

Intermittent exposure to low-concentration paraphenylenediamine can be equivalent to single, higher-dose exposure

JONATHAN M. L. WHITE¹, DAVID A. BASKETTER², CAMILLA K. PEASE², DAVID A. SANDERS² AND JOHN P. MCFADDEN¹

¹St John's Institute of Dermatology, St Thomas' Hospital, London and ²Safety and Environmental Assurance Centre, Unilever, Colworth Park, Sharnbrook, Bedfordshire, UK

Hair dye allergy is an important and increasingly common cause of allergic contact dermatitis. The role of repeated exposure in elicitation of allergy has not previously been extensively studied. We have therefore compared elicitation between single and intermittent exposure to paraphenylenediamine (PPD). 23 subjects known to be allergic to PPD from positive patch tests were exposed to 0.3% and 0.03% PPD, both in petrolatum and water, for 5 min at the same site every day for up to 8 D. In the same subjects, single exposures were also performed at different sites, from 5 to 40 min. Other experiments exposed rat skin to radiolabelled PPD as one-off application or multiple exposures. There were 8 reactions in the cumulative exposure site using 0.3% PPD in aqueous solution. In 7 of these, there was an exact correlation with reaction to the cumulative time needed for repeat exposures to elicit a reaction and the time needed for a reaction to the single exposure. There were no reactions to 0.03% PPD in water or pet under either type of exposure condition. There was also a positive correlation between grade of original reaction in clinic (+++, ++, +) and appearance/intensity of elicitation reactions. In the animal study, cumulative time and single exposure time sites correlated with regards to retention of radiolabelled substance within the skin. This study therefore demonstrates for the first time that, over the time period tested, the allergenic component of PPD accumulates in the skin. Hence, intermittent exposure to lower concentrations of PPD may be equivalent to higher concentration, one-off exposure.

Key words: contact dermatitis; cumulative exposure; elicitation; hair dye allergy; paraphenylenediamine. © Blackwell Munksgaard 2007.

Accepted for publication 7 December 2006

Hair dye allergy is an important and increasingly common cause of allergic contact dermatitis (1). There are 2 ways in which a hair dye can be applied. It can be applied as an infrequent single exposure ('permanent' hair dye) or as a frequently repeated application (usually as a 'temporary' dye or with new forms of application). Previous work by our group has suggested that intermittent short applications could cause elicitation reactions equivalent to the same application time as a single exposure (2). However, there is very little information on the impact of duration of exposure on elicitation and in particular on the impact of repeated short duration exposure on elicitation. Generally, the work that has been undertaken has investigated only a reduction in individual exposure duration. This has been evaluated for isoeugenol (3), methyldibromoglutaronitrile (4) and, as mentioned, paraphenylenediamine (PPD)

(2). There is little information on whether individuals sensitized to an allergen might, in fact, better tolerate repeated short exposures compared with less frequent exposure of a greater magnitude. Indeed, data from studies with methyldibromoglutaronitrile suggest that frequent, short exposures of this preservative in a rinse-off liquid soap can elicit a positive repeated open application test (5). This suggests that allergic subjects may react to frequent exposure compared with less frequent exposure, even if the less frequent exposure is of greater magnitude.

This study compares elicitation reactions in subjects who have been previously patch tested and found to be allergic to PPD, particularly regarding cumulative exposure time compared with single-application times. In conjunction, our experiments compared single-dose application with intermittent applications for a similar

time-frame, estimated by means of accumulated radiolabelled PPD.

Methods

Human study

This research was approved by our hospital ethics committee and the investigations were conducted according to the Declaration of Helsinki principles. 23 subjects were recruited, aged 25–68 years (14 female, 9 male). They had all been previously assessed in the St John's Contact Dermatitis clinic and found to be allergic to PPD. Their reactions were recorded according to ICDRG criteria (6). The levels of reaction (with the greatest reading from either D2 or D4) were + ($n = 7$), ++ ($n = 12$) and +++ ($n = 4$).

Each subject participated in parts A and B of the study.

A: single-dose application. On each successive day, PPD was applied at 0.3% pet (90 mcg/cm²), 0.3% aqueous, 0.03% pet (9 mcg/cm²), 0.03% aqueous at different sites on the upper back, starting at 5 min on D1, then 10 min at D2 and onwards until 40 min application as a single exposure. When a reaction occurred, there was no further application at that concentration and with that base.

B: repeated daily exposure. The same concentrations and base of PPD were used as with single-time application but, on 8 successive days at the same site, there was a 5-min application. Sites of application were delineated by a wide circle made with a water-soluble marker (Schuco International, Tokyo, Japan) to ensure the same site was used. When a reaction occurred, the relevant concentration/base combination was discontinued.

Skin-binding study

Guidelines for use of experimental animals were followed in this study according to the 'Principles of Laboratory Care' formulated by the National Society for Medical Research. 6 female, 6-week-old, Sprague Dawley/Charles River CD (SPF) live rats were obtained from Charles River Laboratories (Sulzfeld, Germany). ¹⁴C-PPD (ring radio-labelled) was obtained from Amersham Pharmacia Biotech, Little Chalfont, UK. Prior to any PPD application, the skin area to be treated was rinsed with a 10% detergent solution. A ¹⁴C-PPD containing prototype 'hair dye' base vehicle (also containing other intermediates and couplers as to mimic in-use conditions) was prepared to yield test item concentration of 3.5 mg PPD/g base vehicle. The PPD was applied to 10 cm² of skin in a freshly prepared prototype base formulation mixed with peroxide developer (1:1 by weight) so that the

amount of PPD applied was approximately 35 µg/cm². Exposure was for 5 min, either as a single application or as 3 daily applications. 3 treatment groups each of $n = 2$ were treated as follows: group 1 was treated with a single 5-min application and killed at 1 D; group 2 was treated with a single 5-min application and killed at 3 D; group 3 was treated with 3 daily 5-min doses and killed at 3 D. After a 5-min dosing, the skin was rinsed with pure water and the treatment site occluded for 1 D. At the killing there was a final rinse and tape strip of the treated site. Over the course of the study, the levels of radioactivity appearing in urine, faeces, tape strips, treated skin, blood, carcass and skin washings were determined using a Packard liquid scintillation counter equipped with d.p.m. and luminescence options (Tri-Carb 2500 TR, Canberra Packard, Meriden, CT, USA) and a mass balance calculated.

Results

There were no reactions to either 0.03% in pet or aqueous solution with either single or repeated application. Individual results in subjects who reacted are given in Table 1. In 7 out of 9 subjects who reacted to 0.3% PPD, there was an exact correlation in exposure time required for elicitation between repeated and single-application exposure. There was also a close correlation between the grade of original reaction and frequency of reaction with 0.3% pet. 4/4 subjects with original +++ patch test reactions also reacted to 0.3% PPD either as a cumulative or single dose up to 40 min, 4/12 ++ patch test reactors responded in a similar way but none of the 7 + patch test reactors responded.

It was noted that 7/9 + patch test reactors still dyed their hair, with only 4/10 ++ reactors and 0/4 +++ reactors still doing so. We did not gather data on usage in this group, although some individuals reported that they had started using semi-permanent dyes.

Skin-binding study

The majority (approximately 80%) of the dose was rinsed from the skin after 5 min exposure. However, when interpreting data from multiple dosing studies, it is important to express the results as the total in micrograms recovered, rather than percentage of dose applied. Data for individual experiments are provided in Table 2.

At 1 D in group 1, after a mean single 319 µg/10 cm² application, 58.9 µg equivalents of PPD was present in the excised, treated skin area, plus a further 1.5 µg equivalents recovered from the tape-stripped skin (i.e. separated stratum corneum).

Table 1. Dose–response relationship of paraphenylenediamine: intermittent and continuous exposure. This shows an exact correlation in exposure time required for elicitation between repeated and single-application exposure.

Age/Sex	Still dyes hair?	Intermittent		Continuous	
		0.3% aq	0.3% pet	0.3% aq	0.3% pet
54F	No	+ (10m)	?+ (25m)	–	–
54M	No	++ (20m)	–	++ (20m)	++ (40m)
32F	No	+++ (10m)	–	+ (10m)	++ (40m)
38F	No	++ (10m)	–	+ (10m)	++ (40m)
50F	No	++ (15m)	++ (40m)	+ (15m)	+++ (40m)
46F	No	+ (20m)	–	+ (20m)	–
47F	Yes	–	++ (10m)	–	–
45M	No	+ (15m)	+ (25m)	+ (15m)	–
47F	No	+ (20m)	–	+ (20m)	+ (40m)

aq, aqueous; F, female; M, male; m, minutes.

At 3 D in group 2, after a mean single 319 $\mu\text{g}/10\text{ cm}^2$ application, 52.0 μg equivalents of PPD was present in the excised, treated skin area plus a further 1.4 μg equivalents recovered from the tape-stripped skin.

At 3 D in group 3, after 3 daily doses (No. 5: dose 1 = 310 $\mu\text{g}/10\text{ cm}^2$; dose 2 = 294 $\mu\text{g}/10\text{ cm}^2$; dose 3 = 338 $\mu\text{g}/10\text{ cm}^2$) (No. 6: dose 1 = 279 $\mu\text{g}/10\text{ cm}^2$; dose 2 = 314 $\mu\text{g}/10\text{ cm}^2$; dose 3 = 3158 $\mu\text{g}/10\text{ cm}^2$); a mean of 138.0 μg equivalents of PPD remained present in the excised, treated area plus a further 3.9 μg equivalents of PPD recovered from the tape-stripped skin.

The total amount of penetrated and excreted radioactivity was as follows:

- group 1: 0.89 μg equivalents of PPD
- group 2: 0.92 μg equivalents of PPD
- group 3: 5.1 μg equivalents of PPD.

The overall recoveries of the total applied radioactive dose in the 3 groups were 102.7%, 102.6%, and 102.6%, respectively.

Discussion

This study has shown that applications of PPD applied to the same site repeatedly over 8 D will cause an elicitation reaction approximating to

a single-application equivalent to the aggregation of repeated time exposure. This is corroborated by skin-binding data in group 3, showing accumulation of radiolabelled PPD (or its polymers or metabolites) on repeated skin application. The data from group 1 and 2 also show that following a single exposure, the level of PPD equivalent in skin at 1 D and 3 D is the same, indicating that there is persistence of PPD in the skin following PPD removal. This may have clinical relevance when considering repeated applications of PPD as a hair dye, or indeed other allergens, applied over a short time period, i.e. hours/days. However, whether PPD will cause an allergic reaction in sensitized individuals would depend upon other factors such as pre-existing scalp dermatitis, the agent within which PPD is applied and length of time for which PPD is applied.

The observations that repeated exposures to PPD may summate and persist could have important consequences. Hair-dyeing instructions currently recommend a skin allergy test performed by the consumer prior to use. Concentrations ranging from 0.1 to 1.5% are used, depending on the darkness of the shade (7). If the skin allergy test is performed before each use, a consumer may perform approximately 6 tests per year, with a possibility of sensitization. Alternatively, patients

Table 2. Microgram equivalents of radioactive material recovered, from each animal per group, and as an average per group

	Excreted	Treated skin	Total absorbed	Total non-absorbed	Total recovered
Group 1A	0.88	66.77	67.64	241.92	309.56
Group 1B	0.90	49.25	50.15	277.58	327.73
Group 1 average	0.89 $\pm$ 0.02	58.01 $\pm$ 12.39	58.90 $\pm$ 12.37	259.75 $\pm$ 25.22	318.65 $\pm$ 12.85
Group 2A	1.27	57.34	58.61	257.47	316.08
Group 2B	0.57	44.79	45.37	273.02	318.38
Group 2 average	0.92 $\pm$ 0.49	51.07 $\pm$ 8.87	51.99 $\pm$ 9.36	265.25 $\pm$ 10.99	317.23 $\pm$ 1.63
Group 3A	4.83	133.01	137.84	809.74	947.58
Group 3B	5.37	144.30	149.67	767.02	916.69
Group 3 average	5.10 $\pm$ 0.38	138.65 $\pm$ 7.99	143.76 $\pm$ 8.37	788.38 $\pm$ 30.21	932.14 $\pm$ 21.84

Groups 1 and 2 show persistence of PPD in the skin after a single application. Group 3 shows that PPD accumulates in the skin after multiple applications.

who are already sensitized to PPD may develop allergic contact dermatitis with the use of very low PPD concentrations (such as those seen with the skin allergy test) if repeatedly exposed. This phenomenon has already been demonstrated with isoeugenol (3) and methyldibromoglutaronitrile (5) and seems likely to occur with PPD as well.

The allergens in PPD reactions are unknown. They have been suggested to include PPD, one of the polymers of PPD such as Bandrowski's base, or metabolites, such as benzoquinone (8), although the evidence for this is controversial (9, 10). PPD in water was more efficient in eliciting reactions within the time periods studied here. This demonstrates that the base within which the hair dye is presented may be of importance. However this study did not show that PPD in water is a more efficient agent for patch test substances, as the time in question is different (2 D).

This study also shows that patients with a + reaction to PPD in patch test clinics are more likely to continue to dye their hair whilst ++ reactors tend to stop. This area of contact dermatitis, with the strength of patch test reactions inferring different clinical behaviour, is an understudied area (11).

After single or after 3 repeated dermal treatments *in vivo* with PPD in a prototype base vehicle, the majority of the administered dose (>80%) was rinsed from the skin after 5 min. At the killing there were still considerable amounts of the dose located in the tape-stripped treated skin. In terms of absolute amounts (microgram equivalent), there were approximately 3-fold higher levels present in the skin treated 3 times. It is interesting to note that in group 3, there was 5 times more systemically available and excreted PPD equivalents than in group 2, which is more than a direct 1:1 increase in systemic absorption with respect to the 3 times higher total dose. The consequences of this finding in relation to skin allergy remain unknown.

In summary, we have previously shown that there is a dose–time relationship for elicitation thresholds in PPD allergic reactions (2, 12); however, this current study shows that the 'time' element can be divided over the course of 8 D, but still give a similar elicitation threshold. After repeated dermal treatments, the amount of the allergic component of PPD within the skin accumulated in proportion to the number of treatments. These observations are of significance for

both the definition of elicitation thresholds as well as for more general considerations of skin sensitization risk assessment. This study therefore demonstrates for the first time that, over the time period tested, the allergenic component of PPD accumulates in the skin. Hence, smaller cumulative time exposure is equivalent to larger, single-time exposure.

References

1. Patel S, Basketter D A, Jeffries D et al. Patch test frequency to para-phenylenediamine: follow-up over the last 6 years. *Contact Dermatitis* 2007; 56: 35–7.
2. Hextall J M, Alagaratnam N J, Glendenning A K et al. Dose-time relationships for elicitation of contact allergy of para-phenylenediamine. *Contact Dermatitis* 2002; 47: 96–99.
3. Andersen K E, Johansen J D, Bruze M et al. The time-dose response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001; 170: 166–171.
4. Jensen C D, Johansen J D, Menne T, Andersen K E. Methyldibromo glutaronitrile contact allergy: effect of single versus repeated daily exposure. *Contact Dermatitis* 2005; 52: 88–92.
5. Jensen C D, Johansen J D, Menne T, Andersen K E. Methyldibromoglutaronitrile in rinse-off products causes allergic contact dermatitis: an experimental study. *Br J Dermatol* 2004; 150: 90–95.
6. Fregert S. *Manual of Contact Dermatitis*. Copenhagen, Denmark: Munksgaard, 1981.
7. Krasteva M, Cottin M, Cristaudo A et al. Sensitivity and specificity of the consumer open allergy test as a method of prediction of contact dermatitis to hair dyes. *Eur J Dermatol* 2005; 15: 18–25.
8. Krasteva M, Nicolas J-F, Chabeau G et al. Dissociation of allergenic and immunogenic functions in contact sensitivity to paraphenylenediamine. *Int Arch Allergy Immunol* 1993; 102: 200–204.
9. Basketter D A, Lidén C. Further investigation of the pro-hapten concept: reactions to benzene derivatives in man. *Contact Dermatitis* 1992; 27: 90–97.
10. White J M L, Kullavanijaya P, Duangdeedee I et al. p-phenylenediamine allergy: the role of Bandrowski's base. *Clin Exp Allergy* 2006; 36: 1289–1293.
11. Ho S G, Basketter D A, Jeffries D et al. Analysis of paraphenylenediamine allergic patients in relation to strength of patch test reaction. *Br J Dermatol* 2005; 153: 364–367.
12. McFadden J P, Wakelin S H, Holloway D B, Basketter D A. The effect of patch-test duration on the elicitation of paraphenylenediamine contact allergy. *Contact Dermatitis* 1998; 39: 79–81.

Address:

Contact Clinic, St John's Institute of Dermatology
St Thomas' Hospital, Lambeth Palace Road
London SE1 7EH
England, UK
Tel: 0044 20 7188 1613
Fax: 0044 20 7188 7473
e-mail: jonathan.white@gstt.nhs.uk or
jonathanmlwhite@hotmail.com