

Tolerance to a Hair Dye Product Containing 2-Methoxymethyl-*P*-Phenylenediamine in an Ethnically Diverse Population of *P*-Phenylenediamine-Allergic Individuals

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Background: Allergic contact dermatitis after exposure to *p*-phenylenediamine (PPD)-containing hair dye products is a common and important clinical problem. Because there is a high rate of cross-elicitation of allergic contact dermatitis to other important hair dye products (such as *p*-toluene diamine and other aminophenol hair dyes) in PPD-allergic patients, safer alternative dyes with excellent hair coloring options are needed.

Objective: This study aimed to study tolerance to Me-PPD in a PPD-allergic cohort.

Methods: Twenty ethnically diverse volunteers with a history of contact dermatitis to hair dyes or other PPD-containing chemicals and positive patch test results to 1% PPD in petrolatum were recruited to study their immediate and delayed skin reactivity to PPD, vehicle control, and 2-methoxy-methyl-PPD (Me-PPD) using the allergy alert test (simulating hair dyeing conditions) on volar forearm skin. This test is a short-contact open patch test.

Conclusions: The Me-PPD may offer a safer alternative for PPD-allergic patients with an absent or reduced elicitation response in the allergy alert test simulating hair dye use conditions. The absent or reduced response to Me-PPD diagnosed using the allergy alert test has been shown to help reduce the possibility of moderate to severe cross-elicitation reactions among consumers during hair dyeing.

Allergic contact dermatitis (ACD) to PPD is an important clinical problem because the frequency of positive patch test results to 1% PPD in petrolatum in a large series of dermatitis patients has been in the range of 4% to 5% of prevalence.^{1–4} This has been extrapolated to approximately 1% of the general population.⁵ Sensitization to PPD is considered to occur mainly by exposure to hair dyes, but can also occur by exposure to leather (eg, shoes), fur, textiles (eg, undergarment elastic) or industrial rubber products (eg, grips of tools or bicycles, tires, hoses, tubes), contaminated street henna tattoos, and printer ink.⁶

For PPD-allergic individuals who acquire allergy to this dye because of exposure to hair dye products that contain PPD, selection of safer alternatives that exhibit excellent hair color performance has been challenging. Other parasubstituted benzene

compounds (para-toluene diamine [PTD], *p*-aminophenol, or 2-nitro-PPD as well as some azo dyes) can also cross-elicit ACD in PPD-allergic patients.^{7–9} For example, concomitant reactions to PTD occur in 50% of patients with positive patch test results to PPD.⁷ Conversely, in PTD-sensitized patients, cross-elicitation to PPD occurs in 81% with patch testing.⁷ This limits the safe alternatives that exhibit excellent hair color performance in PPD- or PTD-allergic patients who want to continue to dye their hair.

2-Methoxy-methyl-PPD (Me-PPD) is a recently developed PPD derivative that was created by the introduction of a methoxymethyl side chain into PPD.¹⁰ The resulting molecule is a hair dye precursor that exhibits excellent hair coloring performance when used with couplers such as resorcinol and oxidizing agents (ie, hydrogen peroxide).¹¹ Preclinical studies with this PPD derivative revealed that it exhibited only moderate sensitizing properties (EC3 value on local lymph node assay): 4.3% for Me-PPD versus 0.1% or 0.17% for PPD (considered an extreme sensitizer) or PTD (considered a strong sensitizer), respectively.¹⁰ These preclinical data translated well in a clinical study of a group of PPD-allergic patients using an allergy alert test that simulates hair dyeing conditions on the surface of the skin (short-contact open patch test), typically volar skin of the forearm.^{12,13} A recent study performed at the University Medical Center Groningen in the Netherlands involved 30 subjects with patch test–proven PPD allergy from hair dye use. When exposed to

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Me-PPD under conditions simulating hair dyeing, cross-elicitation to Me-PPD occurred in only 30% of this population, with the remaining 70% of these PPD-allergic subjects exhibiting tolerance to this novel hair dye.¹³ Because this study was completed in Europe, the PPD-allergic volunteers were exclusively white and type I and II Fitzpatrick skin types.

The effects of ethnicity on ACD to specific allergens is not well studied. One study examined effects of race and ethnicity and patterns of patch test reactivity.¹⁴ There was a statistically significant higher rate of ACD to PPD in blacks (10.6%) compared with whites (8%).¹⁴ The reason for these differences was postulated to be related to hair dyeing practices and exposure to higher concentrations of PPD related to darker hair shades and possibly related to the N-acetylation (N-acetyltransferase 1 [NAT1] and 2 [NAT2]) capacities (metabolism of PPD). The purpose of this study is to determine whether tolerance to Me-PPD translated to an ethnically diverse population such as found in downtown Baltimore in which approximately 70% of the inhabitants are African American.¹⁵

Herein, we report that a population of 20 patch test–proven PPD-allergic patients (predominantly from hair dye exposure) who are ethnically diverse also exhibits a tolerance to Me-PPD under conditions simulating hair-dyeing. Seventy percent of this PPD-allergic population was tolerant of Me-PPD, and this was strongly influenced by the strength of their diagnostic patch test. The Me-PPD may be a safer alternative in PPD-allergic patients (after dermatologic testing to demonstrate tolerance to this chemical dye) even in ethnically diverse patients who tend to use darker shades of hair dye (ie, higher concentrations of PPD) to color their hair. It also suggests that cross-elicitation to Me-PPD in PPD-allergic patients is no different than that found in white

individuals despite differences in ethnicity and skin type. These data also suggest that hair dyeing practices (ie, exposures to higher concentrations of PPD), rather than fundamental differences in the immunobiology of PPD allergy, may be the reasons for a higher rate of sensitization to PPD in diverse ethnic populations.

MATERIALS AND METHODS

The PPD-Allergic Volunteers

Our inclusion criteria included healthy men and women aged between 18 and 79 years of skin types with a history of a positive patch test result to PPD. Exclusion criteria included history of severe systemic allergic reaction (such as anaphylaxis); the use of topical corticosteroids or immunosuppressive agents (on the arms) 1 month before visit 1; the use of systemic corticosteroids or immunosuppressive agents 3 months before visit 1; the use of antihistamines 1 week before visit 1; known or suspected history of drug or alcohol abuse within the past 6 months; scarring, infection, or skin disease in the area being patch tested; pregnant women or nursing mothers; and life-threatening illnesses.

Twenty volunteers (N = 20; 3 men, 17 women) were recruited who had a history of ACD to PPD based on a positive standard patch test result (1% PPD under a FINN Chamber [Chemotechnique Diagnostics, Vellinge, Sweden]) and problems (dermatitis and itching) using hair dye, darkroom work, or printer ink. These volunteers were recruited from our patch test clinic at the University of Maryland Baltimore, Department of Dermatology, as well as referrals from community dermatologists. The characteristics of the 20 volunteers enrolled in this study are summarized in Table 1. Open

TABLE 1. Characteristics of Volunteers

Volunteer Identification	Skin Type	Ethnicity	Age/Sex	History
1	IV	African American	55/female	Hair dye intolerance
2	V	African American	65/male	Darkroom work
3	V	African American	66/male	Hair dye intolerance
4	V	African American	62/female	Hair dye intolerance
5	V	Indian	62/male	Hair dye intolerance
6	V	African American	63/female	Hair dye intolerance
7	V	African American	52/female	Hair dye intolerance
8	II	White	64/female	Hair dye intolerance
9	IV	African American	58/female	Hair dye intolerance
10	II	White	72/female	Hair dye intolerance
11	IV	Indian	44/female	Hair dye intolerance
12	IV	African American	28/female	Hair dye intolerance
13	IV	African American	67/female	Hair dye intolerance
14	IV	African American	66/female	Hair dye intolerance
15	III	Chinese	58/male	Hair dye intolerance
16	III	Chinese	66/female	Hair dye intolerance
17	III	Chinese	61/female	Hair dye intolerance
18	I	White	66/female	Hair dye intolerance
19	II	White	57/female	Hair dye intolerance
20	IV	African American	49/female	Hair dye intolerance

patch test readings were performed at days 2 and 3 and graded according to the International Contact Dermatitis Research Group (ICDRG) classification system.¹⁶ Among these 20 individuals, 7 were scored as +3, 7 were scored as +2, and 6 were scored as +1, with these reactions to diagnostic patch tests to PPD 1% under a FINN Chamber (see Fig. 3C for a summary of patch test results). Approval for the study was provided by the local institutional review board (protocol no. HRE-HP-00060991-1). Recruitment occurred between January 2015 and November 2016; enrollment was suspended when the target number of subjects ($N = 20$) was achieved. This trial has been registered with ISRCTN registry (Reference no. ISRCTN13869335; www.isrctn.com).

Although the investigators were not blinded to the skin test sites when reading the allergy alert test, there was a dermatologist safety monitor (Dr. Ronald Goldner) who did read the allergy alert test separately and independently from the investigators (A.Z. and A.G.) and was blinded to the placement of the skin test challenge sites. His blinded readings concurred with the study team in all 20 volunteers.

Preparation of Hair Dye Formulation and Epicutaneous Testing

The vehicle (Koleston Perfect formula without fragrance) containing the hair dye precursor (PPD 4% or ME-PPD 4%, free base) and couplers (2-methylresorcinol 1.9% and 2-methyl-5-hydroxyethylaminophenol 1.9%) as well as the hydrogen peroxide-based developer solution (6% w/w Welloxon) were provided by Procter & Gamble (Cincinnati, OH). The couplers were selected based on their negligible sensitization potency as determined in the local lymph node assay, each with an EC₃ greater than 50.^{17,18} The hair dye test product was always freshly prepared by mixing the tint (containing PPD, or Me-PPD, and the couplers) with the hydrogen peroxide solution using a small wooden stick (1:1, 90 μ L each). A 100- μ L aliquot of the final PPD- or Me-PPD-containing product was applied directly to the volar forearm in a 1-cm² marked area (infinite dose, 100 mg/cm²). A dye-free test product was used as a control. Each test site (PPD, vehicle control, and Me-PPD) was separated by 3 cm on the volar forearm. After 30 minutes, the formulations were gently rinsed off with commercial soap and water to simulate hair dyeing conditions. Responses were recorded at 30 minutes after application and on days 2 and 3 and graded according to the ICDRG criteria. The Me-PPD preparation did not contain any detectable PPD (limit of detection < 100 ppm) (personal communication, Dr. Carsten Goebel).

Statistical Analysis

Differences in the strength of reactivity between PPD and Me-PPD were analyzed using the paired Student *t* test (PRISM-Graphpad Software, Irvine, CA). Values of $P < 0.05$ were considered significant.

RESULTS

To determine whether an ethnically diverse group of 20 PPD-allergic volunteers (80% of the volunteers were dark skinned, see Table 1) were tolerant of Me-PPD, we used the allergy alert test

design on the volar forearm¹⁹ to PPD (positive control), vehicle (containing hydrogen peroxide), or Me-PPD. We performed readings at 30 minutes to assess immediate reactivity (urticarial reaction with a wheal and flare). None of the 20 volunteers exhibited immediate reactivity (30 minutes) to PPD, vehicle, or Me-PPD (data not shown). Thus, there was no immediate reactivity to PPD or Me-PPD despite the presence of a range of patch test reactivity to PPD on diagnostic patch testing (+1 to +3 reactivity, see Fig. 3A–C).

Delayed reactivity to Me-PPD was also studied in these same 20 volunteers. Of this population of PPD-allergic individuals, 7 exhibited +3 reactivity to PPD on diagnostic patch testing, 7 exhibited +2 reactivity to PPD on diagnostic patch testing, and 6 exhibited +1 reactivity to PPD on diagnostic patch testing. Because cross-elicitation to Me-PPD was dependent on the strength of PPD patch testing,¹² we clustered our volunteers into subgroups based on their diagnostic patch test PPD reactivity. A time course of skin reactivity of the allergy alert test in volunteer 6 is depicted from time 0 (application of the allergy alert test) through 30 minutes (at 30 minutes, all 3 tests are negative, but staining of the epidermis is apparent at the PPD and Me-PPD sites but not the vehicle site), at 48 hours (in which there is +2 reactivity to PPD but not to vehicle or Me-PPD), and at 72 hours (in which there is +2 reactivity to PPD but not to vehicle or Me-PPD) (Fig. 1A). Cross-elicitation of Me-PPD in PPD-allergic individuals was found predominantly in those with stronger diagnostic patch tests to PPD: there was cross-elicitation in 5 (71%) of 7 of the +3 PPD patch test group; 1 (14%) of 7 of the +2 PPD patch test group; and 0 of 6 of the +1 PPD patch test group (Fig. 1B). This volunteer is considered tolerant of Me-PPD because there is ACD at the PPD allergy alert site but not at the Me-PPD allergy alert test site at 48 and 72 hours (scored negative at both time points). A summary of the frequency of tolerance to Me-PPD versus strength of patch test result is depicted in Figure 1C. Tolerance to Me-PPD is inversely proportional to the strength of the PPD diagnostic patch test: 6 (100%) of 6 of the +1 PPD patch test group were tolerant of Me-PPD; 6 (85%) of 7 of the +2 PPD patch test group were tolerant of Me-PPD; and 2 (29%) of 7 of the +3 PPD patch test group were tolerant of Me-PPD.

Overall, 6 (30%) of 20 of the PPD-allergic volunteers cross-elicited to Me-PPD. Because PPD and Me-PPD allergy alert tests were administered simultaneously, this provided the opportunity to compare the strength of the allergy alert between the 2 chemicals. Figure 2A summarizes the clinical allergy alert scores of the 6 Me-PPD-intolerant volunteers and compares these allergy scores to the PPD test sites. In all 6 cases in which there was cross-elicitation between PPD and Me-PPD, the PPD allergy alert score was stronger than that of the Me-PPD allergy alert score. The mean (SEM) allergy alert scores summarized and the PPD allergy alert score were significantly greater than that of the Me-PPD allergy alert score ($***P < 0.001$, highly significant; paired Student *t* test) (Fig. 2B).

Figure 3 depicts a detailed summary of the PPD allergy alert scores and the Me-PPD scores in all 20 volunteers. Figure 3A summarizes allergy alert test results to PPD, with volunteers clustered by patch test reactivity. Figure 3B summarizes allergy alert test results to Me-PPD, clustered by patch test reactivity.

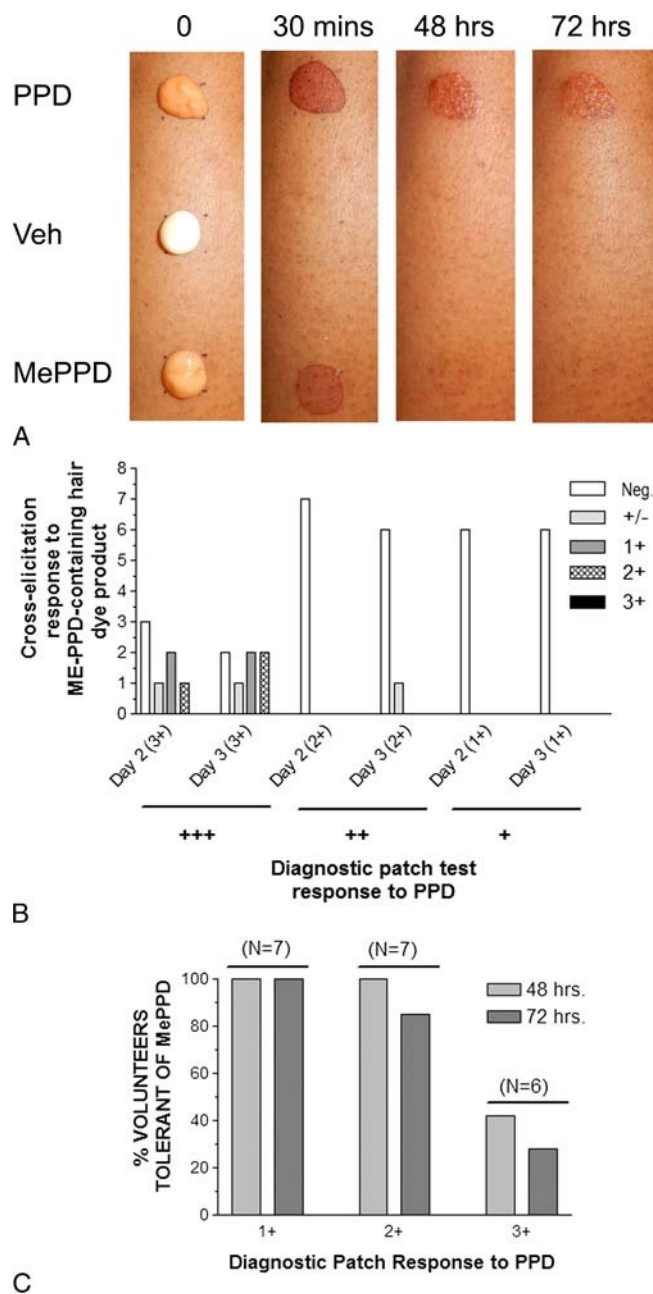


Figure 1. Tolerance to Me-PPD as demonstrated by allergy alert test. A, Time course of allergy alert test on the forearm of volunteer 6. Time 0, 30 minutes, 48, and 72 hours are depicted. This volunteer developed a +2 reaction to PPD but did not react (-) to Me-PPD at 0, 30 minutes, 48, and 72 hours. B, Frequency of cross-elicitation of Me-PPD (2% in hair dye for 30 minutes) in 20 PPD-allergic volunteers clustered by patch test results to PPD (+1 to +3). C, Bar histogram depicting the frequency of tolerance to Me-PPD (nonreactivity (-), or reading on allergy alert test) clustered by strength of diagnostic patch test to PPD.

Figure 3C is a summary of the diagnostic patch test to PPD, clustered by reactivity. This again demonstrates that all Me-PPD cross-elicitation was observed predominantly in the +3 PPD patch test group (5 of 6), with 1 of the 6 cross-elicitations in the +2 PPD patch test group. It also demonstrates that the PPD allergy

alert scores increase with increasing strength of the diagnostic PPD skin test. Of note, most of the volunteers in the +1 PPD diagnostic patch test group were also nonreactive to PPD on allergy alert test.

DISCUSSION

Herein, we describe the study of Me-PPD using the allergy alert test in a cohort of ethnically diverse patients (of which 80% were Fitzpatrick skin types III–VI, see Table 1) reflecting the ethnic profile of Baltimore City, Md.¹⁵ Despite these differences in ethnicity in our population, when compared with the initial study of cross-elicitation of Me-PPD in PPD-allergic volunteers performed in Europe,¹³ our study of tolerance to Me-PPD in PPD-allergic individuals resulted in identical results, with 70% of the PPD-allergic individuals exhibiting tolerance to Me-PPD under hair dye use-like conditions. These data are important because they demonstrate the reproducibility of a low rate of cross-elicitation in a PPD-allergic population and also support the utility and robust nature of the allergy alert test.^{12,13}

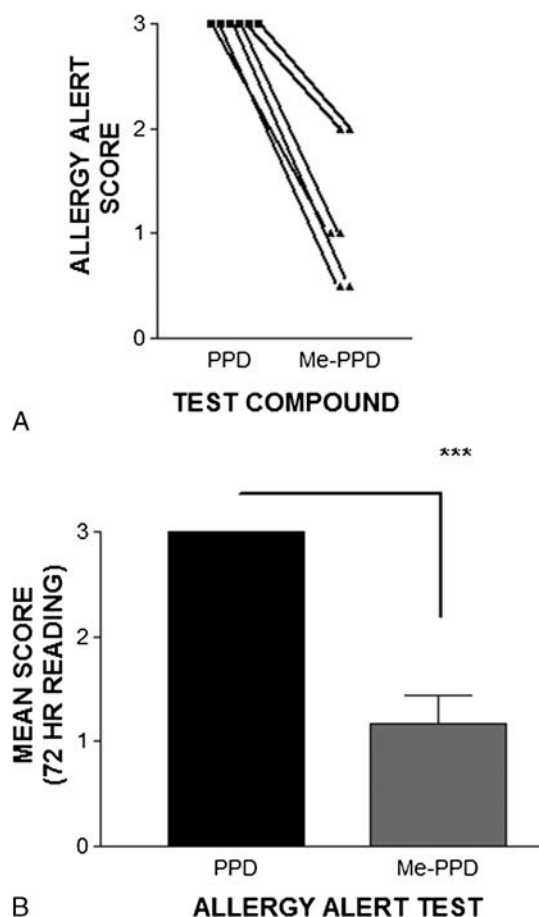


Figure 2. Contact dermatitis to Me-PPD was less severe than PPD using allergy alert test. A, Scatter plot, depicting strength of allergy alert test results to PPD and Me-PPD in 6 volunteers who were not tolerant of Me-PPD (ie, exhibited positive skin test reactivity (+/- to +3) to Me-PPD on allergy alert test. B, Mean allergy alert test reactivity of PPD was significantly stronger than that of Me-PPD (** $P < 0.001$, highly significant; paired Student *t* test).

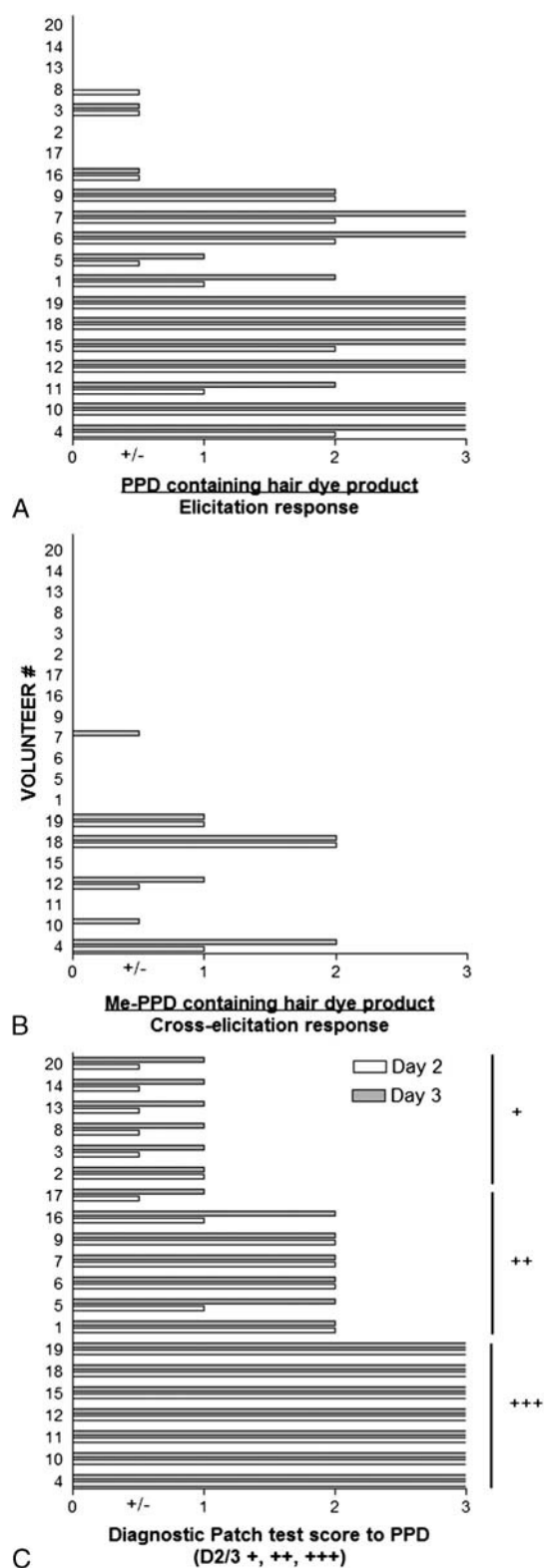


Figure 3. Detailed summary of patch test reactivity and reactivity to PPD and Me-PPD using allergy alert test. Skin reactivity (using ICDRG scoring system) to (A) PPD and (B) Me-PPD on allergy alert test (2% in hair dye product) correlated with the strength of the diagnostic patch test results to PPD (C). Details of test results at 48 and 72 hours in all 20 volunteers are clustered by the strength of their diagnostic patch test response to PPD. Histograms depict strength of skin reactivity scores from no reactivity (or negative) (0) and, for those with skin reactions, ranging from +/- reactions to +3 reactions.

Previous studies of the effect of skin color and ethnicity demonstrated that in dark-skinned subjects, there is a significantly higher rate of sensitization to PPD as detected by conventional patch testing (10%) compared with whites (8%).¹⁴ There are no studies that examine the effect of skin type and ethnicity on cross-elicitation in PPD-allergic individuals, so our study is the first of its kind to examine this critical issue. We found that patch test reactivity to PPD on diagnostic patch testing has a strong influence on cross-elicitation to Me-PPD (Figs. 1 and 3), which is identical to the results of the European cross-elicitation study in white patients (predominantly type I and II Fitzpatrick skin types).¹³ These data suggest that the immunobiology of PPD allergy and cross-elicitation to structurally related PPD derivatives such as Me-PPD is fundamentally the same in different skin types and ethnicities despite the higher rate of sensitization to PPD in patients of skin of color.¹⁴

The PPD-allergic individuals due to hair dye exposure (from hair coloring practices or, as observed in hair dressers, as occupational dermatitis) have been described, in some cases, to exhibit type I-like symptoms on exposure (rhinitis, wheezing, contact urticaria).²⁰ We therefore examined immediate reactivity to PPD, vehicle, and Me-PPD 30 minutes after the application of the allergy alert test. We did not observe contact urticaria in any of the 20 volunteers in response to PPD allergy alert or Me-PPD allergy alert, even in the subgroup of volunteers with +3 reactivity to PPD on diagnostic skin patch testing (data not shown). We cannot exclude the possibility that open application of either PPD or Me-PPD on intact skin using hair dyeing conditions (see “Methods” section) is not sufficiently sensitive to detect contact urticaria, and methods such as prick testing may be necessary to detect type I reactivity to PPD or Me-PPD.²¹

As noted in the European study, cross-elicitation to Me-PPD in PPD-allergic volunteers is strongly influenced by the strength of the allergic reaction to PPD on patch testing.¹³ We also noted that reactivity to the PPD allergy alert test was also strongly influenced by the strength of the allergic reaction to diagnostic patch testing to PPD because we observed reactivity to PPD allergy alert test in 100% of the 7 subjects with +3 reactivity to PPD on diagnostic patch testing; 85% of the 7 subjects with +2 reactivity to PPD on diagnostic skin testing; and only 33% of the 6 subjects with +1 reactivity to PPD on diagnostic skin testing. This is consistent with prior studies of the PPD allergy alert test,^{12,13} which is designed to detect moderate and severe reactions to the use of hair dye products containing PPD.

Lastly, because the exposure conditions to PPD and Me-PPD were identical (both at 2% and in the presence of a coupling agent and H₂O₂; see “Methods” section), it was possible to compare the strength of ACD reactions in those subjects who reacted to both PPD and Me-PPD (Fig. 2). In all subjects, ACD was stronger to PPD allergy alert testing than that of Me-PPD allergy alert testing. Quantitative patch testing with dose-response studies to PPD and Me-PPD in PPD-allergic individuals will be necessary to confirm these results and is currently ongoing (data not shown).

In summary, we demonstrate tolerance to Me-PPD in 70% of PPD-allergic patients when tested under hair dye use-like conditions.

Because ACD to PPD is T-lymphocyte mediated, it is likely that the mechanism for the cutaneous tolerance involves T-lymphocyte tolerance.^{22–25} This most likely involves altered recognition by PPD-specific T-lymphocytes that recognize self-hapten complex.^{26,27} That is, tolerance to Me-PPD may at least in part represent immunologic ignorance in which the T-cell antigen receptor in PPD-specific T-lymphocytes may not recognize Me-PPD because of steric hindrance. There is precedent for this idea because chemical modification of PPD by the addition of 2 methyl groups resulting in 2,5-dimethyl-PPD was not recognized by T-lymphocytes from PPD-allergic individuals.²⁸ In addition, only 1 of 16 PPD-allergic patients reacted to diacetylated PPD on patch,^{29,30} demonstrating that the chemical modification of the PPD molecule can result in T-lymphocyte tolerance in vitro (which is technically T-lymphocyte ignorance, or lack of recognition of a modified antigen) and in vivo (skin tolerance, or lack of patch test reactivity).^{31,32}

The Me-PPD may represent a safer alternative for those consumers and hair dressers who are PPD allergic. It remains to be determined whether the use of Me-PPD for hair dyeing in immunologically naïve subjects (those individuals who have never been exposed to PPD through hair dye practices or at work) will decrease the risk of sensitization to Me-PPD compared with the current risk associated with PPD. Based on the current preclinical data¹⁰ and studies of PPD-allergic volunteers using Me-PPD to dye their hair,¹⁹ it is reasonable to speculate this may occur. It is also of interest to directly compare the rate of cross-elicitation to Me-PPD and PTD in PPD-allergic subjects using allergy alert test because it is known that the rate of cross-elicitation to PTD in PPD-allergic subjects by standard patch testing is as high as 50%.⁷

The limitations of this study are its small sample size (N = 20) and lack of follow-up. It is also possible that we may have missed delayed reactions to PPD or Me-PPD because our readings of the allergy alert test were taken at 48 and 72 hours but not at 96 hours. Lastly, the long-term safety of Me-PPD remains to be determined because our study was a one-time exposure.

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