

Dose–time relationships for elicitation of contact allergy to *para*-phenylenediamine

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Skin-sensitizing chemicals exhibit dose–response relationships for the elicitation of contact dermatitis. Previously, considerable work has been carried out in which the elicitation of allergic skin reaction has been examined as a function of the applied concentration. However, the relationship between exposure time, dose and response has not been explored in any depth. The present work has extended our initial assessment of the relationship between both exposure time and concentration for *para*-phenylenediamine (PPD) in a group of 19 PPD-allergic volunteers. The results clearly demonstrate that a relationship exists between both exposure time and concentration. Positive responses to PPD were directly proportional to exposure time: at 5 min 16% responded; at 15 min, 38%; at 30 min, 50%; and at 120 min, 69%. A similar direct relationship was found between concentration of PPD and response: after 120 min, 22% of patients had responded to 0.01%, and 69% to 1% PPD. All exposures for 1 and 2 min were negative. Subsequent evaluation using repeated 5 min open application testing demonstrated a cumulative effect, as after 8 days 39% of the panel reacted, more than double the number that reacted to a single occluded 5-min treatment. It was noted that there was marked subject variability in exposure time and dose required to elicit an allergic response. These results are of relevance for the general interpretation of patch test data, especially with regard to risk assessment.

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Patch testing provides information vital for the diagnosis of allergic contact dermatitis (ACD) and has been standard practice for many decades. However, the 2-day (2D) patch test employed for this purpose, using generally the maximum nonirritant concentration of a prospective allergen, provides only limited information on the likelihood of a sensitized individual responding to the allergen under more 'real-world' exposure conditions. The needs of optimally effective diagnostic patch testing (reviewed in 1) mean that the impact of concentration, duration of exposure to the allergen, effect of repeated treatment, importance of occlusion and vehicle, and considerations of the relative susceptibility of different skin sites are not normally considered. Nevertheless, it is known that all of these parameters can have some impact on the nature and intensity of the allergic skin response. For example, it is well-recognized that the applied concentration of allergen has an important impact on the degree of reaction (2, 3). In addition, limited

evidence indicates that the duration of exposure has an effect. Whilst a reduction from 2 days to 5 h appeared to have little impact on the diagnosis of nickel allergy (4), we have shown previously that a reduction in the duration of exposure to *para*-phenylenediamine (PPD) from 2 days to 2 h or less causes a substantial reduction in the frequency of positive reactions. In the present work, we have extended these investigations in order to complete fully the matrix of dose–time responses to PPD applied under patch test conditions.

Materials and Methods

Ethical approval was obtained from the Ethical Review Committee at St. Thomas' Hospital. Following this, volunteers who had been previously diagnosed as sensitized to PPD were recruited. Their ages ranged from 17 to 65 years. In the first part of the study (previously reported in brief in 5), 17 volunteers were patch tested to concen-

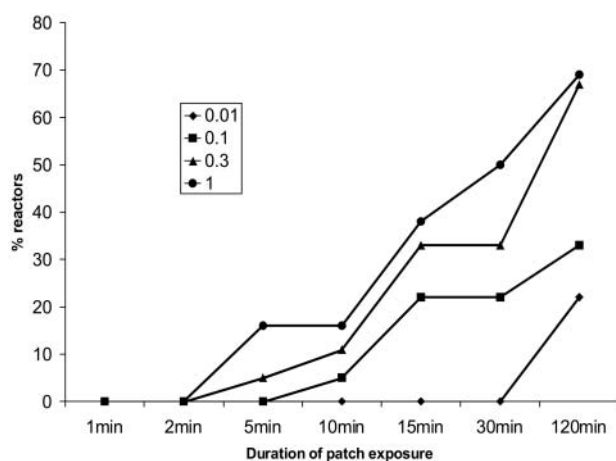


Fig. 1. Results of patch testing with *para*-phenylenediamine (PPD) at different doses and exposure durations. The graph displays the proportion of PPD-allergic individuals who gave positive patch test reactions to treatment with doses of PPD (0.01% to 1.0%) applied for between 1 and 120 min.

trations of PPD (Aldrich, Poole, UK; >99%) at 1, 0.3, 0.1 and 0.01% for 30, 60 and 120 min, respectively. In the second part of the study, reported here in combination with the earlier data, a group of 19 PPD-allergic patients had the same four concentrations of PPD applied for 1-, 2-, 5- and 10-min exposures. All concentrations of PPD were prepared in petrolatum (pet.) and applied to the back using 8-mm Finn Chambers on Scanpor (Epitest, Tuusula, Finland). Skin reactions were recorded at D2 (2 days) and D4 (4 days) using the grading system shown in Table 1.

In the second part of the present study, 18 of the 19 patients agreed to participate in repeated open application testing (ROAT). This involved daily application of 0.2 mL of 1% PPD in pet. to the antecubital fossa for up to 8 days; each application was rubbed onto the skin for 1 min, then left for a further 4 min; excess material was then wiped away. The volunteers were trained to carry out this procedure at the first visit and a card was given to the patient to complete to keep a record of treatments received. If at any time during the ROAT the patient developed an allergic response, then treatment of that individual ceased. The ROAT was assessed using the original grading description

of Hannuksela and Salo (6), which is widely used in industry.

Results

The results showed a direct relationship between both exposure time and concentration of PPD and elicitation of allergic response (Fig. 1). For example, positive response to PPD at 5 min was 16%, at 15 min was 38%, at 30 min was 50% and at 120 min was 69%. Exposures to any concentration of PPD for either 1 or 2 min gave entirely negative results. A similar direct relationship was found between concentration of PPD and elicitation of allergic response. After 120 min exposure, only 22% of patients responded to 0.01% PPD, while 69% reacted to 1% PPD. Unlike standard 2-day occlusion, there was no visible black coloration seen after removal of the PPD patches.

The results of the ROAT are presented in Table 2. In the ROAT, 7/18 (39%) of the subjects reacted to the accumulated effect of 5-min applications of 1% PPD; the intensity of the reaction varied widely, with one individual reacting both quickly and strongly, another quickly and weakly, and others either failing to react or exhibiting only the weaker grades of reaction at the end of the ROAT. The average time to response was approximately 4 days. Interestingly, the subjects who reacted most quickly (patients 1, 16 and 17), but not necessarily more strongly, were those who showed responses to 5- and 10-min patch treatment with PPD.

Discussion

The practice of hair dyeing dates back to antiquity. In many parts of the world natural vegetable dyes are used. However in recent times synthetic dyeing has overtaken this method. PPD is an extremely effective hair dye, which gives hair a black shade and can be mixed with a multiplicity of other dyes to produce a variety of colours. Problems with PPD usage lie not in its efficacy but in its sensitization potential. PPD sensitization remains a persistently frequent phenomenon (7) and this study shows that some sensitized individuals are extremely sensitive to PPD. The hair-dyeing process

Table 1. Scoring scheme

Grade	Description of response
0	No reaction.
?	Doubtful reaction, may be irritant – faint erythema only.
+	Weak allergic reaction – erythema, infiltration, possibly papules.
++	Moderate allergic reaction – erythema, infiltration, papules, vesicles.
+++	Strong allergic reaction – intense erythema and infiltration, coalescing vesicles.

Table 2. Results of the repeated open application testing

Repeated open application testing results		
Patient	Reaction grade	Day reaction first appeared
1	+++	2
2	++	5
3	—	—
4	+	8
5	—	—
6	+	4
7	—	—
8	—	—
9	—	—
10	—	—
11	—	—
12	—	—
13	—	—
14	—	—
15	++	8
16	+	1
17	+++	1
18	—	—

can often involve the oxidation of PPD. It is probable that oxidation products of PPD, as well as the chemical itself, are involved in sensitization reactions. Nevertheless, individuals who have an apparent hair dye-related dermatitis are often allergic to PPD itself.

One aspect that interested us was the extent to which the duration of exposure to an allergen, as well as the concentration applied, would have an effect on the elicitation of ACD. To this end, we initiated a small patch test study using PPD as a model allergen. These initial results confirmed that time was an important factor (5). The data has recently been extended by examination of other allergens, showing that the results, unsurprisingly, are not unique to PPD (8).

The extended PPD data reported herein confirm that both concentration and duration of skin exposure are important variables in the elicitation of an allergic response. For PPD at 1.0%, it seems that exposure under the occlusive conditions of a patch test requires a minimum period of more than 2 min for the accumulation of hapten to be sufficient to trigger an allergic reaction. In fact, just one subject reacted to 1.0% and 0.3% PPD after 5 min of occlusive treatment. However, fully 30-min exposure was necessary for 50% of the PPD-allergic subjects to react. When the concentration was reduced to 0.1%, the response frequency in the panel was approximately one-half of that found with 1.0% PPD.

Interestingly, the results in the ROAT showed that although 5 min of open application of 1.0% PPD was insufficient to produce any but the weakest level of reaction in a single individual, repeated exposure under these conditions did give

rise to skin reactions, a number of which were moderate to strong in intensity. Indeed, the percentage of subjects who responded to 6 days of 5-min ROAT was comparable to the proportion that responded to the 30-min single exposure to the equivalent concentration of PPD. This suggests that accumulated exposure over time may be of considerable importance when interpreting dose-response data for risk assessment purposes. The observation that the subjects who reacted most quickly, but not necessarily more strongly, in the ROAT were those who showed responses to 5- and 10-min patch treatment with PPD is consistent with observations concerning the ability of metabolic enzymes in the skin being a key to PPD allergy (reviewed in 9). Such a result is also consistent with the outcome of the dose-time study carried out with isoeugenol-allergic patients (8).

In summary, we have shown that time, concentration and number of applications (and therefore accumulated dose in the skin) are all important factors in the elicitation of allergic contact reactions to PPD. Superimposed upon this, however, are substantial variations in individual reactivity to a defined stimulus. These factors are likely to be of general importance in the analysis of dose-response data and risk assessment for all chemical contact allergens.

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