Kontaktallergie durch Haarfarben am Beispiel von PPD: Die Rolle von Oxidation, Hautmetabolismus und Exposition für die Induktion einer Immunantwort

(Contact allergy to hair dyes using PPD as example: The role of oxidation, skin metabolism and exposure for the induction of an immune response)

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Commonly used aromatic amine hair dye precursors

Primary Intermediates

- p-phenylenediamine (PPD)
- 2,5-diaminotoluene
- p-aminophenol
- 4-amino-m-cresol

Couplers

- 4-amino-2-hydroxytoluene (AHT)
- m-aminophenol
- Resorcinol
How can a chemical induce contact allergy?

**Initial Contact**
- Foreign molecule
- No symptoms

**Repeated contact** (Elicitation)
- Sensitization
- Redness
- skin rush
- urticaria

**Contact allergy**
Events occurring during Skin Sensitization

Induction

- Oxidation
- Penetration
- Metabolism/Protein binding
- DC activation

Elicitation

- Haptenization
- GP Tests, HRIPT
- LLNA

1° T reaction
What happens to PPD on the skin under assessment conditions of sensitization/elicitation tests? (e.g. LLNA, HRIPT, confirmatory clinical patch test)

To note: above conditions are different from use conditions of hair dyeing
Oxidation of PPD by strong oxidizers

PPD after 30 min ferrocyanide exposure

Top: potential monomeric species corresponding to benzoquinone diimine; Middle: dimeric species; Bottom: trimeric species; peak at 8.67 min corresponds to BB.
Oxidation of PPD by air oxygen

Typical spectrum of PPD auto-oxidation products
Auto-oxidation products of PPD

- Fresh PPD
- Oxidation
- Dimers/Trimers
- Bandrowski's Base
- Oxidized PPD
- BB

Auto-oxidation products of PPD include dimers and trimers of PPD, which are oxidized forms of the molecule. Bandrowski's Base is a product formed through this oxidation process, and it is denoted as BB.
Events occurring during Skin Sensitization

Induction

Haptenization
- Oxidation
- Penetration
- Metabolism/Protein binding
- DC activation
- 1° T reaction

Elicitation

GP Tests, HRIPT
- LLNA
- DC activation

Haptentization
What happens to PPD when arrived in the skin, i.e. epidermis?

Metabolism
Dermal N-acetylation of PPD by keratinocytes (HaCaT) \textit{in vitro}

keratinocyte cell line (HaCaT)

Detection of metablite(s)/parent by HPLC/MS

0 - 24 h

Detection of metablite(s)/parent by HPLC/MS

![Graph](image-url)

PPD (µM)

µM recovered

Millions

cells / ml

Monoacetyl-PPD

Diacetyl-PPD

cells t=0
Major metabolic pathway in the skin is $N$-acetylation:

Human skin ex vivo results:

PPD  Mono-Acetyl-  Di-Acetyl-

Fig. 1. Representative example of kinetic analysis of PPD acetylation in human skin cytosol (■). Time-dependent formation of MAPPD (▲) and DAPPD (●) was measured in the presence of 400 μM PPD as substrate. DAPPD was detected after a 45-min incubation.

$N$-acetylated metabolites of PPD

$N$-acetylation by $NAT-1$

- Fresh PPD

= mono-Acetyl-PPD

= Di-Acetyl-PPD

= Acetylated PPD
**Epidermal N-acetylation of AHT (coupler)**

**HaCaT in vitro**

![Graph showing acetylated form vs. µg substrate/ml](image)

**Human skin ex vivo**

![Bar graph showing µg/cm² in stratum corneum, epidermis, and dermis](image)

**Rat in vivo**

![Bar graph showing % urine metabolites](image)

NAT1

$\text{OH} \quad \text{H}_2\text{N}$

$\text{OH} \quad \text{H}_2\text{N}$
ADME study with AHT in the rat: Metabolism data: Urine

Radioactivity chromatograms after analysis of undiluted urine pools of group 1 (A) i.v., 2 (B) oral low, 3 (C) oral high, 4 (D) dermal low and 5 (E) dermal high (A, B, D= 12.5; C= 500; E = 37.5 mg/kg bw )
N-acetylation of AHT: A dermal first pass effect

Dermally absorbed AHT → Skin → AAHT (N-acetyl AHT) → Liver

Orally dosed AHT → Liver → AHT-O-glucuronide → AAHT-O-glucuronide

Liver → AHT-O-sulfate

> 30% N-acetylation of AHT: A dermal first pass effect
Commonly used aromatic amine hair dye precursors

Conclusion on skin metabolism:

Aromatic amine hair dye precursors (PPD and AHT) undergo epidermal N-acetylation (Dermal first pass effect)
**Events occurring during Skin Sensitization**

**Induction**
- Oxidation
- Penetration
- Metabolism/Protein binding
- DC activation

**Elicitation**
- GP Tests, HRIPT
- Haptenization
- LLNA

**1° T reaction**
Effects of PPD and derivatives when arrived in the skin, i.e. epidermis?

Activation of Dendritic cells (DC)
**N-acetylated metabolites and auto-oxidation products of PPD**

- **N-acetylation by NAT-1**
  - Fresh PPD
  - Oxidation
  - mono-Acetyl-PPD
  - Di-Acetyl-PPD
  - Acetylated PPD
  - Dimers/Trimers
  - oxidized PPD
  - Bandrowski's Base
  - BB
Principle of DC like cells activation

Pool of 4 donors ➔ «Dendritic-like cells» ➔ Exposure to chemical ➔ Analysis

IL-4 & GM-CSF 4 days

Activation markers

+ CD86 measurement by flow cytometry

+ IL-1β /IL-8 and AQP3 measurements by quantitative RT-PCR
Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD

Population CD 86 bright (% of control ± SEM)
Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD

Population CD 86 bright (% of control ± SEM)

IL-1β mRNA (% of control ± SEM)

Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD
Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD
Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD

AQP-3 mRNA (% of control ± SEM)

μM

C. Neg. Fresh PPD Ox. PPD Mono-Acetyl-PPD di-Acetyl-PPD BB
Dose effect analysis after exposure to PPD, ox-PPD, BB, mono and di-Acetyl-PPD

PPD oxidation is a precondition for DC activation/
acetylated PPD has no activating effect
Events occurring during Skin Sensitization

**Induction**
- Haptenization
- Oxidation
- Penetration
- Metabolism/Protein binding
- DC activation

**Elicitation**
- GP Tests, HRIPT
- LLNA
- DC activation
- 1° T reaction
Analysis of the skin sensitization potency of PPD/derivatives:

Local Lymph Node Assay (LLNA)

Exposure conditions support oxidation of PPD to oxidized PPD derivatives
In vivo (LLNA) effects of PPD, BB, Mono- and Di-Acetyl-PPD

Application (OECD 429):
3 daily applications of 250 µg/cm² (1%) PPD = 750 µg/cm² in DMSO/water (80:20)

Local lymph node assay (LLNA)

Based on all available LLNA data (summarized in Baketter 2008) as well as human data the Weight of Evidence No Expected Sensitization Induction Level (WoE NESIL) is 0.1% ~ 25 µg/cm²
How do conditions of sensitization testing compare to PPD exposure during hair dyeing?
Exposure to PPD during hair dyeing:
Basic chemistry of hair coloring

Commercial hair colorants are all based on similar chemistry:
Primary intermediates + couplers, oxidant, high pH, exposure for approx. 30 min

- Uncontrolled auto-oxidation of PPD is prevented
- Reaction with coupler is chemically preferred
- No benzoquinone diimine
- Exposure time approx. 30 min
Skin penetration study to measure actual PPD exposure

Permanent hair dye usage conditions: **Test Product F**

- PPD in Dye solution + Developer solution
- 30 min
- Removal with water/shampoo
- • Surface excess (rinsings)
  • Skin
  • Receptor fluid

Diagnostic patch testing conditions: **Test Patch H**

- PPD
- 48 hours
- Removal with swap
- • Surface excess (pet in chamber/swap)
  • Skin
  • Receptor fluid

Applied dose - surface excess = **Measured exposure level (MEL)**
Exposure measurement with skin samples *in vitro* (OECD 428)

Application of hair dye product containing 2% PPD + coupler for 30 min and rinse off

Surface access

Conductivity readout

Receptor fluid (systemic circulation)

Measured PPD exposure level
Determination of Measured exposure level (MEL) for PPD under hair dyeing and diagnostic patch test conditions

<table>
<thead>
<tr>
<th></th>
<th>Diagnostic patch: Test patch H (1% PPD)</th>
<th>Test Product F (2% PPD)</th>
<th>Product¹ (2% PPD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied dose [µg/cm²]</td>
<td>400</td>
<td>3000</td>
<td>400</td>
</tr>
<tr>
<td>Exposure time [h]</td>
<td>48</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Surface excess/rinsings (%)</td>
<td>49</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Measured Exposure level (MEL)</td>
<td>206</td>
<td>7</td>
<td>15 (22)</td>
</tr>
</tbody>
</table>

¹from Hueber-Becker et al 2004,

Patch test exposure (MEL) more than 10 fold higher
Hazard Assessment Conditions (e.g. LLNA)

Skin surface: pH 5 – 6
Exposure time >48 hours

Auto-oxidation products

Auto-oxidation by O₂

50% penetration

Enzymatic activation? NAT-1 deactivation

DC activation
Skin sensitization

In Use Conditions (Hair dyeing)

Hair, Scalp: pH 9 – 10
Exposure time 30 min.

Controlled oxidation by H₂O₂

<1.3% penetration

Low penetration < 0.05%
non reactive

N-acetylated derivatives
Not sensitizing

Couplers
Conclusions on PPD sensitization potency

- PPD (non-oxidized) is a weak activator of DC in vitro (CD86, II-1β, II-8, AQP3)
- Auto-oxidation is a precondition for relevant DC activation (danger)
- Current (in vivo) assays predominantly consider sensitization potency of PPD auto-oxidation
- Hair dyeing conditions block PPD auto-oxidation and significantly reduce exposure to PPD
- Skin actively N-acetylates PPD to non sensitizing derivatives
- Equilibrium between dermal N-acetylation and oxidative activation of PPD is critical for individual risk of induction of PPD allergy
Publications

**PPD data:**

Skin sensitization to \(p\)-Phenylenediamine: The diverging roles of oxidation and \(N\)-acetylation for dendritic cell activation and the immune response


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**AHT data:**

Skin metabolism of aminophenols: Human keratinocytes as a suitable *in vitro* model to predict the dermal transformation of 4-amino-2-hydroxytoluene *in vivo*


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