Actas Dermosifiliogr. 2011;**102(3)**:206-211

ACTAS
Dermo-Sifiliográficas

Full English text available at
www.elsevier.es/ad

ORIGINAL ARTICLE

Pattern of Contact Sensitization to Paraphenylenediamine and Its Detection in Hair Dyes

J.M. Fernández-Vozmediano,* M. Padilla-Moreno, J.C. Armario-Hita, C. Carranza-Romero

Servicio de Dermatología, Hospital Universitario de Puerto Real, Universidad de Cádiz, Cádiz, Spain

Manuscript received March 25, 2010; accepted for publication March 31, 2010

KEYWORDS
Paraphenylenediamine;
Allergen detection;
Hair dyes

Abstract
Background: One of the greatest challenges in occupational dermatology is the identification of chemical substances used by patients in their work in order to determine their allergenic potential. Numerous techniques have been described for the identification of allergenic compounds. These tests must be sensitive, specific, and safe. We describe a study to detect the presence of paraphenylenediamine (PPD) in hair dyes that are commercially available in Spain.
Material and methods: We undertook an experimental study involving qualitative and semiquantitative detection of PPD in hair dyes sold in Spain. The qualitative technique we used was a previously described colorimetric method involving dilution of the dye with isopropyl alcohol followed by addition of a reagent solution (1g of vanilla in 15ml of isopropyl alcohol and 7.5 ml of hydrochloric acid). A quantitative study was then done in which the dye was extracted in 96% ethanol and subjected to 1-dimensional thin-layer chromatography.
Results: A total of 15 brown and 12 blonde dyes were analyzed. PPD was identified in all of the brown dyes analyzed, irrespective of whether it was indicated (n=12) or not (n=3) in the composition. PPD was found in 6 of the 9 blonde dyes that indicated it in the composition and 2 of the 3 in which it was not indicated. Semiquantitative analysis by thin-layer chromatography revealed that the concentration of PPD in brown hair dyes (mean, 3%) was higher than in blonde dyes (mean, 0.1-0.3%).
Conclusions: The presence of PPD in hair dyes is related to the color of the dye. It is consistently present in darker dyes and at low levels in blonde dyes. This study highlights the clinical and epidemiological importance of identifying allergens in dermatology, particularly in occupational dermatology.

*Corresponding author.
E-mail address: fdezvozmediano@dermasur.com (J.M. Fernández-Vozmediano).

PALABRAS CLAVE
Parafenilendiamina;
Detección de alérgenos;
Tintes capilares

Patrón de sensibilización por contacto a parafenilendiamina y su detección en tintes capilares

Resumen

Introducción: Uno de los retos más difíciles en el campo de la Dermatología laboral es la identificación de las sustancias químicas con las que trabaja el enfermo para poder determinar su capacidad alergénica. Se han descrito múltiples técnicas para poder identificar las distintas sustancias alergénicas contenidas en la composición de las sustancias que maneja el enfermo. Estas pruebas de detección de alérgenos deben ser sensibles, específicas y seguras. Presentamos un estudio experimental cuyo objetivo es detectar la presencia de parafenilendiamina en tintes capilares comercializados en nuestro país.

Material y métodos: Hemos realizado un estudio experimental con el fin de realizar una detección cualitativa y semicuantitativa de parafenilendiamina en tintes capilares comercializados en nuestro país. Como técnica cualitativa utilizamos la técnica de identificación descrita consistente en la dilución del tinte con alcohol isopropílico y con posterioridad se añade la solución reactiva (1 g de vainilla en 15 ml de alcohol isopropílico y 7,5 ml de ácido clorhídrico). Esta prueba da una respuesta colorimétrica que indica la presencia o no del alérgeno en el tinte capilar. Con posterioridad hemos procedido a realizar un estudio cualitativo mediante la extracción del colorante con etanol 96º, seguido de una cromatografía en capa fina monodimensional.

Resultados: Se estudiaron un total de 15 tintes de color castaño o moreno y 12 tintes de color rubio. Este estudio nos permitió identificar la PPD en todos los tintes capilares morenos estudiados, con independencia de que su presencia estuviera indicada (n = 12) o no (n = 3) en la composición del tinte. Comprobamos la presencia de PPD en 6 de los 9 tintes rubios que indicaban la presencia de PPD en su composición y en 2 de los 3 que no la indicaban. La valoración semicuantitativa mediante cromatografía de capa fina nos permitió confirmar que la concentración de PPD utilizada en los tintes capilares de color moreno era superior (media del 3%) a la de los tintes rubios (media del 0,1-0,3%).

Conclusión: La presencia de PPD en tintes capilares está en relación con la coloración del tinte, siendo constante en los de color oscuro y de intensidad baja en los rubios. Este estudio pone de manifiesto la importancia clínica y epidemiológica que tiene la identificación de alérgenos en la Dermatología, y de forma más concreta, en el campo de la Dermatología laboral.

Introduction

Paraphenylenediamine (PPD) has a high capacity to induce contact sensitization. This agent has several applications, including as an ingredient of the coloring used in hair and fabric dyes, rubber, lacquers, leather, eye shadow, and shoe polish. It has also been used as an antioxidant in plastics, printing ink, fax machines, photographic products, and liquid for x-ray film, as well as in lithography.[1] The main contact allergen in hairdressing is PPD, followed by its derivatives para-aminodiphenylamine (PAD), o-nitro-p-phenylenediamine (ONPPD), and paratoluenediamine (PTD). Sensitization to PPD derivatives could be due to cross-reactions between them.[2] Given the high frequency of sensitizations, different methods have been designed to identify the presence of PPD and determine their relevance. Such methods, as in the detection of any allergen, must be sensitive, specific, and safe.

We present a study performed to ascertain the frequency and relevance of positive reactions to PPD and to define the characteristics of patients at risk of active sensitization. We then present the results of a qualitative and semiquantitative study of the presence of PPD in hair dyes.

Materials and Methods

Phase 1. Epidemiologic Study

We performed a retrospective 10-year epidemiologic study. During the study period, patch tests were performed using the standard series from the Spanish Contact Dermatitis and Skin Allergy Research Group (GEIDAC) (Table 1) on 1878 patients who attended the Skin Allergy Clinic of the Dermatology Department of Hospital Universitario de Puerto Real, Puerto Real, Spain with suspected allergic contact dermatitis. The allergens (Chemotechnique) were applied in a Finn chamber. Readings were taken at 48 and 96 hours.

Table 1 Allergens Included in the Standard Series

Lanolin alcohol, 30%

Peru balsam, 25%
Caine mix, 7%
Carba mix, 3%
Cobalt chloride, 1%
Colophony, 20%
Potassium dichromate, 0.5%
Epoxy resin, 1%
Ethylenediamine, 1%
Formaldehyde, 1%
Fragrance mix, 8%
Kathon CG, 0.02%
Mercapto mix, 1%
Mercaptobenzothiazole, 2%
Mercury, 0.5%
Neomycin sulfate, 20%
Nickel sulfate, 5%
Palladium dichloride, 1%
Paraben mix, 16%
p-Phenylenediamine, 1%
p-Phenylenediamine mix, 1%
p-tert-Butylphenol-formaldehyde resin, 1%

Quaternium-15, 1%
Quinoline mix, 16%
Thiomersal, 0.6%
Thiuram mix, 1%

Phase 2. Detection of Paraphenylenediamine in Hair Dyes

We performed an experimental study for qualitative and semiquantitative detection of PPD in hair dyes sold in Spain. We used 27 hair dyes (15 dark-colored [brown and black] and 12 blonde). The instruction sheet indicated the presence of PPD in 12 of the 15 dark-colored dyes and in 9 of the 12 blonde dyes. Twenty-six were widely used by the general public and are easily obtained in supermarkets; the advertising literature of the remaining dye explained that it was available from pharmacies, as evidence of its quality and harmlessness (Table 2).

Qualitative Method

We used a colorimetric assay to detect PPD. First, the study dye was diluted with isopropyl alcohol 1:1 (v/v) and a reagent solution (1 g of vanilla in 15 mL of isopropyl alcohol) added. Shortly before the analysis, 7.5 mL of concentrated hydrochloric acid was added. The control solution was prepared with 0.5 mg of PPD or its salts and 10 mL of NH_1OH. (As an additional quality-control step, a solution of PPD that has been processed in the same way can be used to compare the tone obtained with that observed with the tested product solution.) The study sample was then diluted with isopropyl alcohol 1:1 (v/v) and a drop of the dilution was placed on a filter paper. After 1 minute, a drop of the reagent solution was added (Figure 1). If the filter paper turned a brick-red color, PPD was present (Figure 2).

Table 2 List of Hair Dyes Used in the Experimental Study for Detection of Paraphenylenediamine

Dark	Blonde
1D - G. Belle Color	1B - G. Belle Color
2D - L. Recital	2B - L. Recital
3D - LL. Color Advance	3R - LL. Color Advance
4D - C. Rojo Fuego	4B - N. Rubio
5D - JFM. Negro	5B - JFM Castaño
6D - F. Negro	6B - F. Rubio
7D - N. Negro	7B - C. Eugene perma
8D - G. Ilumia	8B - G. Nutrisse
9D - L. Casting	9B - L. Excellence
10D - LL. Baño de color	
11D - G. Nutrisse	
12D - L. Excellence	
13D - C. Eugene perma	
Did not indicate PPD	
1D - S. Country	1B - G. Ilumia
2D - W. Kolesti	2B - W. Kolestin
3D - S. Brillance	3B - S. Brillance

Semiquantitative Method

We then performed a semiquantitative study by extracting the coloring agent with 96% ethanol, followed by 1-dimensional thin-layer chromatography to identify the coloring agent. Prior to chromatography, 3 g of homogenized study liquid or cream or various concentrations of PPD (control solution) were added to a tube containing 300 mg of ascorbic acid (the color changed). A few drops of ammonia along with 96% ethanol were added to each tube to make up a 10-mL solution. The tube was sealed and centrifuged at 4000 rpm for 10 minutes. The resulting solution was separated into 2 phases.

In the next stage of the process, 1 µL of each study solution was added to a silica chromatography plate. The solutions were taken from the supernatant and from each of the PPD concentrations used as controls and spotted at several points. The silica plate was placed in a

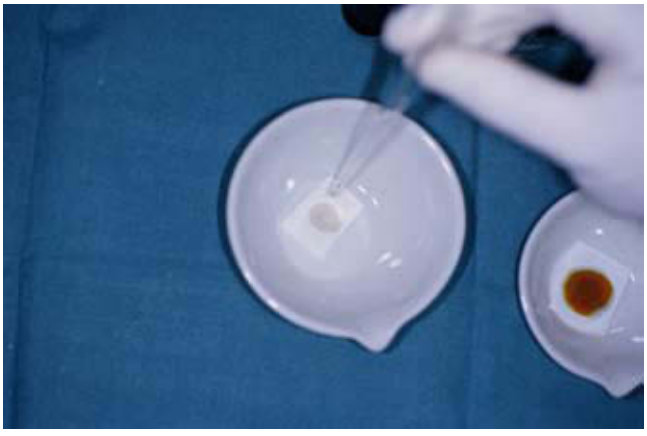

Figure 1 Qualitative method. Addition of the reagent solution to the study solution.

Figure 2 Qualitative method. Result of the study solution and comparison with the control. PPD indicates paraphenylenediamine.

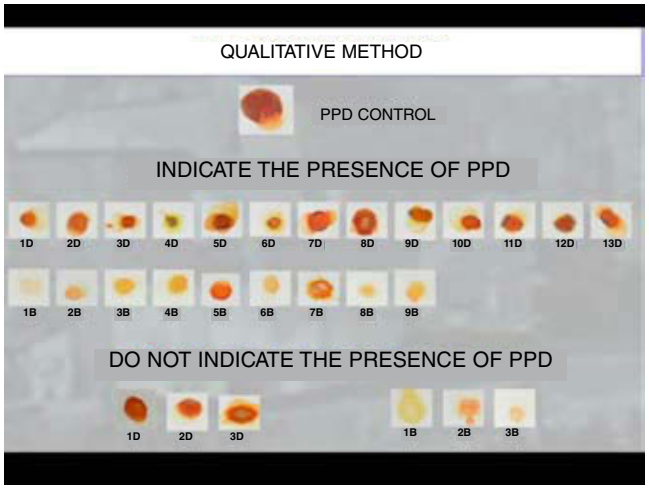

Figure 3 Results of the qualitative method for detection of paraphenylenediamine. PPD, indicates paraphenylenediamine.

recipient saturated with the appropriate solvent (acetone-chloroform-toluene [35:25:40]) and left to develop at room temperature in darkness until the solvent front advanced 15 cm. Finally, the plate was removed, dried, and sprayed with a developing fluid.

The allergens were identified and quantified by comparing the reference values and colors obtained for the sample with those of the reference substances used. Semiquantitative analysis involved visual comparison of the intensity of the substance in the chromatographic image with a reference range of known concentrations.

Results

Epidemiologic Study

Of the 1878 patients studied, 60.96% (1145) had at least 1 positive response. Of these, 50 (4.36%) were positive for PPD; we observed present relevance in 33 cases (66.6%). The PPD-positive group comprised 38 women (76%) and 12 men (24%), and age ranged between 17 and 81 years, with a mean (95% confidence interval [CI]) of 38.75 (4.48) years. The most common sites of the lesions were the hands (56%), followed, at some distance, by diffuse sites (21%) and the scalp (13%). The high frequency of lesions on the scalp could be associated with the use of hair dyes containing PPD. The allergens that most commonly presented positive results in the PPD-positive group were nickel sulfate (24%), cobalt chloride (9%), caine mix (9%), and palladium dichloride (8%); these were similar to those reported for the general population. The high percentage of positive results to Peru balsam (7%) and fragrance mix (7%) is noteworthy. Most of the patients studied were homemakers (44%). The most relevant occupations were hairdressing (14%) and hotel and catering (12%). We found a statistically significant association between a positive PPD test result and hairdressing ($P<.01$; odds ratio [OR], 8.49; 95% CI, 3.60-19.97).

Experimental Study

We studied 15 dark-colored dyes and 12 blonde dyes and identified PPD in all the dark-colored dyes, irrespective of whether its presence was indicated on the package insert (12) or not (3). We verified the presence of PPD in 6 of the 9 blonde dyes, which indicated the presence of PPD among its ingredients and in 2 of the 3 that did not (Figure 3). Semiquantitative evaluation using thin-layer chromatography confirmed that the concentration of PPD used in the dark-colored dyes was greater (0.5%-3.0%) than that used in the blonde dyes (0.1%-1.0%) (Figure 4).

Discussion

PPD is a lightly colored compound that acts as a primary intermediate in hair dyes. It is oxidized by hydrogen peroxide and is polymerized in air. PPD continues to

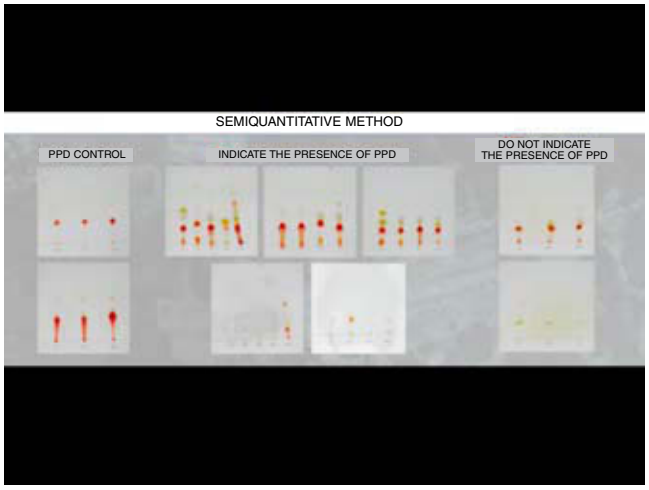

Figure 4 Results of the semiquantitative method for detection of paraphenylenediamine.

be a common and relevant allergen. The frequency of sensitization to PPD decreased in 1985 and 1988; however, these findings were due to the false negatives arising from the use of 0.5% PPD dihydrochloride in petroleum jelly in the test chamber.[3] Once this was replaced by 1% PPD in petroleum jelly, the positivity indexes for PPD were around 3.2%,[4] although a recent study from the North American Contact Dermatitis Group showed them to be 6.8%,[5] and in other countries they were as much as 11.5%.[6] In our series, 4.36% of patients with a positive response had positive reactions to PPD. A larger number of positive reactions to PPD have been observed with the Finn chamber than with the True Test panel.[7] There have also been reports, although less frequent, of contact urticaria,[8] contact leukoderma,[9] and lichenoid eruptions[10] caused by PPD.

Most cases of sensitization to PPD are due to contact with hair dyes, both among hairdressers and the general public. Our study confirmed this observation. A study performed to evaluate the frequency of positive results to PPD and its derivatives revealed that 30 of 66 hairdressers (45.4%) were sensitized to PPD.[11] The frequency of sensitization to PPD derivatives was somewhat lower, around 7.2% to PTD, 4.5% to PAD, and 3% to ONPPD. No cross-reactions with PPD have yet been observed with a commercially available mousse formulation containing coloring agents (Arianor Dyes, Williams Co., United Kingdom).[12] Occupational contact sensitization among hairdressers can also be produced by nickel sulfate, preservatives (methylisothiazolinone, formaldehyde), surfactants (cocamidopropyl betaine, hydrolyzed animal proteins), and ingredients of perfume.[13]

Among hairdressers, sensitization occurs in the early stages of their working life, forcing most to leave the profession. The factors favoring a reaction include previous irritant dermatitis of the hands caused by detergents, lotions, and moisture. In fact, irritant dermatitis is the most common occupational skin disease in hairdressing.[14] On the other hand, stylists or professionals working with hair dyes are less likely to be sensitized to hair dyes: skin irritation seems to be less marked, as they do not perform other tasks such as hairwashing.[15] Atopic dermatitis does not favor sensitization, and, under the same conditions of exposure, sensitization to PPD is similar in atopic and nonatopic patients.[16]

Few studies have analyzed the presence of PPD in hair dyes, and most that have employed complex quantitative detection systems based on liquid chromatography[17] or on gas chromatography–mass spectrometry.[18] PPD has also been detected in biologic fluids using ion analysis, although these systems are limited to the field of forensic medicine.[19] In our experience, the qualitative and quantitative methods based on thin-layer liquid chromatography we used in the present study are simple and easy to apply in daily practice, providing useful results that enable us to confirm the relevance of patch testing.[20]

These tests allowed us to demonstrate the presence of PPD in hair dyes, which is associated with the intensity of the color of the dye. The presence of PPD was constant in the dark-colored dyes, yet much scarcer in the blonde dyes. Not all the packages indicate the presence of PPD; however, we observed that all the dark-colored dyes and some of the blonde dyes did provide this information. In blonde dyes, we detected PPD in a dye that did not indicate its presence (Wella) and in 2 that did (Color Cream and Just for Men Brown).

Conclusions

PPD continues to be a common allergen in Spain; therefore, it should remain in the standard series. The typical patient is a woman with hand eczema who works as a hairdresser or is a frequent client of hairdressers and who is allergic to hair dyes. We found a statistically significant association between hairdressing and sensitization to PPD ($P<.01$; OR, 8.49; 95% CI, 3.60-19.97).

The present study reveals the clinical and epidemiological relevance of identifying allergens in dermatology, especially in occupational dermatology. International laws should strictly regulate the information provided with cosmetic products.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Liden C, Brehmer-Andersson E. Occupational dermatoses from colour developing agents. Acta Derm Venereol. 1988;68: 514-22.
2. MacKie BS, Mackie LE. Cross sensitization in dermatitis due to hair dyes. Aust J Dermatol. 1964;7:189.
3. Dooms-Goosens A, Scheper RJ, Andersen KE. Comparative patch testing with PPD-base and PPD-dihydrochloride: human and animal data compiled by the European Environmental and Contact Dermatitis Research Group. In: Frosch PJ, Dooms-Goosens A, Lachappelle JM., Rycroft RJG, Sheper RJ, editors. Current topics in contact dermatitis. Berlin: Springer; 1989. p. 281-5.
4. Armstrong DKB, Jones AB, Smith HR, Ross JS, White IR, Rycroft RJG, et al. Occupational sensitization to p-phenylenediamine: a 17-year review. Contact Dermatitis. 1999;41:348-9.
5. Marks JG, Belsito DV, DeLeo VA, Fowler JF, Fransway AF, Maibach HI, et al. North American Contact Dermatitis Group patch test results for the detection of delayed-type hypersensitivity to topical allergens. J Am Acad Dermatol. 1998;38:911-8.
6. Sharma VK, Chakrabarti A. Common contact sensitizers in Chandigarh, India. A study of 200 patients with the European standard series. Contact Dermatitis. 1998;38:127-31.
7. Goh CL. Comparative study of TRUE test and Finn Chamber patch test techniques in Singapore. Contact Dermatitis. 1992;27:84-9.
8. Edwards EK, Edwards EK. Contact urticaria and allergic contact dermatitis caused by paraphenylenediamine. Cutis. 1984;34: 87-8.
9. Taylor JS, Maibach HI, Fisher A, Bergfeld WF. Contact leukoderma associated with the use of hair colors. Cutis. 1993;52:273-80.
10. Sharma VK, Mandal SK, Sethuraman G, Bakshi NA. Paraphenylenediamine-induced lichenoid eruptions. Contact Dermatitis. 1999;41:40-1.

11. Lynde CW, Mitchell JC. Patch test results in 66 hairdressers 1973-1981. Contact Dermatitis. 1982;8:302-7.
12. Maibach HI. Paraphenylenediamine-free hair coloring mousse. Contact Dermatitis. 1986;15:103.
13. Frosch PJ, Burrows D, Camarasa JG, Dooms-Goossens A, Ducombs G, Lahti A, et al. Allergic reactions to a hairdressers' series: results from 9 European centres. Contact Dermatitis. 1993;28:180-3.
14. Borelli S, Moormann J, Dungemann H, Manok M. Ergebnisse einer vierjährigen Untersuchungsreihe bei Berufsanfangern des Friseurgewerbes. Berufsdermatosen. 1965;13:216-38.
15. Nethercott JR, MacPherson M, Choi BCK, Nixon P. Contact dermatitis in hairdressers. Contact Dermatitis. 1986;14:73-9.
16. Sutthipisal N, McFadden JP, Cronin E. Sensitization in atopic and non-atopic hairdressers with hand eczema. Contact Dermatitis. 1993;29:206-9.
17. Andrisano V, Cavrini V, Summer P, Passuti S. Determination of impurities in oxidation hair dyes as raw materials by liquid chromatography (HPLC). Int J Cosmet Sci. 1995;17: 53-60.
18. Di Gioia ML, Leggio A, Le Pera A, Liguori A, Napoli A, Perri F, et al. Determination by gas chromatography/mass spectrometry of p-phenylenediamine in hair dyes after conversion to an imine derivative. J Chromatogr A. 2005;25:143-8.
19. Stambouli A, Bellimam MA, El Karni N, Bouayoun T, El Bouri A. Optimization of an analytical method for detecting paraphenylendiamine (PPD) by GC/MS-iontrap in biological liquids. Forensic Sci Int. 2004;146:S87-92.
20. Koh D, Tan C, Ng SK, Tan BT, Leow YH, Goh CL. Screening for p-phenylenediamine (PPD) in hair-care products by thin layer chromatography (TCL). Contact Dermatitis. 2000;43: 182-3.