

Experimental study on skin sensitization potencies and cross-reactivities of hair-dye-related chemicals in guinea pigs

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In screening patch testing of hairdressers with occupational contact dermatitis, multiple positive reactions to hair dye-related chemicals, such as p-phenylenediamine (PPD), p-toluenediamine · 2HCl (PTD) and p-aminophenol (PAP), a fabric dye p-aminoazobenzene (PAB), and a tar dye Sudan III, were frequently encountered. To investigate individual skin sensitization potency and the cross-reactivities among above chemicals, a guinea pig maximization test with the above 5 chemicals was performed. In each group, 6 animals were induced with one of the chemicals at 0.1% concentration by intradermal injection and at 1.0% by topical application. The animals were challenged with all 5 chemicals in concentrations of dilution by 10 from 0.1% to 0.001%. Under the conditions of 0.1% challenges, similar sensitization potencies were observed in PPD (6/6), PTD (6/6), PAP (5/6) and PAB (6/6) groups, but no positive reactions were elicited in the Sudan III group. The cross-reactivities to PPD were confirmed in the animals challenged with PTD (6/6), PAP (6/6), PAB (6/6) and Sudan III (3/6). In the PTD-induced group, positive responses to cross-challenges were elicited by PPD (5/6), PAP (3/6), PAB (5/6) and Sudan III (1/6). The cross-reactivities to PAP were observed only with PPD (2/5) and PAB (5/5). PAB-induced animals responded only to PPD (1/6). The results indicate that all these chemicals except Sudan III are strong sensitizers. Their cross-reactivities are different in sensitized conditions, respectively. The cross-reactivities to PPD were higher than those to PTD, PAP and PAB.

Key words: sensitization potency; cross-reactivity; p-phenylenediamine; hair dye chemicals; guinea pig maximization test. © Munksgaard, 2000.

Accepted for publication 29 November 1999

We reported previously that in screening patch testing of hairdressers with occupational contact dermatitis, positive reactions to hair dye-related chemicals, such as p-phenylenediamine (PPD), p-toluenediamine · 2HCl (PTD) and p-aminophenol (PAP), a fabric dye p-aminoazobenzene (PAB) and a cosmetic tar dye Sudan III, were frequently observed (1–5). In our recent report (1), among the PPD-positive subjects, 93% had 2 or more positive reactions to hair dye-related chemicals. Whether the positive reactions were induced by primary sensitization or cross-reactions was hard to decide in humans (6). As hairdressers are exposed to many chemicals, it is difficult to identify specific

causative chemicals. To explain the reason for multiple positive reactions in hairdressers' patch testing, it is necessary to clarify the sensitization potential of each chemical and cross-reactivities among them. This study aimed at evaluating the skin sensitization potencies of the above 5 chemicals and the cross-reactivities among them, using the guinea pig maximization test.

Materials and Methods

Animals

Female Hartley strain albino guinea pigs (Nippon Bio Supp. Center, Tokyo) were introduced at 4

weeks of age. After 1 week of acclimatization, animals that were healthy and weighed 300 – 350 g were selected for experiments. The animals were housed in groups of 3 in stainless steel wire-mesh cages in a room kept at a constant room temperature of $23 \pm 2^\circ\text{C}$, a relative humidity of $55 \pm 5\%$ and an air exchange rate of $15 \times/\text{h}$. The room was artificially illuminated for 12 h daily. They had free access to tap water and a solid diet (RC-4, Oriental Yeast Co., Ltd., Osaka). The animals were randomly assigned to test and control groups. Hair was removed with an electric clipper 24 h before treatments and shaved with an electric shaver 2 h before treatments and readings. All animals were weighed $1 \times$ a week. Japanese laws concerning the protection and control of animals, standards related to care and management of experimental animals, and the Guidelines on Experimental Animals set out by Nagoya University School of Medicine, were strictly followed throughout the experiments.

Test chemicals

PPD (97% purity), PAP (98% purity) and PAB (98% purity) were purchased from Wako Pure Chemical Industries Ltd., Osaka, PTD (98% purity) from Tokyo Chemical Industry Co., Ltd., Tokyo, and purified Sudan III (99% purity) from Kishi Kasai Co., Ltd., Yokohama.

Decision of optimal concentrations and vehicles

The optimal vehicles and concentrations for intradermal and topical inductions and challenges were investigated preliminarily. For intradermal injections, 0.1% of PPD and PTD in saline and 0.1% of PAP and PAB in olive oil were selected. For topical applications, 1.0% in petrolatum was decided upon. These concentrations and vehicles were confirmed to be appreciable enough to induce a sensitization without producing toxic effects in animals. Although open applications with 10% of the above 4 chemicals had not evoked irritant reactions in non-sensitized animals, 10% and 1% caused positive responses too strong to be correctly read in sensitized animals. Therefore, 0.1% of PPD or PAB in acetone and 0.1% of PTD or PAP in acetone: distilled water (1:1) were selected as optimal challenge concentrations. The challenge concentrations elicited responses at around 3 points of the mean response (summation of numerical scoring / total number of animals) without exciting reactions. As purified Sudan III is not soluble in olive oil and acetone to the extent of more than 0.1%, the highest soluble concentrations were used for intradermal induction and challenge, respectively.

Procedure

The procedure of the Magnusson & Kligman (7) guinea pig maximization test (GPMT) was followed for induction. Open applications were used for the challenge (8). 6 animals were included in each test group and each control group. All the chemicals were prepared in w/w concentration. The animals in each test group were induced with one of the 5 chemicals tested, respectively, and challenged with all 5 chemicals and vehicle. The sensitization potentials and cross-reactivities were investigated by the pairs of same or different chemicals in induction and challenge. The animals in each control group (naive control) were treated in the same way as the corresponding animals in the test group, except the procedure that the tested chemical was not administered during the induction stage.

Induction. On day 0, a row of 3 injections was given on each side of the 2×4 cm clipped neck: (1) 0.1 ml emulsified mixture of Freund's complete adjuvant (FCA: Iatron Co., Ltd., Tokyo) and saline (1:1); (2) 0.1 ml of 0.1% chemicals (PPD and PTD were dissolved in saline, PAP, PAB and Sudan III in olive oil); (3) 0.1 ml of the 0.1% test substance in FCA and saline mixture (1:1).

In order to enhance the sensitization, sodium lauryl sulfate 10% in petrolatum was applied on induction area for 24 h before the topical induction. On day 7, 0.5 g of 1.0% test chemicals in petrolatum were occlusively applied for 48 h. The samples were covered with lint pads which were lining with Blenderm tape (3 M, ST Paul, MN, USA). The region patched was then bandaged with Erastopore No. 75 tape (Nichiban Co., Ltd., Osaka) to fix the patched materials tightly.

Challenge. On day 21, the flank regions of the animals were clipped and shaved. 0.03 ml aliquots of test chemicals in acetone or acetone and dis-

Table 1. Evaluation of skin reaction (scale of scoring)

Skin reaction	Value
Erythema & eschar formation	
(a) no erythema	0
(b) very slight erythema (barely perceptible)	1
(c) well-defined erythema	2
(d) moderate to severe erythema	3
(e) severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation	
(a) no edema	0
(b) very slight edema (barely perceptible)	1
(c) slight edema (edges of area well-defined by definite raising)	2
(d) moderate edema (raised approximately 1 mm)	3
(e) severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

Table 2. Sensitization potencies of the chemicals

Challenge (%)	PPD	PTD	PAP	PAB	Sudan III
0.1	6/6	6/6	5/6	6/6	0/6
0.01	5/6	5/6	2/6	2/6	0/6
0.001	0/6	0/6	0/6	1/6	0/6

Table 3. Cross-reactivities to PPD

Challenge (%)	PTD	PAP	PAB	Sudan III
0.1	6/6	6/6		3/6
0.01	0/6	1/6	6/6	1/6
0.001	0/6	0/6	6/6	0/6

Table 4. Cross-reactivities to PTD

Challenge (%)	PPD	PAP	PAB	Sudan III
0.1	5/6	3/6	5/6	1/6
0.01	2/6	0/6	5/6	1/6
0.001	0/6	0/6	1/6	0/6

Table 5. Cross-reactivities to PAP

Challenge (%)	PPD	PTD	PAB	Sudan III
0.1	2/5	0/5	5/5	0/5
0.01	0/5	0/5	3/5	0/5
0.001	0/5		0/5	0/5

Table 6. Cross-reactivities to PAB

Challenge (%)	PPD	PTD	PAP	Sudan III
0.1	1/6	0/6	0/6	0/6
0.01	0/6			0/6
0.001				0/6

tilled water mixture (1:1) were applied on the shaved area for challenge. The materials were left for 24 h. Then the materials were carefully removed by octamethyl cyclotetra siloxane. The treated sites were marked by black indelible ink. The concentrations of the chemicals for challenge were 0.1%, 0.01% and 0.001% for detection of sensitization potencies and cross-reactivities.

Readings. Reactions were read at 24, 48 and 72 h after the challenge applications, according to the Draize's criteria (Table 1). The summation of numerical scoring more than 2 was judged as a positive reaction (9).

Results

The results were shown in Table 2 to Table 6. During the period of the experiment, the body weight gain of the animals was not significantly different to the control groups. The reactions usually appeared at the 24 h reading and increased at the 48

h and at the 72 h readings. The peak response was adopted at 48 h after the challenge application. No positive responses were found in either the naive control or the vehicle control.

Sensitization potencies

When the challenge concentration was 0.1%, the positive reactions were observed in all of the 6 animals in the groups induced with PPD, PTD, PAB, and 5/6 in the group induced with PAP. The lowest challenge concentrations to elicit positive reactions were 0.01% of PPD (5/6), PTD (5/6) and PAP (2/6) and 0.001% of PAB (1/6). In Sudan III-induced animals, no positive reaction was elicited at 0.1% concentrations (Table 2). Fig. 1 shows a positive reaction of PPD at 0.1% challenge concentration in the animal sensitized with PPD.

Cross-reactivities

The cross-reactivity to PPD was confirmed in the animals challenged with PTD (6/6), PAP (6/6),

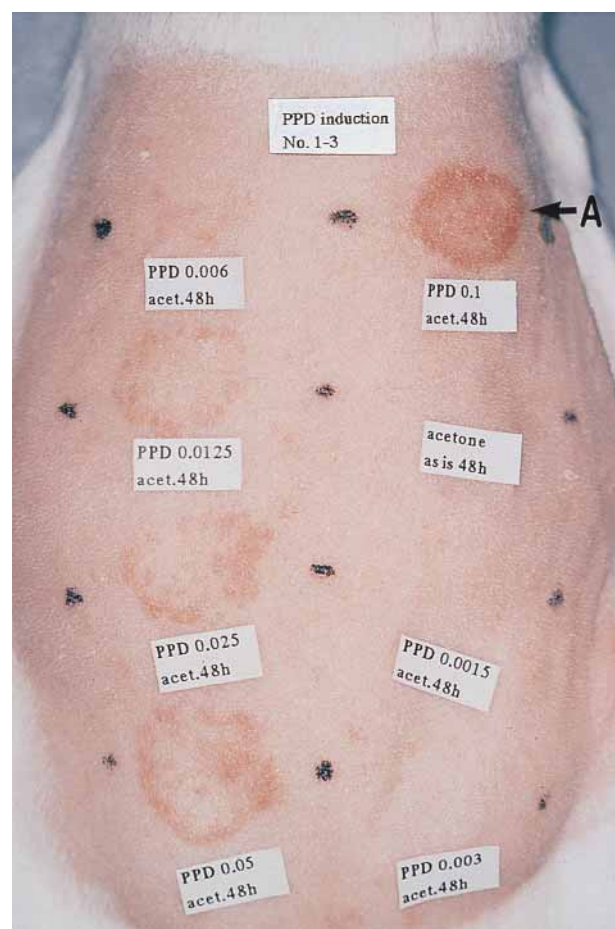


Fig. 1. Positive reaction of PPD at 0.1% challenge concentration (A, arrow) elicited in the guinea pig sensitized by 0.1% PPD of intradermal injection and 1.0% PPD of topical application.

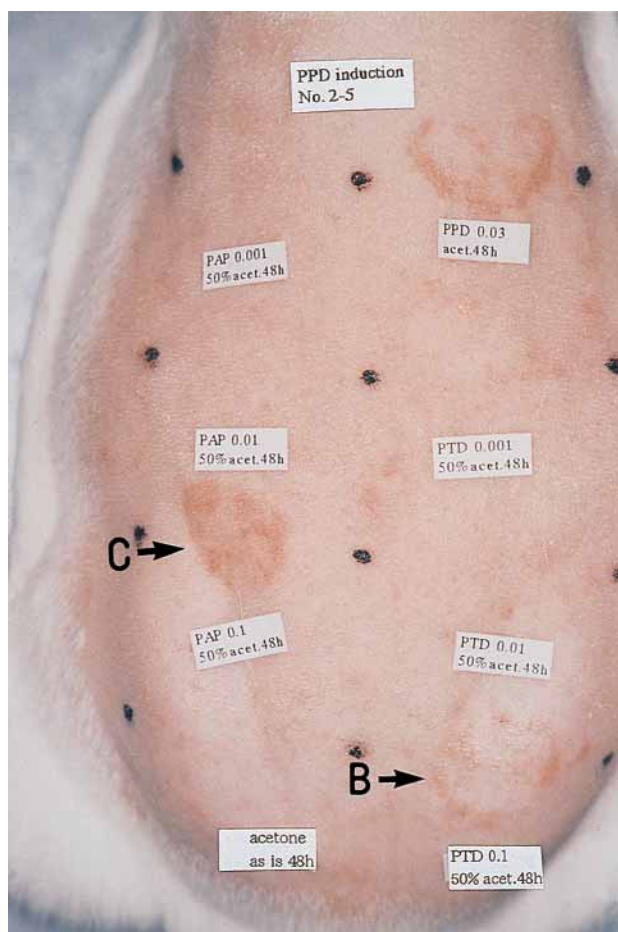


Fig. 2. Cross-reactions of 0.1% PTD (B) and 0.1% PAP (C) in the animal sensitized with PPD.

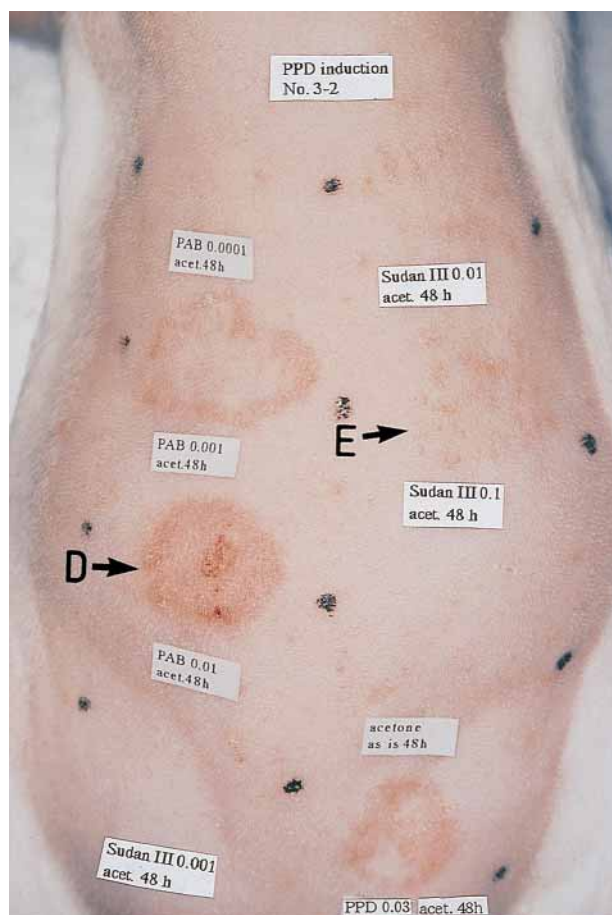


Fig. 3. Cross-reactions of 0.01% PAB (D) and 0.1% Sudan III (E) in the animal sensitized with PPD.

PAB (6/6) and Sudan III (3/6) at 0.1% concentrations. The lowest concentrations of positive cross-reactions to PPD were 0.1% of PTD (6/6), 0.01% of PAP (1/6) and Sudan III (1/6) and 0.001% of PAB (6/6) (Table 3). In the PTD-induced group, the animals showed positive reactions when they were challenged with PPD (5/6), PAP (3/6), PAB (5/6) and Sudan III (1/6) at 0.1% concentration. The lowest concentrations of positive cross-reactions to PTD were 0.01% of PPD (2/6) and Sudan III (1/6), 0.1% of PAP (3/6) and 0.001% of PAB (1/6) (Table 4). The cross-reactivity to PAP were observed in the animals challenged with PPD (2/5) and PAB (5/5) at 0.1% concentration. The lowest concentrations of positive cross-reactions to PAP were 0.1% of PPD and 0.01% of PAB (3/5) (Table 5). PAB-induced animals responded only by the challenge of PPD (1/6) at 0.1% concentration (Table 6). Fig. 2 shows cross-reactions of PTD and PAP in the animal sensitized with PPD. Fig. 3 shows cross-reactions of PAB and Sudan III in the animal sensitized with PPD.

Discussion

The present study indicated that the grade of skin sensitization potencies of these chemicals are $PPD = PTD = PAB > PAP \gg \text{Sudan III}$. Their cross-reactivities are different among the chemicals investigated. The PPD-sensitized group was confirmed to be the most susceptible to elicit positive reactions in the cross-challenges among the chemicals tested. The 2nd was the PTD-sensitized group and the 3rd was the PAP-sensitized group. The intensity of cross-reactivities to PPD are $PAB > PAP \approx PTD > \text{Sudan III}$. PAB frequently responded to the groups induced by PPD, PTD and PAP. However, the cross-reactivity to PAB was observed only by PPD. Sudan III demonstrated cross-reactivities only to PPD and PTD. The figure of patch test results of hairdressers with occupational contact dermatitis (1) was similar to the results of this animal experiment. The consistency of the clinical observations with animal studies indicates that the positive reactions to multiple chemicals in the hair-

dressers' patch testing were caused by cross-reaction.

It has been proposed that strong sensitization potency of chemicals was indicated by a high sensitization rate in an animal model, particularly when the test concentrations are low (10). In our study, the sensitization rates of PPD, PTD, PAP and PAB were all 6/6 or 5/6 under the conditions of 0.1% intradermal and 1% topical inductions and 0.1% challenges. These 4 chemicals were considered strong sensitizers. Under the identical conditions of induction and challenge, Sudan III failed to give positive reactions. This does not mean that Sudan III is not a sensitizer. In fact, a Sudan III positive reaction was seen in human patch testing with and without a PPD positive reaction (1, 3, 5).

It was postulated that cross-sensitization is based on the formation of common or similar haptens (11). It has been confirmed that the PPD oxidation product, Bandrowski's base (BB), play an important role in its sensitization (12–14). The chemicals tested in the present study have similar structure. PAB and Sudan III might be reduced in vivo into PPD and so forth (15, 16). In the PPD-induced group, the positive reactions to PTD, PAP and PAB in cross-challenges were 6/6, and 3/6 with Sudan III. The cross-reactive frequencies in the PPD-induced group were higher than those in other groups. Our results suggest that PPD is a strong sensitizer and, once sensitized to PPD, there is a strong possibility of cross-reaction to the other 4 chemicals.

It is known that animals treated with FCA in the GPMT are in a generalized state of hyperreactivity (17). In this state, doses of substances that normally cause no reaction may provoke an inflammatory response that might be confused with an allergic reaction. The challenge at the maximum nonirritating concentration identified in preliminary studies on non-FCA treated guinea pigs might produce irritant reactions that would be read falsely as positive allergic reactions (9, 17). Consequently, 1/10 of the highest induction concentration including intradermal and topical inductions was used for the highest challenge concentration in this study. The evaluations at 0.1% challenge concentration and the lowest challenge concentration of dilution by 10 could thus reflect the profile of skin sensitization potencies and cross-reactivities among these chemicals.

In summary, this study provided a comprehensive description of the patterns of sensitization potencies and cross-reactivities among PPD, PTD, PAP, PAB and Sudan III. These chemicals, except for Sudan III, are strong sensitizers. Their cross-reactivities are different in respectively sensitized conditions. The cross-reactive responses of the ani-

mals tested to PPD were higher than those to PTD, PAP and PAB. The results suggested that the multiple positive reactions of these chemicals in human patch testing were caused by cross-reaction to PPD.

Acknowledgements

The staff at the Research Laboratories of Nippon Menard Cosmetic Co., Ltd., provided expert animal care and technical assistance. Comments from Dr. Tomonori Katada were highly appreciated.

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