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A SEARCH FOR CHEMICALS RESPONSIBLE FOR CONTACT DERMATITIS CAUSED BY HOUSEHOLD PRODUCTS - I

N-isopropyl-N'-phenyl-p-phenylenediamine in Heavy Duty Rubber Gloves

Masa-aki KANIWA[1], Shigeo KOJIMA[1], Akitada NAKAMURA[1], and Masaru ISHIHARA[2]

1. INTRODUCTION

Among the health hazards caused by chemicals present in household products, a major category is contact dermatitis[1)] and a variety of chemicals have been reported to be responsible[2), 3)].

Search for allergens in contact dermatitis is usually done by doing patch tests, simultaneously with diagnosis and treatment.

.....****### is meaningful only for a particular patient and cannot be substituted by animal testing. We have to, therefore, quickly select the chemicals that will be used in the patch test and apply the test with the consent of the patient. When we question the manufacturers regarding chemicals present in their product that might cause contact dermatitis, they are usually not very cooperative. Analysing the chemical constitution of the product will take a long time. The doctors therefore, ... ****####### to obtain information regarding the chemicals present in the household products. Due to this, often the chemical used in the patch test and the chemical present in the product do not in the end match.

To overcome such problems, it is important to obtain information regarding chemicals used in household products prior to carrying out the test by making enquiries with the manufacturers or by carrying out

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[1]National Institute of Hygiene Sciences, 1-18-1 Kamiyoga, Setagaya-ku Tokyo ~~158~~ 158.

[2]School of Medicine, Toho Univeristy, 6-11-1 Ohmorinishi, Ohta-ku, Tokyo 143

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chemical analyses. This will enable the doctors to use this information while selecting candidate chemicals for the patch tests. The search for the allergen should be carried out through patch tests and chemical analyses of various household products and case reports should be maintained in files so that they can be consulted when needed.

We investigated a case of allergic contact dermatitis caused by a pair of heavy duty gloves. After carrying out investigations along the lines mentioned above we came to the conclusion that the allergen was N-isopropyl-N'-phenyl-p-phenylendiamine (IPPD) which has been used as an anti-oxidant in the product. The results of this investigation are reported here.

2. A BRIEF OUTLINE OF THE PRESENT CASE OF CONTACT DERMATITIS

The patient is a 48-year-old male who works as a tester of the pressure resistance of pressurized gas containers. He has been doing this work since about 10 years ago. He had dermatitis on his hands since the middle of April 1980. He consulted us in early August of the same year. He had been using three types of gloves in his work. These were, thick rubber gloves, suede gloves, and vinyl chloride gloves with cloth inner lining.

3. TEST METHODS

3. 1 Test samples

Among the different types of gloves that the patient had been wearing, we selected the rubber gloves for further analysis because it gave positive results in the patch test (See Table II). These gloves were caramel coloured and were elastic. Fig. 1 shows one of the gloves.

3. 2 Reagents used

Table I lists the names of rubber additives used in our experiments,

their abbreviations, trade names, and the names of the manufacturers. They were used without any purification. In the case of other chemicals, in principle we used 'guarenteed reagents', or 'extra pure' grades ~~if the former was not~~ or 'Japan Pharmacopia' grade if guarenteed reagents were not available.

3. 3 Equipment used

A Shimadzu IR-400 infrared spectrophotometer, a Shimadzu GC-4BM·PFE gas chromatograph (with hydrogen flame ionization detector), and a Shimadzu LKB-7000 gas chromatograph-mass spectrometer (GC-MS) were used.

3. 4 Patch test

The patch test for the rubber additives was done by the standard method. Each compound was mixed with white vaseline to the extent of ~~100% and applied on~~ 1.0% and applied on the patient using a fin chamber manufactured by Taisho Seiyaku Co. Ltd. In the case of samples 4-7 of Table II, standard patch test samples manufactured by the Hollister Co. were used.

3. 5 Confirmation of the identity of the ~~compounds~~ glove material

The ~~structure~~ identity of the polymer compounds was confirmed by comparing the IR spectrum of the polymer component obtained by a standard method[4a], with the standard spectra[5].

3. 6 Analysis of anti-oxidants

3. 6. 1 Extraction - About 2 g of sample that had been cut thinly and further ~~sliced~~ minced was placed in a 50 ml centrifuge tube. To this was added 20 ml of 1:1 acetone-chloroform mixture and the mxiture was ~~she~~ shaken for 30 min at room temperature. The extract was then separated using a pipette. After extracting four times, the extracts were combined and

of standard IPPD was spotted on ~~each~~ the plate. The plate~~s~~ ~~were~~ was developed up to 10 cm using chloroform-benzene (10:9) mixture. After drying, the plate~~s~~ ~~were~~ was examined under UV light of 254 nm and the region having the same Rf value (0.8) as the standard IPPD sample was scrapped out. The silica gel in the regions immedately above and below this region also were separately scraped out. Each sample was then extracted with methanol, the solvent evaporated off in a rotary evaporator and the residue dissolved in 0.5 ml of benzene for use in GC and GC-MS. ~~The~~ These fractions were designated, from the upper towards the lower side of the TLC plate, as fractions I, II, and III.

3. 6. 4 Qualitative and quantitative analyses by GC and GC-MS - The fractions obtained in 3.6.2 and 3.6.3 were ~~poured~~ injected into GC columns under the conditions given below and their gas chromatograms were compared with those of standard anti-oxidant samples. Quantitative determination was done by the absolute calibration curve method.

GC Conditions: 3% SE-30 on Gaschrom Q (80-100 mesh); 3 mm inner dia. and 1.5 m length glass column; column temperature 150-275°C (10°C/min); injection and detector temperature 280°C; carrier gas nitrogen 60 ml/min; hydrogen flame ionization detector, hydrogen 0.8 kg/cm^2, air 1.0 kg/cm^2.

GC-MS conditions: GC conditions were the same as given above. But helium (40ml/min) was used as the carrier gas. MS conditions: Ionization voltage 70 eV, Ionization current 60 microA, Ion acceleration voltage 3.0 kV, Temperature of ion source and separator 250°C.

4. RESULTS

4. 1 Patch test

Table II gives the results obtained in the patch test. Among the three types of gloves worn by the patient, only the rubber gloves

showed a strong positive reaction. The reaction persisted for more than two weeks. Among the rubber additives tested, i.e. 9 types of vulcanization accelerators and 2 types of anti-oxidants, IPPD alone showed a strong positive result. Among the Hollister standard patch test samples, only PPD_{mix} showed a strongly positive result. p-phenylenediamine (PPDA) was negative. The subject's reaction to IPPD and PPD_{mix} persited for more than 2 weeks as in the case of the rubber glove. So, the reaction was diagnosed as contact allergy.

4. 2 Confirmation of the identity of the glove material

As shown in Fig. 2, the polymer component of the rubber glove was identified .....****###### , Since and since the other absorption peaks almost coincided with those of standard natural rubber sample, this glove was confirmed to be made of natural rubber.

4. 3 Analysis of anti-oxidant

Since the patient showed a negative reaction to all the vulcanization accelerators and a positive reaction to IPPD, an anti-oxidant, we selected eight typical anti-oxidants including IPPD for analysis. These are IPPD, PANA, HBANA, PBNA, ETMDQ, BHT, SP, and TPP.

Usually, for the extraction of rubber additives such as anti-oxidants[4), 6)], room temperature shaking extraction, soxhlet extraction, refluxing, etc are employed. Benzene, acetonitrile, acetone-chloroform mixture, acetone, ethanol, methanol, etc are employed as the solvent. It is yet to be determined as to which of the methods of extraction is most suited. In the present study, we employed the room temperature shaking extraction with a mixture of acetone and chloroform. .....******###### For analysing anti-oxidants, we examined the advantages and disadvantages of four types of GC columns (Versamid 900, Carbowax 20M, Apiezon L, & SE-30).

These were selected on the basis of the review [6f] by Wheeler. We found that the SE-30 column was the best both with respect to separation of the peaks and detection sensitivity. Therefore, this column was used.

Fig 3 shows the gas chromatogram of the authentic anti-oxidants. All the substances showed symmetrical and clear peaks. As can be seen from Fig3(b), SP was found to be a mixture of 4 components. These were designated as SP- 1 to SP-4 in a sequence of increasing retention times. The 8 anti-oxidants separated well except for overlapping of the IPPD and the PANA peaks.

The relationship between the height of the peak and the amount of substance injected was linear and the line passed through the origin. So, quantitative analysis was done using the peak height by the absolute calibration curve method.

The chromatogram of the rubber extract had many unknown peaks (Fig. 5(a)). Thus, there was not much practical utility in calculating the detection limit of the authentic substance at S/N=2. Therefore, here we ....**** where the peak height was 0.5 cm or more under the conditions mentioned below. Adjusting the quantity of the sample obtained ....**** to 0.5 ml, 5 micro litre was injected into the GC column. Under the conditions FID sensitivity 10^3 MOhm, and range 0.16 V, the lower limit of quantitative detection was 10 micro g/g for HBANA, and about 2 micro g/g for SP and TPP. For the others, this was about 1 micro g/g.

4. 3. 2 Gas chromatography

The gas chromatograms of the rubber extract were so complex that sometimes it was difficult to analyze. We felt that some purification was needed before GC analysis. We, therefore, studied the elution

behaviour of the antioxidants in silica gel column chromatography.

Fig. 4 shows the results obtained when 500 micro g of each antioxidant was adsorbed on the column and the column eluted according to the method given in 6.3.2. The numerals in parentheses ~~prepr~~ represent recovery . BHT was eluted out in Fraction A. PANA, PBNA, SP-2, SP-4, and HBANA came out in Fraction B. Fraction C contained some of the HBANA, the ETMDQ, SP-1, and SP-3. ~~In~~ Fraction D contained IPPD and TPP. The IPPD and PANA peaks overlapped in the gas chromatogram (see section 4. 3. 1). However, in column chromatography tney were easily separated and caused no problem in analysis.

Next, we examined the elution behaviour of these antioxidants when rubber was also present. For this purpose, four anti-oxidants (IPPD, PBNA, ETMDQ, and BHT), which differ in chemical structure and in elution behaviour in column chromatography, were used. 500 micro g each of these anti-oxidants were mixed in about 1 g of ~~isopreplylene-rubber~~ isoprene rubber extract that did not contain any anti-oxidants. The mixture was then subjected to the procedure of Section 3. 6. 2. The elution behaviour of the four anti-oxidants did not show any change. Recovery of IPPD was 102%, PBNA was 100%, ETMDQ was 88%, and BHT 83%. These were quite satisfactory.

4. 4 Analysis of the heavy duty rubber glove

The rubber glove that gave positive reaction in the patch test was processed as described in Section 3. 6. 1 to obtain a sample. The gas chromatoram of this sample is shown in Fig. 5 (a). Fig. 5 (b) ~~and~~ to Fig. 5 (f) are gas chromatograms of ~~two~~ fractions of the sample separated by the method of 3. 6. 2. Broken lines in Fig. 5 indicate the retention times of anti-oxidants that are expected to be eluted in the particular

fractions.

In Fraction D (Fig. 5 (e)), a peak coinciding with IPPD was present. When this was analysed quantitatively as IPPD, its concentration was found to be 177 micro g /g .

In order to confirm that it is IPPD, Fraction D was purified by TLC according to the procedure of 3.6.3. Chromatograms shown in Fig. 6 (a) to Fig. 6(c) correspond to TLC fractions I-III illustrated in Fig. 6 (d). The ~~gas~~ chromatogram of Fraction II (Fig 6 (b)), which had the same Rf as authentic IPPD in TLC, ~~and it~~ showed a peak corresponding to the retention time of IPPD.

The peaks that corresponded to IPPD in Fraction D and the TLC fraction II were analysed for their mass spectra by GC-MS. .....***##### They showed almost the same pattern and intensity ratio as authentic IPPD ~~(Fig. 8)~~. In Fig. 7 (c) , the mass spectrum showed lesser ion peaks in the low molecular weight region compared to Fig. 7 (b) . Thus it is getting closer to the mass spectrum of the authentic IPPD sample. This suggests that the purification by TLC after the column chromatography was effective.

Fraction B (Fig. 5 (c)) had peaks corresponding to SP-2 and SP-4, while fraction C (Fig. 5 (d)) had peaks corresponding to SP-1 and and SP-3. When quantitative analysis of SP-2 was carried out in terms of SP-~~2~~ its concentration was found to be 1,600 micro g / g.

To confirm this, the mass spectra of the concerned peaks of fractions B and C were measured by GC-MS. The peak corresponding to SP-1 showed the main ion fragment at m/z 198 (M^+), 183, 165. The peaks corresponding to SP-2 and SP-3 had the ion fragment at m/z 302 (M^+), 287, 210, 198, and 105 ~~whiel~~ while the peak corresponding to SP-4

had them at m/z 406(M^+), 391, 314, 302, 105. Almost all of these showed the same patterns and intentsity ratios as authentic SP-1 to SP-4. .....******* #####. Fig. 8. ---- *** ###

Other anti-oxidants were confirmed to be absent by means of GC and GC-MS.

As discussed above, the rubber glove that caused the contact dermatitis was found to be made of natural rubber containing IPPD to the extent of 177 micro g/g, and SP to the extent of 1600 micro g/g but with no trace of PANA, HBANA, PBNA, ETMDQ, or TPP.

5. DISCUSSION

We started our investivagions by confirming the identity of the material from which the rubber glove was made. Only after confirming that it was made of natural rubber did we undertake further investigations (see 4.2).

Patch tests were conducted with 9 vulcanization accelerators and 2 anti-oxidants (Table II). In these tests, we followed the scheme laid down by Household Articles Safety Unit of the Ministry of Health and Welfare. All the vulcanization accelerators tested were found to be negative. Therefore further analysis was done only with the anti-oxidants including IPPD. Presently, more than 30 different [7] major anti-oxidants are being produced. We selected 8 most commonly used anti-oxidants.

As discussed in 4. 4, as a result of analyses the presente of IPPD and SP was detected. According to the practical examples of compounding given in a source book [4(b)], a vulcanization accelerator or an anti-oxidant is normally compounded to the extent of about 1%. For instance, IPPD is added in tyres, belts, and other industrial products to the extent of 1-2% [7), 8)]. However, they get decomposed during vulcanization and during usage of the product. So the amount added

cannot be always recovered [6a]. In our study, the amount of IPPD detected was 177 micro g/g and that of SP was 1600 micro g/g. These are quite low ~~when the~~ compared to the standard amounts of these additives used. This is quite expected.

Because of the polluting (staining) and colouring properties of IPPD, rubber products to which this additive is added tend to become blackish. Because of this, it is not used in products other than black coloured ones.[7] The rubber glove analysed in the present study was of a blackish brown colour and, therefore, there was no need to worry about whether the addition of IPPD would spoil its appearance. The use of IPPD is quite appropriate in such a product. SP has no ~~polluting~~ staining or colouring property. Because of this, the latter is a more widely used anti-oxidant.

In the patch test, only IPPD and PPD_{mix} showed a positive reaction (Table II). PPD_{mix} contains N-cyclohexyl-N'-phenyl-p-phenylene-diamine (0.25%) and N,N'-diphenyl-p-phenylenediamine (0.25%), in addition to IPPD (0.1%). In the patch test, the concentration of IPPD used was 1.0%.

The amount of IPPD detected, i.e. 177 micro g/g, was as low as about 1/5th of the IPPD concentration in the PPD mix and about 1/50th of the concentration ~~using~~ of IPPD used in the patch test. However, it is quite possible that when the IPPD got eluted out of the glove by sweat and ~~gets~~ got concentrated on the skin, this led to the dermatitis. We could have conducted patch tests with IPPD concentrations of less than 1.0%. However, this could not be done because we could not get the patients consent for this.

The reaction to the rubber glove, IPPD, and the PPD_{mix} in the patch test persisted in an identical fashion for more than 2 weeks.

Therefore, it was diagnosed as an allergic reaction.

The patient showed positive reaction towards IPPD but did not react to PPDA. Fisher[3a] had reported that "a cross reaction can occur between IPPD and PPDA", and Cronin[2a] had shown that about 1/3rd of the patients reacting to IPPD ~~showe~~ had a positive reaction to IPPD also. We believe that our subject was one for whom IPPD and PPDA did not have cross reaction.

On the basis of the above results, we concluded that allergic contact dermatitis observed in this patient was caused atleast partly by the anti-oxidant IPPD present in the heavy duty rubber glove used by him..

Some authors have reported [2b), 3b), 9)] that phenolic compounds can cause contact dermatitis. Since SP is a phenolic anti-oxidant, there is a possibility of SP being a causative agent. However, since we did not carry out the patch test with SP this could not be confirmed.

Literature cited

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