Study of Allergenic Substances in Latex Products for Clinical Use

KUMIKO SAKATA¹, SANAE OHFUSA¹, TOMOKO CHIBA¹, JUN TERUI¹ and MASAKATSU SAKATA²

Abstract: The allergenic substances (dithiocarbamate compounds (DTC) and latex protein) in several latex products (examination gloves, surgical gloves and rubber dams) were determined, and several skin tests were performed. The DTC-type accelerators detected in 15 latex samples were Zinc-dibutylldithiocarbamate (ZDBC) (8 samples), Zinc diethyldithiocarbamate (ZDEC) (4 samples), and Zinc pentamethylenedithiocarbamate (ZPC) (4 samples). Zinc dimethylthiocarbamate (ZDMC), which is considered the most sensitive chemical of the DTC-type accelerators, was not detected in any sample. The amount of latex protein in the 15 samples varied from 96 ng/g to 4133 ng/g. The rubber dam had the lowest protein level. The surgical gloves had a moderate amount of protein, less than 1000 ng/g. The examination gloves had a wide range of protein: the highest values were 20~30 times the lowest values. The positive reaction rate of a patch test with a piece of latex glove on the human subjects was 2.5% (2/81). An additional patch test with petrolatum, containing the same kind of DTC compounds as in each positive latex glove, showed no positive reaction. Four subjects (4.9%) had a positive reaction to a prick test.

Key words: Allergy, Latex gloves, Latex protein, Dithiocarbamate, Skin tests

Introduction

The issues of viral cross-infection and personal protection in medical care have led to a significant increase in glove usage over the past several years¹,². In current dental practice, most dental professionals routinely wear latex gloves³. Along with the increase in the use of latex gloves, allergic reactions to their ingredients have increased⁴,⁵. Reported reactions have ranged from contact dermatitis to contact urticaria and systemic anaphylaxis. Two different allergic mechanisms are involved; delayed-type allergy (contact dermatitis) and immediate-type allergy (contact urticaria). The main allergenic substances of delayed-type latex allergy are thought to be some accelerators and other latex additives⁶,⁷, and for immediate-type latex allergy a protein derived from natural latex was recently suggested⁸,⁹. Various skin tests and immunological tests are performed to diagnose these latex allergies⁴,⁵,¹⁰.

There are many reports about latex allergies caused by surgical gloves, which are regulated as a medical device by Pharmaceutical Affairs Law in Japan. However, most of the gloves used in dental practice are examination gloves, the quality of which is not regulated by the law, and few studies on examination gloves have been carried out.

In the present study, the allergenic substances (dithiocarbamate compounds (DTC) used as an accelerator, and latex protein) of several latex products, including examination gloves, surgical gloves and rubber dams was determined, and several skin tests for latex allergy in human volunteers, dental students was performed at our university.

Materials and Methods

Samples: Twelve examination gloves (A-L), 2 surgical gloves (M, N), and 1 rubber dam (O) were used (Table 1).

Determination of allergenic substances
1) Qualitative analysis of DTC compounds

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The structure and names of the DTC studied compounds are given in Table 2. ZPC was generously provided by Dr. Akitada Nakamura, National Institute of Hygienic Sciences. The other DTC compounds and reagents were analytical grade.

The analytical procedure reported by Kaniwa et al. was used for analysis of the DTC compounds. The samples (0.5 g) were cut into fine pieces and extracted 5 times with 5 ml of acetone-chloroform (1 : 1). After evaporation of the solvent, the residue was dissolved in 1.5 ml of dichloromethane followed by shaking with 1 M cobalt chloride. The resulting Co-complexes of DTC compounds were analyzed with reversed phase high performance liquid chromatography (HITACHI L 6200) on a Wakosil II 5C18-100 column using methanol-water (80 : 20—100 : 0).

2) Quantitative analysis of latex protein

Samples (2 g) were cut into small pieces (1×1 cm) and extracted with 10 ml of phosphate buffered saline (pH 7.4) for 2 hr. After removing rubber pieces, the residual solution was centrifuged (3000 rpm, 10 min), and the supernatant was analyzed by the bicinchonic acid (BCA) method with colorimetry reported by Yagami et al.12.

Skin tests

The volunteers were informed about the purpose and the risk of the tests, and they provided their medical histories. The volunteers with any history of asthma or dermatological symptoms were excluded, and skin tests were performed on the 81 remaining subjects.

1) Patch test

Four examination gloves (A-D) and 2 surgical gloves (M, N) were tested. Small pieces (1×1 cm) of each of the glove samples were placed on the forearm of each subject and held in place with bandages. After 48 hr, the allergic reaction was checked according to the methods of the International Contact Dermatitis Research Group (ICDRG). The subjects who had positive or false positive results were given an additional patch test with petrolatum ointments, containing the same ingredient as the DTC compounds, and held in place with a special bandage (Miniplaster®).

2) Prick test

Two examination gloves (A, B) and 2 surgical gloves (M, N) were tested. Four hundred mg of freshly-cut glove pieces (1×1 cm) were incubated for 30 min in 5 ml of sterile physiological saline. After removing the latex pieces, the incubation fluid was used as a solution for the prick test. Physiological saline and histamine solution (10 mg/ml) were used as the negative and positive controls respectively. A drop of test solution was administered to the subject’s forearm and the prick was made with a special lancet through the drop, and the solution was gently wiped off with blotting paper.

Table 2 Structure and Name of Dithiocarbamate (DTC) Compounds

<table>
<thead>
<tr>
<th>R₁</th>
<th>N</th>
<th>C</th>
<th>S</th>
<th>Zn</th>
<th>S</th>
<th>C</th>
<th>N</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc Dimethylthiuram Disulfide</td>
<td>ZDMC : R₁ = R₂ = -Me</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Zinc Diethylthiuram Disulfide</td>
<td>ZDEC : R₁ = R₂ = -Et</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Zinc Dibutylthiuram Disulfide</td>
<td>ZDBC : R₁ = R₂ = -Bu</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc Ethylphenylthiuram Disulfide</td>
<td>ZEPD : R₁ = -Et, R₂ = -C₆H₅</td>
<td></td>
<td></td>
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<tr>
<td>Zinc Pentamethylenedithiuram Disulfide</td>
<td>ZPD : R₁ = R₂ = -C₅H₁₀</td>
<td></td>
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<td></td>
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</table>

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Manufacture</th>
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<tbody>
<tr>
<td>Hibiki 1,100</td>
<td>Hibiki</td>
</tr>
<tr>
<td>Denafit</td>
<td>Yoshida</td>
</tr>
<tr>
<td>Latex Clean Hand</td>
<td>Iwatsuki</td>
</tr>
<tr>
<td>Dental Glove</td>
<td>G C</td>
</tr>
<tr>
<td>Disposable Latex Glove</td>
<td>Asahi Emerse</td>
</tr>
<tr>
<td>Latex Dental Glove</td>
<td>Dunlop</td>
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<tr>
<td>Latex Dental Glove</td>
<td>Baxter</td>
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<tr>
<td>Biogel D</td>
<td>Okamoto</td>
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<td>Latex Glove</td>
<td>Shofu</td>
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<tr>
<td>Quali-Touch Latex Glove</td>
<td>Smart Practice</td>
</tr>
<tr>
<td>New Ascott Glove</td>
<td>Taipras</td>
</tr>
<tr>
<td>Washi Lagex Glove</td>
<td>Kisui</td>
</tr>
</tbody>
</table>

(The Surgical Glove) Readiwrap-N | Sankyo |
(The Hypoallergenic Surgical Glove) Sensi-Derm | Toray Medical |
(The Rubber Dam) Rubber Dam Sheet Thin | Miles Ivory |
paper. Wheal reactions were measured 15 min after application in the major and minor axes and divided by two. The reaction was considered positive if it was at least 1/2 the figure for the histamine reaction.

### Results

**Determination of allergenic substances**

The analytical results of the DTC compounds and latex protein in the 15 samples are shown in Table 3.

1) Qualitative analysis of DTC compounds

Though there were no DTC compounds detected in 2 examination gloves (H, K) and 1 surgical glove (N), one, two, or three kinds of DTC compounds were detected in each of the other 12 samples. ZDBC was detected in 8 samples, ZDEC or ZPC were detected in 4 samples, and zinc ethylphenyl-dithiocarbamate (ZEPC) was only detected in the rubber dam. ZDMC, recognized as the most sensitive of the accelerators, was not detected in any sample.

2) Quantitative analysis of latex protein

The amount of latex protein in the 15 samples varied from 96 to 4133 µg/g. The rubber dam contained the least amount of protein. Four examination gloves (B, D, H, K) and 2 surgical gloves (M, N) contained a moderate amount of protein, 100-1000 µg/g. Other examination gloves (A, E, I, L) contained the largest amounts of protein, which were 20 to 30 times higher than the lowest amounts.

**Skin tests**

The results of the patch tests and prick tests are given in Table 4. Two of the subjects who underwent the patch test and four of the subjects who underwent the prick test had positive reactions. The positive reactions were weak, and no subject showed a severe reaction such as anaphylaxis.

1) Patch test

Of the 2 subjects who had positive reactions, one had positive reaction to samples B and D and the other to sample D. The total positive rate was 2.5 % (2/81). Additional patch tests with petrolatum ointment containing the same ingredients as the positive gloves were performed with those 2 subjects, and the results were negative.

2) Prick test

Four subjects showed positive reactions which made a total positive rate of 4.9 % (4/81). There was no relationship between the positive reactions and the latex protein amount in each sample.
Table 4 Results of Skin Tests

<table>
<thead>
<tr>
<th>Allergenic Substance</th>
<th>Kinds of Containing DTC Compound</th>
<th>Protein Amount (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZDEC</td>
<td>ZPC</td>
</tr>
<tr>
<td>Sample Student</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>No. 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. 2</td>
<td>-</td>
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<td>No. 3</td>
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<td>No. 4</td>
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<td>No. 5</td>
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<td>No. 6</td>
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</table>

Discussion

In a preliminary survey on the usage of latex gloves in dental hospitals of private dental schools, it was observed that glove-wearing during clinical practice was regulated in all such hospitals. Various kinds and brands of gloves were in use in those hospitals; 19 examination gloves, 9 surgical gloves, and 12 plastic gloves were in use, and one hospital used about 5 kinds of gloves simultaneously on the average. Although in dental practice examination gloves are mainly used, the gloves are not regulated for quality, in contrast to surgical gloves. Information about the quality of examination gloves is limited, and only one report about the prevalence of perforations and permeability of these gloves is available14).

In 1933, Downing reported seven cases of rubber glove dermatitis, and he noted some symptoms which are common to urticaria15). In 1979, a woman who reacted to her household rubber gloves was the subject of the first report of latex allergy in the medical literature16). Since then, many case reports have been published, and the study of allergenic substances has progressed17-24).

Two types of allergic reactions to latex have been described. Delayed-type allergy is caused from chemical additives in the manufacturing process, especially the antioxidants, which prevent rubber deterioration, and the accelerators, which speed up speed up vulcanization. There are two types antioxidants, amine-type and phenol-type compounds. The phenol-type antioxidants are used in surgical gloves, and their sensitization activity is low. There are three types of accelerators, thiazole-type, thiram-type, and dithiocarbamate (DTC)-type compounds. Since DTC compounds are used in surgical gloves25), the DTC accelerators were analyzed in examination gloves.

In the present study, 3 samples contained no DTC accelerators, but other latex products contained 1-3 kinds of DTC compounds. The appearance rates of DTC accelerators in 12 examination gloves were ZDMC and ZEPC: 0%, ZDEC: 33.3%, ZDBC: 58.2%, and ZPC: 25.0%. The appearance rates in surgical gloves reported by Nakamura were ZDMC: 16.7%, ZEPC: 4.2%, ZDEC: 29.1%, ZDBC: 37.5%, and ZPC: 62.5%26). The differences in the appearance rates the two studies is attributable to the quality of gloves, which has improved with changes in the quality of the accelerators, because it was reported that ZDMC is a strong sensitizer in all DTC compounds, and that ZPC causes methemoglobinemia. Sample N is proposed as a hypoallergenic glove, in which no DTC accelerator was detected.

Recently, deaths caused by anaphylactic reaction to latex medical devices have been reported in the United States. Thereafter the Ministry of Welfare of Japan alerted Japanese health care professionals to the risk27). The causative substances are thought to be latex protein and derivatives thereof, so many studies, in which quantitative analysis of latex protein is carried out, are now being performed.

Although the total proteins were less than 100 µg/g and 1000 µg/g in the rubber dam and surgical gloves respectively, the protein level in the examination gloves varied from 100 µg/g to 4000 µg/g. The latex protein in surgical gloves and household gloves has been reported to be 73-543 µg/g and 1380 µg/g respectively28). This indicates that examination gloves contain the same amount of protein as or more than surgical gloves. There had been several reports on allergy to glove powder29,30), but now it is considered that cornstarch powder used to
bind latex protein may be the cause.33) Since the amount of antigenic protein in latex gloves should be reduced to prevent further sensitization and allergic reactions, only good-quality examination gloves should be used to minimize the risk of latex allergy.

Therefore, skin tests and immunological tests are important for the diagnosis of latex allergy. A patch test using a piece of the latex product is useful for diagnosis of a delayed-type allergy. The positive rate of patch tests for latex allergies is reported to be 1.3-6.4%, but if the latex contains ZDMC, the positive rate reaches 11.5%25). In the present study, there were no latex products containing ZDMC; therefore, the positive rate of the patch test was 2.8%, agreeing with the previous report. For the subjects who reacted positively to the latex piece, an additional patch test was performed and there were no positive reactions. Such a positive reaction is thought not to be due to the chemical additives themselves, but to the decomposed substances of DTC compounds used during the manufacturing process7,27).

The diagnostic methods for immediate-type allergy are skin tests and immunological tests. Quantification of the IgE amount and the radio-allergosorbent test (RAST) for latex antigens may be performed as immunological tests, but they are not common because they require a sample of the patient’s blood, and their results do not correlate well with the results of a prick test28). Therefore, the prick test is preferred by general practitioners29).

The positive rate in the prick test for latex allergy was reported to be 0.8% in the general population and 2.9% in health care professionals, of which dentists were 13.8%, surgeons were 7.5%, surgical nurses were 5.6%, and the other health care professionals were 1.3%30). In the present study, the positive rate in the prick test in the dental student volunteers was 4.9%, which was higher than the rate reported for health care professionals as a group, but lower than for dentists. It is thought that the duration of the exposure was too short to be sensitized because the students started to wear gloves almost 1 year before in preclinical practice.

The safety of latex products is important not only for health care professionals, but also for patients to be treated with latex products. One study reported that contact dermatitis and urticaria occurred with a rubber dem or latex gloves worn by a dentist during dental treatment.30)

Choice and use of lower allergen latex glove should lessen exposure of latex sensitized patients and health care professionals to latex allergen.

Conclusion

1. In the analysis of DTC compounds in latex gloves, ZDMC, recognized as the most sensitive accelerator, was not detected in any sample. Other DTC compounds contained in latex gloves were the same as the previous reports. The amounts of the latex protein in examination gloves varied from each other and these were the same as or more than those of surgical gloves.

2. In the results of skin tests for volunteers of dental students in our university, the positive rate of patch tests was the same as the previous reports. The positive rate of prick tests was higher than the rate reported for health care professionals, but lower than for dentists.

It is hoped that this study will be helpful in considering safe use of latex gloves in dental practice.

Acknowledgments

We thank Dr. Akitada Nakamura, National Institute of Hygienic Sciences, who gave us the standard compounds for the DTC accelerator and information about latex allergy, as well as the directors of pharmacy, at 17 private dental college and university hospitals in Japan, who cooperated in the investigation of latex glove use. This study was supported by a grant from Health Sciences University of Hokkaido.

References


6) Kaniwa M.: Chemical approach to contact dermatitis caused by household products 3. EISEI KAGAKU 1986 32: 197-211.


8) Fuchs T., et al: Immediate reactions to
31) Fisher A. A.: Contact urticaria and anaphylactoid reaction due to corn starch surgical glove powder. Contact Dermatitis 1986 16: 224~225.
医療用ゴム製品中のアレルギー原因物質

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検査用・手術用ゴム手袋、ラバーダムなどのゴム製品中のアレルギー原因物質の検査と皮膚テストが実施された。15点の試料中の DTC 系化合物は、ZDBC が 8 点、ZDEC と ZPC が各 4 点から検出された。DTC 化合物の中で最も感作性が高いとされている ZDMC が検出されたものはない。15点の試料中のゴム蛋白の量は96～4133μg/g とばらついていたが、ラバーダム中の蛋白量が最も少なく、手術用ゴム手袋中の蛋白量は1000μg/g 以下で中程度であった。検査用ゴム手袋中の蛋白量は少ないものと多いものにあわせ20～30倍の聞きがあった。歯科学生によるゴム手袋片を用いたパッチテストでは、陽性率は2.5%であったが、陰性を示したゴム手袋片に含有されている DTC 系化合物の軟膏を用いたパッチテストの結果では、陽性を示したものはいなかった。ゴム片の抽出液を用いたブリックテストでは4名が陽性で、その割合は4.8%であった。