (Cl₂) gas (Eka Nobel, Sweden) in deionized water for 0.5, 2, and 5 min
- 3) treatment of the balloons for 1 min with Cl₂ gas (Eka Nobel, Sweden), dissolved in deionized water at 0.0025, 0.025, and 0.25% concentrations
- 4) treatment of the balloons for 60 min with a solution of the enzyme savinase 16.0 LDX (Novo Nordisk, Denmark), dissolved in deionized water (pH 8) at a temperature of 20°C, at final concentrations of 1, 10, and 100 mg/ml.

As reference, untreated balloons, unpowdered balloons, and powdered (Agenasorb 9023, Stärke-Gesellschaft M.B.H, Germany) balloons from the same production line were used.

Methods

Extract preparation. The MC balloons were cut into 2×2 mm pieces and extracted 1/10 w/v in 0.15 M saline preserved with 0.02% NaN₃ for 4 h at room temperature (approximately 20°C), followed by 37°C overnight. After mixing by vertical rotation for 15 min, the extracts were filtered through paper filter type IF, Munktell, Sweden, and stored at -20°C pending analyses. The raw latex and FDA latex reference were examined as is.

Protein assays. The protein concentration of five or six randomly selected lots for each of the 4 years was measured by the modified Lowry method (8, 9). In this method, the protein-containing extracts are treated with sodium deoxycholate (DOC), trichloroacetic acid (TCA), and phosphotungstic acid (PTA). This procedure is intended to precipitate the proteins before the original Lowry method is applied. Assay sensitivity was calculated to be higher than 2 mg/l, as this corresponded to the mean response in negative samples + 3 SD (*n*=5).

In extracts of 14 lots of MC balloons, the protein concentration was measured by the Kjeldahl method (10) as part of the routine production control by the manufacturer. The results are given in percent nitrogen.

Latex IgE antibody inhibition test (latex EAI assay). An EAI assay (11) was used to measure the allergen content of the latex extracts. In this method, the capacity of the latex allergens to block IgE antibodies to latex, resulting in a reduction in the level of remaining free IgE antibodies as measured by the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden), is compared to a reference.

A serum pool from three adult patients who were clinically allergic to latex was used. The patients were selected as being positive in SDS-PAGE and IgE immunoblotting to major latex-allergen components. In the EAI assay, the pool was incubated with three 10-fold dilutions of each latex extract. With a negative control (0.15 M saline), the assay showed no background problems. Good parallelism between different samples and reference was obtained. The inhibitory capacity of an extract was determined at 50% inhibition and expressed in units/ml, in relation to the arbitrary value of 1000 units of a standard preparation of cytosol from *H. brasiliensis* without ammonia. With this method, 0.03 U/ml (1 SD=0.007 U/ml) of latex allergen could be accurately quantitated (50% inhibition) and down to 0.02 U/ml (30% inhibition) detected. If the allergen content was too low to bring about 50% inhibition, the inhibition values were presented as percent inhibition of undiluted

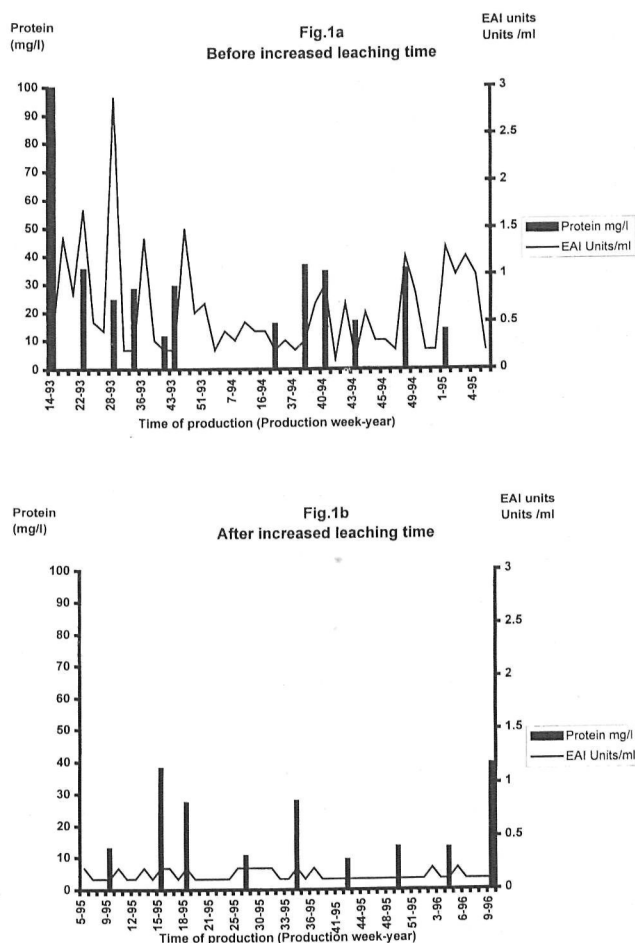


Fig. 1. Latex allergen concentration (solid line) expressed in EAI units/ml compared to protein concentration (solid bars) expressed in mg/ml measured by modified Lowry method in extracts of MC balloons produced April 1993 to March 1996. a) Values in extracts before leaching time were increased from 10 to 20 min and temperature raised from 40°C to 50°C. b) After increased leaching time and temperature.

extract. The interassay variation was 11%, estimated from one positive control in 10 runs.

Results

MC balloons from regular (33 months) production

Latex-allergen content. The latex-allergen content of extracts of balloons from the regular production collected from April 1993 to March 1996 (33 months) varied between 0.1 and 2.9 U/ml (Fig. 1). The corresponding value for the original batch of raw latex compound used by the producer in 1995 was 105 U/ml. The allergen content of the FDA E8 extract (reference) was 960 U/ml.

Protein concentration. The values for randomly selected lots each year, measured by the modified Lowry method, varied between 9.3 and 100 mg/l

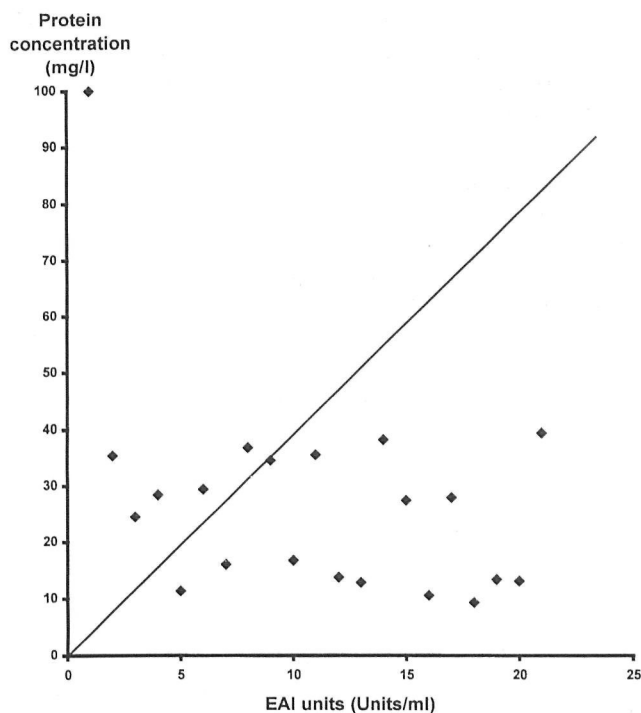


Fig. 2. Correlation between protein concentration measured by modified Lowry method and corresponding EAI-inhibition values (U/ml) of extracts of 21 MC balloons ($r=0.085$).

(Fig. 1). The reported protein concentrations of 14 lots from 1993 and 1994 estimated by the Kjeldahl method varied between 0.21 and 0.38% nitrogen.

Correlation between protein and allergen content. No correlation between the protein and EAI-inhibition values was found; $r=0.085$ (Fig. 2).

Measurements of specially treated MC balloons

Latex-allergen content. The allergen content of all the extracts from specially treated MC balloons was too low to reach 50% inhibition in the EAI assay necessary for calculation of latex-allergen units. The allergen concentration was therefore presented as percent inhibition (Fig. 3) and compared to that of the regular MC balloons used as reference. All four procedures resulted in significant reduction of allergen content. Another 10 min of leaching or the addition of proteolytic enzyme to a concentration higher than 10 mg/ml reduced the EAI inhibition to background levels (<20%). Even the mildest treatment with chlorine gas seemed to wipe out the allergen activity completely (Fig. 3).

Protein concentration. The protein values for specially treated MC balloons, measured with the

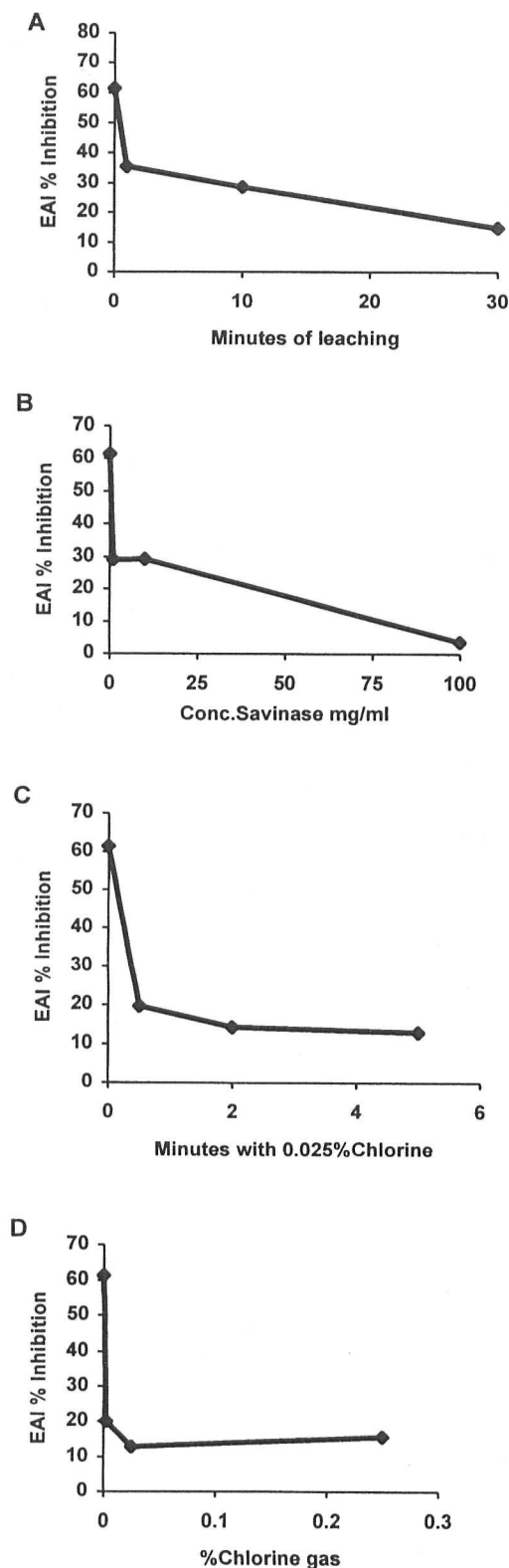


Fig. 3. EAI percent inhibition values of extracts of untreated MC balloons compared to those treated by four different allergen-reducing techniques. A) Extra leaching, B) savinase treatment, C and D) chlorination at different times and concentrations.

modified Lowry method, varied between 4.2 and 4.9 mg/l for additional water leaching and proteolytic enzyme procedures. The regular MC balloon (untreated) value was 7.8 mg/l.

Discussion

Most, if not all, medical products of NRL may contain latex allergens. These are a great hazard, especially for patients exposed intravascularly. Even if, at present, no safe lower limit of latex-allergen content can be defined, it is urgently necessary to minimize the latex-allergen exposure in medical situations. In this study, we show that cooperation between manufacturers and immunologic research laboratories may facilitate the development of techniques for reducing the allergen content in medical devices made from NRL.

Discussion between rubber manufacturers, clinicians, and immunologists has hitherto dealt mostly with the allergic problems caused by latex medical gloves. The standardization work on latex products, especially medical gloves, includes not only their barrier function, but also their biocompatibility and content of allergenic proteins. Manufacturers are trying hard to produce low-allergenic gloves and also to find methods to monitor their allergen content. The methods sought are also of great interest to manufacturers of many other medical latex products.

To measure the many latex allergens of clinical significance, we have to rely on immunologic methods using human sera from patients clinically and serologically allergic to NRL. In the future, however, it is hoped that methods using monoclonal antibodies specific for epitopes characteristic of allergens can be developed. Since latex allergens contain several major and minor allergenic proteins, much work remains to be done before techniques using monoclonal antibodies can replace current methods of allergen tracing, which depend on human serum. The present study employed the EAI-inhibition test to measure the latex-allergen content in MC balloons. The technique is an immunochemical assay for demonstration and quantification of allergens (requiring the use of human IgE antibodies to NRL).

The test has hitherto been used mostly for allergen examination in single objects. Now, in our study, this exclusive method has been used on a regular, long-term basis in an urgent industrial development project, where samples of latex MC balloons were examined continuously for 3 years.

During the first 20 of the 33 months, when the latex-allergen concentration in the balloons was monitored, it varied between 0.1 and 3 units/ml, showing great variation between production lots.

On the basis of these data, it was decided to increase the leaching time, a modification which resulted in the allergen content appearing to stabilize at a low level. Leaching is a well-known method of reducing allergen levels (12), and the study confirms that increased leaching results in a stable reduction of latex-allergen content.

However, with the aim of further reducing or even eliminating measurable latex allergens in the MC balloons, four experimental methods were tested. These procedures – further extended water leaching, savinase leaching, chlorination at different times, and chlorination with different concentrations – are well known in the rubber industry for destruction of protein structures. We found all methods to be effective in reducing latex-allergen concentrations to levels below the measuring range of the sensitive EAI assay. However, from the point of view of both producers and customers, the introduction of chemicals into the production process also presents potential risks. Chlorination presents an occupational hazard and also implies an environmental risk. Any residual chlorine in the final product may also be a potential risk for the patient – the customer. The proteolytic enzyme savinase introduces a new, potentially allergenic protein into the manufacturing process (13). Considering these risk factors, the manufacturer decided to rely on further extended water leaching of the MC balloons.

How far the protein concentration in an extract of a latex product reflects the real allergen content of the product is still under debate (14, 15). The proteins found in such an extract could be derived from the latex sap itself. But other proteins may be added during the manufacturing process; e.g., casein as in the case of glove manufacturing (16). Even if the proteins measured are all derived from the latex cytosol, allergens constitute only an extremely small proportion. Therefore, it is not surprising that in this study no correlation was seen between the allergen concentration measured by the EAI test and the protein content measured by the modified Lowry assays.

Another point of importance is the vulcanizing and antioxidizing chemicals used in the production of NRL products. They remain present in the finished product, and also in extracts thereof. These chemicals may interfere in the assays used to measure proteins in NRL extracts, a fact which also makes the results difficult to interpret.

In our study, the increased leaching seemed to reduce the allergen content, but the total protein content remained at original levels. Obviously, the level of the extractable protein does not reflect the real allergen content of the latex extracts, a hypothesis that fits very well with the fact that allergens

in general are medium-sized (mol. mass 10–50 kDa) glycoproteins readily soluble in water.

The amount of allergenic protein needed to sensitize, i.e., to stimulate IgE antibody production, and to provoke allergic symptoms is extremely low. To measure these very small but potentially clinically significant amounts of allergen in products, methods of very high sensitivity and specificity are required. Since consumers are increasingly aware of the risk of latex allergy, they will demand NRL qualities with the smallest possible allergen contamination.

Even if no safe lower limit of latex allergen content can be determined, EAI inhibition may be a valuable tool for monitoring the latex-allergen concentration in NRL products. Since NRL in medical devices cannot today be replaced by synthetic elastomers, it is valuable for consumers if manufacturers can label the allergenicity of their products.

Repeated analyses with the EAI test during production of NRL medical devices can provide the manufacturer with basic data for such labeling. The EAI-inhibition test is also a valuable tool in the development of production procedures that will result in low-allergen latex products.

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