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Received Date : 30-Apr-2014

Revised Date : 04-Sep-2014

Accepted Date : 07-Sep-2014

Article type : Cutaneous allergy

Cross-elicitation responses to 2-methoxymethyl-*p*-phenylenediamine under hair dye use conditions in *p*-phenylenediamine-allergic individuals

Running head: Responses to 2-methoxymethyl-*p*-phenylenediamine in allergic subjects

article keywords: permanent hair dyeing, cross-elicitation, *p*-phenylenediamine, 2-methoxymethyl-*p*-phenylenediamine, allergic contact dermatitis, simulated hair dye use conditions

B. Blömeke¹, L. M. Pot², P.-J. Coenraads², J. Hennen¹, M. Kock³, C. Goebel³

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¹Dept. of Environmental Toxicology, University Trier, Trier, Germany; ²Dermatology Dept., University Medical Center Groningen, University of Groningen, The Netherlands; ³The Procter & Gamble Co., Central Product Safety, Darmstadt, Germany

corresponding author: Prof. Dr. Brunhilde Blömeke

¹Department of Environmental Toxicology, University Trier, Universitätsring 15, 54296 Trier, Germany

phone: 0049-651-201-3781

Fax: 0049-651-201-3780

e-mail address: bloemeke@uni-trier.de

Funding sources:

The project was supported in part by The Procter & Gamble Co., Central Product Safety, Darmstadt, Germany, the Womens Professor Programme of the Federal Ministry of Education and Research (Germany) (J. H.) and the Foundation Environmental & Occupational Dermatology (SMAD), Netherlands (L. M. P.).

Conflict of interest:

The authors Carsten Goebel and Monika Kock are employees of the Procter & Gamble Company. The hair dye ingredient studied in this paper is currently used in commercial products marketed by the Procter & Gamble Company. The authors Jenny Hennen, Laura M. Pot, Pieter-Jan Coenraads and Brunhilde Blömeke participated as experts in skin sensitization. The authors alone are responsible for the content and writing of the paper.

Bulleled statements:

What's already known about this topic?

2-Methoxymethyl-*p*-phenylenediamine is a new *p*-phenylenediamine (PPD) derivative with reduced skin sensitization properties.

What does this study add?

Exposure to a hair dye test product containing 2-methoxymethyl-*p*-phenylenediamine on the forearm for 30 min indicated a concern for 9/30 (30%) of PPD-allergic individuals, while 70% were negative. This response rate is significantly lower than we previously reported for PPD under simulated hair dye use conditions (84%).

Abstract

Background: Factors influencing elicitation responses in individuals allergic to *p*-phenylenediamine (PPD) related to hair dyeing are not well understood.

Objectives: Investigation of the elicitation response to the new, less-sensitizing PPD-alternative 2-methoxymethyl-*p*-phenylenediamine (ME-PPD) under simulated hair dye use conditions.

Patients/Methods: The cross-elicitation response to ME-PPD (2% in a hair dye test product for 30 min on forearm and rinsing) was analysed in 30 PPD-allergic individuals with diagnostic patch test grades +, ++ or +++ according to the classification of the International Contact Dermatitis Research Group at day 2 and day 3.

Results: Cross-reactivity to the ME-PPD containing hair dye test product was elicited in 9/30 (30%) while 70% were negative (-). Cross-reactivity was elicited in 2 of 4 cases with grade +++, 3 of 10 with grade ++, and 4 of 16 with grade +. Under identical conditions, PPD was previously found to elicit 21/27 of these PPD-allergic individuals. In 18 of these 21 individuals, either the strength of the cross-elicitation response to ME-PPD was decreased or no response occurred.

Conclusions: Under simulated hair dye use conditions a significantly lower degree of cross-elicitation to ME-PPD (9 of 30 PPD-allergic individuals) was observed than previously reported for PPD (84%). Additionally, a decreased cross-elicitation strength was observed across all three patch test grades likely reflecting the reduced skin sensitization properties of ME-PPD. Consequently, careful dermatological evaluation is required to assess cross-reactivity to ME-PPD in hair-dye allergic patients.

Introduction

2-Methoxymethyl-*p*-phenylenediamine (ME-PPD) is a recently developed *p*-phenylenediamine derivative ¹. The introduction of a methoxymethyl side chain into PPD yielded a hair dye precursor with excellent hair coloring performance when used together with couplers (e.g. *m*-aminophenol) and an oxidizing agent (e.g. hydrogen peroxide) in hair dyes ². Furthermore, ME-PPD has significantly reduced skin sensitizing properties compared to PPD or the structurally related compound *p*-toluylenediamine (PTD) ¹. Chemical structures are depicted in Fig. 1. For instance, the effective concentration of ME-PPD necessary to induce an immune response 3-fold above vehicle control (EC3 value) in the local lymph node assay (LLNA) was 4.3%, indicating a moderate skin sensitizing potency compared to values of 0.1 and 0.17% equivalent to strong ³ or extreme ⁴ potencies for PPD and PTD, respectively. Furthermore, assessment of the skin sensitizing potency under consumer hair dye usage conditions through a quantitative risk assessment (QRA) indicated a much lower likelihood of ME-PPD to induce skin sensitization compared to PPD or PTD ¹.

The introduction of a less skin sensitizing substitute for PPD and PTD is justified by several additional aspects. PPD and PTD are considered the most important allergens associated with hair dye-related allergic contact dermatitis. A recent study confirmed sensitization to PPD in 4.5% of a total of 2939 tested eczema patients, 2.8% reacted to PTD, and about 55% of these cases mentioned dying hair as likely cause of their sensitization ⁵.

Furthermore, apart from sensitization to either compound, a positive relationship of an elicitation response to PPD on patch testing and concomitant reactions to other chemically related (*para*-substituted benzene) components of oxidative hair colours can be observed. For instance, individuals sensitized to PPD may also cross-react to PTD, *m*-aminophenol, *p*-aminophenol, or 2-nitro-PPD ⁶ as well as to some azo dyes ⁷. This cross-elicitation has been extensively studied for individuals with sensitization to PPD. Recently, Søsted and colleagues ⁵ reported concomitant reactions to PTD in 50% of PPD patch test positive cases (67 of 133), while PPD responses in those sensitized to PTD were found in 81%. Concomitant reactions to the precursor *p*-aminophenol were found in 35% of the PPD-sensitized cases, while simultaneous reactions to *m*-aminophenol were 18% in PPD- and 27% in PTD-sensitized individuals. Also a high degree of concordance (>80%) with PPD was detected among those with a positive patch test reaction to the aminophenols. Thus, usage of hair dye products containing PPD or PTD is a concern for PPD-allergic individuals.

On the other hand there are chemicals which are tolerated by PPD-sensitized patients. Elicitation responses to resorcinol, in line with its significantly lower sensitization potency in humans ⁸, are rarely found (0.1% in the above mentioned study) despite its presence in 80% of all hair dyes. Another benzene derivative hydroxyethyl-*p*-phenylenediamine sulphate (HE-PPD) has less potential for cross-reactivity. It was reported to be tolerated by a subgroup of PPD-sensitized individuals suspected of having contact dermatitis caused by hair dyes ⁹. The latter study reported that 40 of 216 patients (19.9%) reacted to 1% PPD (free base in pet.) under diagnostic patch test conditions, whereas only 5 of them (2.3%) showed a positive reaction to a comparable amount of HE-PPD sulphate (2%). Differences in the

sensitizing potency between HE-PPD sulphate ¹⁰ and PPD ⁸ or PTD ¹¹ are not likely to have a major impact on the rate of the elicitation response, since all three substances are considered as at least strong sensitizers.

These studies demonstrate that elicitation and cross-elicitation are not only affected by chemical potency. The strength of the individual's sensitization status ¹² is known to influence the cross-elicitation rates as well. The latter was also found for HE-PPD ⁹, since reactions were predominantly found in those highly sensitized to PPD. Finally, other factors including differences in skin absorption and individual metabolism ^{13,14} as well as the induction dose itself ¹⁵ are known to influence the elicitation response.

In order to evaluate the possible risks for hair dye-allergic individuals to develop cross-elicitation reactions to ME-PPD under hair dye use conditions, we investigated whether ME-PPD shows cross-elicitation responses in PPD-allergic individuals with a documented history of hair dye-related allergy and different diagnostic patch test response grades (+, ++, +++).

Materials and Methods

Cases

Individuals (n=30, 4 males, 26 females) with a well documented history of allergic contact dermatitis to PPD based on a positive diagnostic patch test response to PPD (diagnostic PPD TRUE test®, Mekos, Hillerød, Denmark), and previous problems after using hair dye products were included. Patch test readings were performed at day 2 (D2) and day 3 (D3) and graded according to the International Contact Dermatitis Research Group's (ICDRG) classification system ¹⁶. At D3 grades were + (n=16), ++ (n=10) and +++ (n=4). Among these 30 individuals were 27 individuals (+, n=14; ++, n=10; +++, n=3) who were previously tested for their elicitation response to a simulated hair dye product containing 2% PPD

under identical conditions as described by us ^{17,18}. Approval of the study was given by the local ethics committee.

Preparation of hair dye formulation and epicutaneous testing

The vehicle (Koleston Perfect formula without fragrance) containing the hair dye precursor (4% ME-PPD, free base) and couplers (1.9% 2-methylresorcinol and 1.9% 2-methyl-5-hydroxyethylaminophenol), as well as the hydrogen peroxide solution (6% (w/w) Welloxon) were provided by P&G. The couplers were selected based on their negligible sensitization potency as determined in the local lymph node assay, each with an effective concentration (EC₃) > 50 ^{19,20}. The hair dye test product was always freshly prepared by mixing the tint (containing ME-PPD and the couplers) with the hydrogen peroxide solution using a small wooden stick (1:1, 90 µl each). An amount of 100 µl of the finished ME-PPD containing product was applied to the filter paper of the van der Bend Chambers® (Brielle, the Netherlands, 1 cm²) using a pipette (infinite dose, 100 mg/cm²). A dye-free test product was used as control. The filled chambers were removed from the tape and directly placed on the skin of the lower arms. The chambers were additionally resecured in the same position with 2 small stripes of tape (3M) across the plastic connections attached to the chamber (occlusion). After 30 min the formulations were gently rinsed off with a commercial shampoo and water to simulate hair dyeing use conditions.

Responses were recorded at D2 and D3 and graded according to the ICDRG criteria.

Results

Cross-elicitation responses to ME-PPD applied under hair dye use conditions in PPD-allergic individuals

The potential of ME-PPD to cross-elicit allergic contact dermatitis was assessed on the skin of 30 individuals who were patch test positive to PPD and who had experienced hair dye-related allergic contact dermatitis in the past. Fig. 2 summarizes the results following a 30 min exposure to ME-PPD in a simulated hair dye product, scored at D2 and D3. Of the 30 individuals (4 males and 26 females) tested, 9 reacted to ME-PPD corresponding to a response rate of approximately 30%. Of the 4 individuals with a +++ patch test grade to PPD, 2 reacted; 3 of the 10 individuals with a ++ patch test grade to PPD reacted, and 4 of the 16 individuals with a + patch test grade to PPD reacted. No reactions were observed when the basic hair dye formula without dyes was applied for 30 min as control.

To include the strength of the cross-elicitation responses to ME-PPD at D2 and D3, results in Fig. 2 were depicted according to the grade of the diagnostic patch test response to PPD at D3. In the group of individuals with a +++ diagnostic patch test response to PPD (n=4), only 2 responded to ME-PPD, with at least ++. In the group of individuals with a ++ diagnostic patch test response to PPD (n=10), 1 responded with a ++ reaction to ME-PPD both at D2 and D3, while 2 were graded + or ? at either reading time. In the group of individuals with a + diagnostic patch test response to PPD (n=16), 3 responded to ME-PPD with + or ? at either reading time, while for 1 the strength increased to ++ at D3.

Among the 30 individuals tested for their cross-elicitation response to ME-PPD, 27 individuals were previously tested by us for their elicitation response to an identical hair dye test product containing 2% PPD instead of ME-PPD ¹⁷. Of those 27 individuals, 21 (78%) reacted to the PPD containing hair dye test product. Fig. 3 provides the elicitation responses at D2 and D3 to PPD under hair dye use conditions of these 21 individuals compared to their cross-elicitation responses to ME-PPD under identical conditions. Six of the 27 individuals

tested with both were negative to either PPD or ME-PPD containing hair dye test product (data not shown). Application of the ME-PPD containing hair dye test product yielded positive responses in 8 of these 21 (38%) cases. In addition, a reduced strength of the cross-elicitation responses was found after application of ME-PPD containing hair dye test product compared to PPD in 18 of the 21 cases (see Fig. 3).

Discussion

In this paper, we studied the potential of a ME-PPD containing hair dye test product (2% ME-PPD applied on the volar forearm under occlusion for 30 min) to elicit a cross-reaction in PPD-allergic individuals. For that purpose, 30 individuals were selected with a PPD-related contact allergy corresponding to a history of hair dye product usage and a documented analysis of their patch test response to PPD upon diagnosis.

We observed that 9 of these 30 individuals reacted to ME-PPD, corresponding to a response rate of approximately 30%. This result indicates that ME-PPD is able to elicit a cross-reaction under simulated hair dye use conditions in PPD-allergic individuals with a history of hair dye-related allergic contact dermatitis. The sensitivity of this exposure scenario was previously studied by us ^{17,18} using PPD and found to elicit a reaction in 20/20 individuals with a moderate or strong allergy against PPD based on diagnostic patch test grades (with grades ++ to +++), while sensitivity was 44% in subjects (8/18) weakly sensitized (+) to PPD.

Due to the fact that ME-PPD is a new molecule, we consider the responses as true cross-reaction ²¹, since co-sensitization can be excluded as our tests were performed prior to its market introduction. The observed cross-reaction rate of 30% for ME-PPD is lower than the reported concomitant reactions to PPD of approx. 75% in PPD patch test positive individuals ^{5,22}. In order to further evaluate the observed cross-reactivity between the new molecule ME-PPD and PPD we performed a more detailed analysis in a subgroup of 27 individuals who had been exposed to both molecules. PPD exposure of these individuals in our previous study ¹⁷ was identical to the ME-PPD exposure in the present study, i.e. we evaluated the response upon exposure to the hair dye product applied for 30 min with maximum realistic

compound concentrations of 2%. In that previous study, 6 of the 27 individuals did not react to PPD and did not react to ME-PPD. A possible explanation is that due to the rinse off conditions of hair dyeing the individual elicitation threshold has not been reached. This is in line with the well described dependency of elicitation responses to PPD on exposure duration and dose ^{23,24}. Of the 21 individuals that reacted to the PPD containing hair dye product in our previous study 8 cross-reacted to the ME-PPD containing hair dye product, but the majority with a reduced strength of their elicitation responses (see Fig. 3). The reduction observed occurred across all patch test categories, indicating an attenuation of the (cross-) elicitation response likely due to the structural alteration of PPD by the methoxymethyl side chain.

Multiple mechanisms of T cell receptor (TCR) cross-reactivity are known ²⁵. Molecular mimicry, in which cross-reactive ligands share key structural and chemical features, is a mechanism commonly used to explain TCR cross-reactivity caused by allergen specific T cells recognizing different chemicals. Accordingly, modification of the basic *para*-structure of the PPD molecule by ring substitutes has been frequently reported to affect induction and elicitation of skin sensitization ^{21,26-29}. For PTD and *p*-aminophenol, the cross-reactivity rate with PPD was 100% when tested under defined conditions in guinea pigs ³⁰ indicating that in the case of PTD the introduction of a methyl side chain into PPD or, in the case of *p*-aminophenol, the exchange of an amino-group by a hydroxyl-group does not represent a relevant modification with respect to cross-reactivity. This view is further supported by studies using PPD-reactive T cell clones (generated by sensitizing mice to a PPD-haptenated self protein) demonstrating that in addition to PPD also *p*-aminophenol and PPD-self oxidation products including Bandrowski's base were recognized by some of the T cell clones ²⁸. Accordingly, the observed cross-reactivity in the responders may be explained (1) by T cells incapable of distinguishing among the different haptens formed by PPD and ME-PPD, respectively; and/or (2) by T cells that react to a hapten formed by a common self-oxidation product of PPD and ME-PPD.

Conversely, the observed limited cross-elicitation to ME-PPD indicates a more impactful alteration of the *para*-structure leading to a reduced rate of T cell recognition. This is in line with the observation that lymphocytes from PPD-allergic patients specifically recognized protein modifications caused by PPD but did not recognize a modified PPD molecule containing two additional methyl-groups in the benzene ring, i.e. 2,5-dimethyl-PPD³¹. Likewise, only 1 of 16 PPD-allergic individuals reacted to diacetylated PPD, i.e. the addition of an acetyl-group to each amino-group of PPD^{13,32}. These findings suggest that addition of the methoxymethyl side chain creates a hapten structure less easily recognized by T cells of PPD allergic patients than PPD itself.

In summary, our data indicate that under simulated hair colouring conditions a significantly lower degree of cross-elicitation to ME-PPD (30%) was observed than previously reported for PPD (84%). Furthermore, the observed decreased strength of the elicitation response to ME-PPD across all three patch test grades may reflect the reduced skin sensitization properties possibly including limited T cell recognition. Consequently, careful dermatological evaluation is required to assess cross-reactivity to ME-PPD in hair dye-allergic patients.

Acknowledgement:

We thank Wim Schaefer MD for performing the patch tests on the PPD-allergic individuals.

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Figure Legends

Fig. 1: Chemical structures of the hair dye primary intermediates (a) *p*-phenylenediamine, (b) *p*-toluylenediamine and (c) 2-methoxymethyl-*p*-phenylenediamine.

Fig. 2: Cross-elicitation responses of individuals with documented history of hair dye-related allergic contact dermatitis following occlusive exposure to 100 $\mu\text{g}/\text{cm}^2$ 2-methoxymethyl-*p*-phenylenediamine (ME-PPD) containing hair dye product for 30 min (containing 2% ME-PPD, n=30). Panelists' test results are grouped according to the strength of previous diagnostic patch test response to *p*-phenylenediamine (PPD) (1% in petrolatum) at reading day 3 (D3).

Fig. 3: Strength of elicitation responses to a PPD containing hair dye product and cross-elicitation responses to a ME-PPD containing hair dye product in individuals with a well documented history of hair dye-related allergic contact dermatitis. Responses of positively responding individuals to a PPD containing hair dye product (21 of 27) after occlusive exposure to 100 or 150 $\mu\text{g}/\text{cm}^2$ hair dye product containing 2% PPD (as described by us¹) were tested for cross-elicitation responses to occlusive exposure to 100 $\mu\text{g}/\text{cm}^2$ ME-PPD containing hair dye product (containing 2% ME-PPD) for 30 min. Individuals' responses are grouped according to their conventional diagnostic patch test response to PPD (1% in petrolatum) at reading day 3 (D3).



