Allergic Contact Dermatitis to p-phenylenediamine and Some of its Reaction Products

E. Young*, E. Zimerson, C. Svedman, M. Bruze

Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, Malmö, Sweden

*Corresponding author: Dr. Ewa Young Department of Occupational and Environmental Dermatology Skåne University Hospital 205 02 Malmö, Sweden, Tel: +46-40-336516; Fax: +46-40-331192; Email: ewa.young@med.lu.se

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Abstract

Background
Contact allergy to hair dyes is prevalent among hairdressers and their consumers. The potent sensitizer p-phenylenediamine (PPD) is a common colour substance in oxidizing permanent hair dyes. The complex hair dye cocktail consists of many ingredients. When the hair dye is used oxidative agents are added and PPD can be transformed into new substances. The substances, being formed and applied to the hair, when PPD is used in hair dyes, are partly unknown.

Objectives
The aim of this study was to investigate contact allergic responses to oxidized substances of PPD and some of its reaction products in PPD-positive patients.

Methods
The methods used were high-performance liquid chromatography (HPLC), patch testing, thin layer chromatography (TLC) and patch testing with TLC strips. Purity analysis were made of PPD and its trimer Bandrowski’s base (BB) using HPLC. 14 patients, previously tested PPD-positive, participated in the investigation. They were patch tested with dilution series of PPD and BB, as well as with TLC strips of oxidized PPD. Reaction patterns were compared.

Results
Of the 14 previously PPD-positive patients, 13 were repeatedly PPD-positive. 7/13 (54 %) reacted to BB. On the TLC strips, oxidized PPD was divided into 4 visible spots. The 7 BB-positive patients reacted to one or more of the TLC spots in various patterns.

Conclusion
The results indicate the presence of several possible sensitizers formed during oxidation of PPD and that PPD-sensitized patients might react to these substances in various patterns.

Keywords: Bandrowski’s Base; Contact Allergy; Hair Dye; Oxidation Products; P-Phenylenediamine; Ppd; Thin Layer Chromatography
### Introduction

Contact allergy to oxidative permanent hair dyes is common among hairdressers and their consumers [1,2]. Exposure to hair dye products means exposure to a complex mixture of substances that apart from the actual dye substances also includes perfumes, surface active agents and many other functional additives. The potent sensitizer p-phenylenediamine (PPD, Figure 1) is still a standard colour substance in oxidative hair dyes [3-5]. The hairdressers and the consumers are also exposed to a cocktail of other irritating substances or contact allergens in hair dyes [6,7]. Due to the strong sensitizing capacity especially of PPD and other similar colour substances [3], efforts are made to find substitutes with a less allergenic potency [8,9].

<table>
<thead>
<tr>
<th>Substance</th>
<th>CAS no.</th>
<th>Molecular Weight</th>
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<tbody>
<tr>
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<td>108</td>
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<tr>
<td>Bandrowski’s base</td>
<td>200-48-27-5</td>
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</table>

**Figure 1.** Chemical structures, Chemical Abstracts Services (CAS) numbers and molecular weights of p-phenylenediamine (PPD), and Bandrowski’s base (BB).

PPD was introduced as a marker for hair dye allergy in 1939, and it still remains the clinically most relevant one [10]. Most hair dye allergic consumers and hairdressers have been exposed to, and developed contact allergy to PPD. Some of these might have been sensitized to another similar substance causing cross reactivity to PPD. The mechanisms of induction and elicitation of contact allergy by PPD are not well understood. PPD can be transformed into new substances by processes such as oxidation, metabolism and reaction with other chemicals [11,12] and these substances might be important in connection to hair dye and PPD allergy.

An oxidative hair dye contains 3 main functional components, a precursor, a coupler and an oxidizing agent. The colour crème, containing a precursor and one or more couplers, is mixed with the oxidizing agent just before application to the hair. The solution of the oxidizing agent, is often called the developer, and contains hydrogen peroxide. After mixing of a PPD based oxidative hair dye PPD oxidation products are formed, which then can react with the coupler(s) creating the desired color. Certain couplers and oxidation products of PPD have been suggested to be involved in hair dye and PPD-related contact allergies [13]. One substance that can be formed by oxidizing PPD (in the absence of a coupler) is the trimer Bandrowski’s base (BB; N,N’-bis(4-aminophenyl)-2,5-diamino-1,4-quinonediimine, Figure 1). BB has earlier been pointed out as one substance possibly responsible for PPD-related contact allergy [14-16].

In vitro studies have been used to investigate some allergens formed in hair dyeing with PPD [17]. Among animal study models, the guinea pig maximisation test is used for investigations of cross reactivity [18]. However, patch testing in men is the best method for detecting allergies to new substances that have sensitized exposed individuals. New allergens among the oxidation products of PPD may be established by patch testing in patients allergic to PPD. It is then necessary to separate the substances found in the mixed hair dye. By using thin layer chromatography (TLC), complex mixtures of substances can often be separated. The more substances, the more complicated the separation.

When a mixture of substances is separated on a thin layer chromatogram, the substances move according to their physical and chemical properties. The different substances can be visualised, as distinct spots on the chromatogram. We wanted to find a way to look at the possible allergens formed when PPD is used in hair dyes. Since, PPD in hair dyes is used with couplers and hydrogen peroxide, the most clinically relevant way would be to use the thin layer chromatogram formed when all the three substances are mixed. However, in this study we chose to look at the oxidation products formed in the mixture of PPD and hydrogen peroxide. This makes it easy to study the oxidation products formed as they are formed in higher concentrations and are easier to identify in this less complex mixture. It is relevant to study the mixture of PPD and hydrogen peroxide, because the development of the same oxidation products also could occur in a complete hair dye.

Furthermore, individuals may be exposed to high concentrations of PPD in temporary tattoos, so called black henna tattoos. No couplers are added in black henna tattoos. The dark shade in these tattoos is due to a mixture of PPD oxidation products, possibly formed by auto-oxidation due to air contact. Some non-European hair dye products in powder form contain henna, PPD and an oxidizing agent also in powder form (i.e barium peroxide), but no coupler [19]. It is highly likely that products for black henna tattoos and the henna hair dyes with an oxidizer will expose the user to relatively high doses of PPD oxidation products.

When patch testing with PPD, the test leaves a dark spot on the
skin due to oxidation products formed from contact with air oxygen. Due to this oxidation process patch tests with PPD may also contain PPD oxidation products.

The aim of the present study was to investigate the importance of oxidation products of PPD and the role of BB in PPD-related contact allergy. The oxidation products have been separated using TLC and TLC strips of oxidized PPD have been patch tested in PPD sensitized patients.

Materials and Methods

Chemicals and test material

The following chemicals were used: Acetone (≥99.5%, Scharlau Chemie SA, Sentmenat, Spain and 99.9%, VWR, Fontenay-sous-Bois, France), BB (ICN Biomedicals Inc, Aurora, Ohio, USA), n-butylamine (Acros, Fisher Scientific, Gothenburg, Sweden), distilled water (Millipore, Q-Guard 1, Molsheim, France), ethanol (Kemetyl, Haninge, Sweden), n-heptane (VWR, Fontenay-sous-Bois, France), hydrogen peroxide (H₂O₂, 30 % w/w, Merck KGaA, Darmstadt, Germany), petrolatum (pet, Vaselinum album, Snow White Quality E from Apotheke Produktion & Laboratorier, Göteborg, Sweden) and PPD (>99%, Sigma-Aldrich, St Louis, MO, USA). Silica gel plastic roll (TLC plastic roll 500 x 20cm with silica gel 60F254, Merck KGaA), Finn chambers, diameter: 8 mm (Epitest OY, Tuusula, Finland) on Scanpor tape (Norgeplaster A/s, Vennesla, Norway).

Patch test preparation

Dilutions of PPD and BB were prepared. The BB dilutions were equimolar to the PPD concentrations. A stock solution of 1.0 % w/v PPD in acetone was prepared and further diluted into the test concentrations (Table 1). A stock solution of 0.29 % w/v BB in acetone was prepared and further diluted into the lower test concentrations. Being non-soluble in acetone 2.9 %, w/w BB was mixed with petrolatum (Table 1). The dilution series were stored in a refrigerated environment for a maximum of one week. New stocks were prepared several times during the test period.

Table 1. Results from patch testing with p-phenylenediamine and Bandrowski’s base, as well as testing with thin-layer chromatogram strips from oxidized p-phenylenediamine.

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* = vehicle is acetone and concentration % w/v except for 2.9% w/w of Bandrowski’s base prepared in petrolatum; # = test reaction stronger day 7 than day 3/4. The strongest reaction is noted in the table.

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The TLC sheets were inspected in daylight and under UV radiation at 254 and 365 nm. All detectable spots were marked with a pencil. The spots were numbered from 1 to 4, where number 1 indicates the site of application. The TLC sheets were cut into strips about 2 x 16 cm, with a band of spots on each (Figure 3).

Figure 3. Examples of test reactions on day 3 to thin layer chromatograms of oxidized p-phenylenediamine 5 µl in two test patients. Left patient 10 and right patient 13.

Patch testing

The dilution series of PPD and BB, were applied on the backs of the patients with Finn chambers, diameter: 8 mm. 15 µl of each dilution was used [24] except for 2.9% BB, which was patch tested in petrolatum, 20 mg [25]. Patch tests were removed after 2 days, D2.

Patch tests were evaluated and scored after 3 or 4 days (D3/4) and after one week (D7). The tests were considered positive if there was at least a + reaction registered, according to the criteria of the International Contact Dermatitis Research group (ICDRG), corresponding to the location of the Finn Chamber [26]. The highest initial test concentration of PPD was 0.1 %. The most PPD sensitized patients had their highest test concentration of PPD lowered to 0.01 %. If a patient presented with negative reaction on D3/4 the higher concentrations up to PPD 1.0 % were tested. Thus these reactions were only read once, on D3/4 after patch test application (Table 1).
The site of contact to the spots on the TLC strip, were marked out on the patients’ back as well as the edges of the TLC strip. The chromatograms were applied with Scanpor tape and removed on D2. Initially, the patients were tested with TLC strips with 5 µl and 25 µl of an oxidized PPD solution. A patient who presented with a negative reaction to these TLC strips on D3/4 was tested with a 50 µl TLC strip which was read on D3/4 only.

The composition of the individual thin layer chromatogram must be taken into account when reading patch test reactions to TLC strips. The amount of possible different allergens, and their distribution area, are unknown. The whole skin area that has been covered with the TLC sheet and where the substances have eluted will be evaluated. This means that an area where there is no visible spot can be positive. The reason can be that the chemical is not detectable in the light used. The chemical, which the patient reacts to, might move too slowly through the silica gel and leave a track of molecules behind that a patient with high reactivity can respond to.

The TLC strips are prepared in duplicates and a copy of the TLC strip serves as test protocol. Possible reactions can be correlated to the exact site of the TLC strip, which can then be used for identification of the correct spot. Infiltration and redness in the area corresponding to where the substances have been eluted will be regarded a one plus reaction. If the reaction also has papules, it will be regarded a two plus reaction. If there are intense redness, papules and even vesicles it will be regarded a three plus reaction.

Patients

Fourteen female patients, mean age 53 years (range 20-77), were included in the study. They were previously tested with the baseline series at the Occupational and Environmental Dermatology Department in Malmö, because of eczema and suspected contact allergy, and found positive to PPD in the past 10 years. PPD allergy was due to professional exposure (hairdressers) or exposure as consumer of hair dye and/or black henna tattoo.

Controls

15 consecutively patch tested dermatitis patients negative to PPD, or other hair dye allergens, were patch tested with BB 0.29% w/v in acetone and served as controls.

Statistics

For comparison of test patients and controls the two sided Fisher’s Exact Test was used. A p-value < 0.05 was considered to be significant. Data analysis was performed with the statistical software SPSS version 22.

Chemical analysis

The purity of the raw material of BB and PPD used for patch testing was investigated by high-performance liquid chromatography (HPLC). The HPLC system consisted of a P4000 quaternary pump, an UV 6000 diode array detector and an AS3000 autosampler (Thermo Finnigan, San José, CA, USA). The system was software-controlled and monitored by Chromelone 7 (Thermo Scientific Inc, Waltham, MA, U.S.A.). The Hypersil column (4.6 mm i.d. x 250 mm) (Thermo Scientific) was packed with 120 Å, 5 µm silica. The detector scanned the eluent in the range 190 to 400 nm and chromatograms recorded at 254 nm were used for detection and measurements. The injection volume was 20 µl and the flow rate 1.0 ml/min. Elution was isocratic with a mobile phase consisting of ethanol:heptan:water: n-butylamine 25:74.475:0.5:0.025 % (v/v) which was prepared by mixing 250 ml ethanol, 5 ml water, 25 µl n-butylamine then adding heptane up to 1000 ml. Freshly prepared ethanol samples of PPD and BB in 0.10% and 0.04%, respectively, were analyzed with regard to contamination of each other in the raw material.

Ethics

This study was approved by the Regional Ethical Review Board, Lund, Sweden (2007, No. 327/2007) and conducted in accordance with the ethical standards specified in the Declaration of Helsinki. Informed written consent was obtained from all subjects.

Results

Analytical results

HPLC analysis showed <0.2% BB in the PPD and 0.3% PPD in the BB used.

Patch test results of dilution series

The results are summarized in Table 1. Thirteen, out of 14 previously PPD-positive patients, reacted to PPD in at least 1 concentration and 7 reacted to BB in at least 1 concentration. Reactions to PPD were to concentrations in the range 1.0-0.0010 % w/v and to BB in the range 2.9-0.029%. Of those patients who reacted to both PPD and BB, 6 patients had reactions to 0.10 % PPD or lower and one patient to PPD 1.0%. Those who did not react to BB reacted to PPD 1.0 % only and one of those patients did not react to PPD at all (6 of 6 versus 1 of 8; p=0.0047, Fisher’s exact test, two-sided).

Among the test patients 5/14 reacted to BB 0.29% or lower and none of the 15 controls reacted to BB 0.29% (5/14 versus 0/15; p = 0.017).
The results are summarized in Table 1 and Figure 2. Figure 2 shows the reactions to the spots of the TLC strips with all three volumes (5, 25 and 50 µl) used of oxidized PPD. In Table 1 the reactions are shown separately. Seven patients reacted to one or more of the TLC spots. In total, of the 13 patients who were PPD-positive on patch testing with dilution series, 7/13 (54%) had a positive reaction to BB and the same 7/13 (54%) had a positive reaction to one or more of the TLC spots. Eight patients, the ones that did not have a reaction to the thin layer chromatograms with 5 µl or 25 µl on D3/4 were also tested with thin layer chromatograms of 50 µl. Patient number 9 was only tested with thin layer chromatogram with 5 µl because of her high reactivity.

Of the seven patients positive to the thin layer chromatogram of oxidized PPD, 6 reacted to spot number 4, 2 reacted to spot number 3, 5 reacted to spot number 2 and 1 reacted to the spot of application, spot number 1. They reacted to the 4 spots in different combinations. One reacted to all 4 spots. (Table 1 and Figure 3). Figure 3 shows an overview of the different reactivity patterns. Figure 4 shows two examples of the patients’ reactions to 5 µl oxidized PPD.

**Figure 4.** Thin layer chromatogram. From the left BB 0.29 % in acetone, PPD 0.1 % in acetone and oxidized PPD.

**Discussion**

The marketed oxidative hair dye products may contain various combinations of more than 100 different precursors and couplers [3]. When ingredients in oxidative hair dyes on the Swedish market were examined, as many as 98% were found to contain potent skin sensitizers, but only 16% were found to contain PPD, according to the labelling [4]. Nevertheless, PPD appears to be a good marker for hair dye contact allergy identifying the majority of positive reactions to other known hair dye allergens [10]. When PPD is patch tested it is applied to the skin without the presence of the many ingredients in a hair dye. Such ingredients in the cream and developer of the typical oxidative hair dye, are greases, couplers and precursors.

The aim of epicutaneous testing is to provoke the patient’s skin with the substances that they have been previously exposed to, and to elicit a contact allergic reaction if the patient is sensitized. When an individual has a positive reaction to a substance and a simultaneous reaction to another tested chemical, this might be due to concomitant sensitization to multiple allergens. Besides, it can be due to so called cross reactivity. Cross reactivity is when an individual by getting sensitized to a substance also acquires immunological reactivity to another substance. These two substances might be similar in structure and thus not distinguished by the immune system or they can form the same hapten after metabolism. Cross reactivity to PPD might be an explanation to why PPD serves well as a marker for hair dye allergy, but little is known about this cross reaction pattern.

Investigating the reactions to substances formed in permanent oxidative hair dyes, is of importance for developing preventive measures, such as recommendations for protective gloves for handling of hair dyes. In the present study we explore the partly unknown allergens which are formed, when the dye and developer have been mixed, and applied on the hair. To approach this, a simplified oxidized PPD model was developed, in order to investigate a part of the hair-dyeing process.

The oxidized PPD model used, enables study of possible contact allergic responses to the substances formed in the hair dye by the reaction between PPD and hydrogen peroxide. Patch testing with the oxidized PPD solution separated on thin layer chromatograms allows to differentiate between separate reactions to several substances, as opposed to testing with the solution as such. Moreover, the oxidized PPD used in the present study is closer to real life exposure, than patch testing with unoxidized PPD. Although, simplified in comparison to a hair dye it gives relevant findings in the investigation of PPD contact allergy.

As shown by the results, the PPD-sensitized patients in the present study react in different patterns to the spots, suggesting the presence of several allergens. The 4 TLC spots may
consist of one or several substances each. Thus the patients may theoretically test positive to a spot that contains one or more possible sensitizing compounds, i.e. oxidation products and other derivatives of PPD. Also the presence of sensitizing contaminants must be considered.

The test patients were also patch tested with BB, a trimer of PPD formed through oxidation in the absence of hair dye couplers [14,15]. In a previous study where 43 PPD-positive patients were patch-tested with BB, seven (16%) were positive to either 0.1% or 1.0 % BB [15]. These results show a lower prevalence compared to the present study, where 6 patients of 14 (43%) reacted to BB 0.29% or lower.

We chose to test BB in equimolar concentrations to PPD, hence the highest concentration tested was 2.9%, equimolar to 1% PPD. In total 7 (50%) individuals hypersensitive to PPD tested positive to BB. Figure 4 shows a thin layer chromatogram where BB, PPD and oxidized PPD are eluted for comparison. This chromatogram is eluted in the same system and for the same time as oxidized PPD. The main BB spot is close in colour, brown, and location, although slightly above, spot number 2 of oxidized PPD (Figure 4). The main spot of PPD is also similar in colour and slightly lower than spot number 2. The proximity might imply that PPD may contain BB and vice versa.

The association between the patients’ reactions to BB and their reactions to the thin layer chromatograms also indicates the possibility of impurities of BB in PPD and the opposite. It could also indicate cross reactivity between the two substances. The analytical HPLC analysis showed, however, only <0.2% BB in the freshly prepared PPD solution and 0.3% PPD in the BB used. It is unlikely that the contamination demonstrated has any significance for the patch test results to PPD and BB. The concentration of BB in PPD may increase depending on time, solvent and temperature.

Figure 5. Diagram showing relationship between positive concentration to PPD and positive reaction to BB in any concentration tested. On the y-axis the percentage of patients positive to BB is shown and on the x-axis the patients are grouped according to the lowest positive PPD concentration (%).

The relationship between positive reaction to PPD and positive reaction to BB is shown in figure 5. 1/7 patients who tested negative to PPD or positive to only 1.0 % PPD was positive to BB. 3/4 patients who tested positive down to 0.1% PPD were positive to BB and 3/3 patients who tested positive to 0.01% PPD or lower were positive to BB. These figures suggest the possibility of cross reactivity between the two substances. Observing the patterns of test responses to the tested TLC strips does not only provide us with valuable clues about what happens to PPD in a mixed hair dye cream. It does give information about the unoxidized PPD in patch tests on the skin, where it is oxidized by air oxygen and where it may be metabolized on the skin by bacteria [27] and furthermore in the skin [11,28]. Additionally, after skin penetration, metabolism of PPD may take place within the rest of the body [28].

There may thus be several reasons for the different reaction patterns found when patch-testing with TLC strips. Differences in reactivity to PPD, and the other involved allergens, can partly be explained by individual differences in skin metabolism [29,30]. These differences might explain: why some PPD-sensitized test patients had negative reactions to the TLC strips, others reacted to one or two TLC spots, one previously PPD-positive patient tested negative and why the patient with the strongest reactions to PPD reacted to all 4 TLC spots. The concentrations and amounts of the allergens present on the thin layer chromatogram are not known. More knowledge will be gained when the substances found in the spots are identified and the patients tested with defined doses of these.

Conclusion

The present study demonstrates the presence of multiple PPD associated allergens. This was done by in vivo-patch testing of PPD-sensitized patients, with an oxidized PPD solution, consisting of PPD and hydrogen peroxide, separated with TLC. Additional studies are needed to identify these allergens. To verify their role as contact allergens by patch test, and to further study the complex hair dye process regarding contact allergens.

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Conflict of interest

None declared.

References


23. Malinauskiene L, Zimerson E, Bruze M, Ryberg K, Isaksson M. Textile dyes Disperse Orange 1 and Yellow 3 contain more than one allergen as shown by patch testing with thin-layer chromatograms. Contact Dermatitis 2009;60:270-278.

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