

Assessment of skin sensitization potency of hair dye molecules *in vitro*

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Today, the potential of cosmetic ingredients and products to induce skin sensitization must be determined without the use of animal methods. Therefore, alternative *in vitro* methods that are able to predict the chemicals' relative potencies for skin sensitization are essential for quantitative risk assessment. This is of special importance for reactive molecules needed for certain applications such as hair dyeing. *p*-Phenylenediamine (PPD) and toluene-2,5-diamine (PTD) play a major role in hair dye-associated contact dermatitis (1), and, currently, either PPD or PTD is present in 80–99% of commercially available oxidative hair dyes (2).

Hazard-based characterization classifies PPD and PTD as strong sensitizers on the basis of the murine local lymph node assay (LLNA), whereas its known trimeric PPD oxidation product, Bandrowski's base (BB), is classified as an extreme sensitizer, showing that the increase in potency is related to the degree of PPD autooxidation (3, 4). In contrast, the recently introduced PPD derivative 2-methoxymethyl-*p*-phenylenediamine (ME-PPD) is classified as a moderate sensitizer, as approximately 30- to 40-fold higher concentrations than with PPD or PTD were needed for the induction of sensitization in the LLNA (5).

Here, we investigated whether the different sensitizing potencies determined in the LLNA for PPD, PTD, ME-PPD and BB can also be distinguished by the use of our human cell line-based coculture model. This model consists of human keratinocytes (HaCaT cells) and THP-1 cells as surrogate dendritic cells (DCs), and uses the chemical concentration that induces significant upregulation of the costimulatory molecule CD86 and/or adhesion molecule CD54 on THP-1 cells as a marker for sensitizing potency (6, 7).

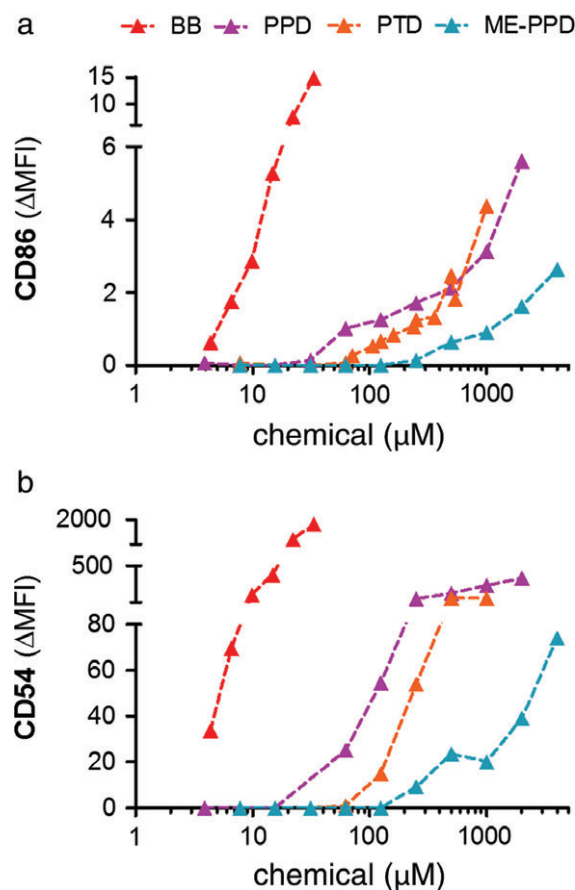


Fig. 1. Comparison of the potential and potency of the hair dye molecules *p*-phenylenediamine (PPD), toluene-2,5-diamine (PTD), and 2-methoxymethyl-*p*-phenylenediamine (ME-PPD), and the PPD autooxidation product Bandrowski's base (BB), for activation of dendritic cells, measured as upregulation of CD86 and/or CD54 on cocultured THP-1 cells. THP-1 cells were exposed to chemicals in coculture with HaCaT keratinocytes for 24 h, and upregulation of CD86 (a) and CD54 (b) was then analysed on viable THP-1 cells via flow cytometry (one representative experiment from $n \geq 3$). MFI, mean fluorescence intensity.

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Table 1. Skin sensitization potency of hair dye molecules *in vitro* (COCAT) and *in vivo* (LLNA)

Chemical	COCAT ECΔ (mM, mean ± SEM, n ≥ 3)	LLNA EC3 (%)
BB	0.007 ± 0.0004	0.02
PPD	0.1 ± 0.01	0.1
PTD	0.2 ± 0.03	0.3
ME-PPD	2.6 ± 0.2	4.3

COCAT, cocultured activation test; BB, Bandrowski's base; LLNA, local lymph node assay; ME-PPD, 2-methoxymethyl-*p*-phenylenediamine; PPD, *p*-phenylenediamine; PTD, toluene-2, 5-diamine; SEM, standard error of the mean.

Materials and Methods

Expression levels of CD86 and CD54 on cocultured THP-1 cells

THP-1 cells were exposed to PPD (Sigma Aldrich, Taufkirchen, Germany), PTD (Merck, Darmstadt, Germany), BB (ICN Biomedicals, Aurora, OH, USA) or ME-PPD (provided by C. Goebel, P&G, Darmstadt, Germany) in direct coculture with confluent HaCaT cells (HaCaT/THP-1 ratio of 2:3) for 24 h. Expression of CD86 and CD54 was analysed on viable THP-1 cells (propidium iodide exclusion) via flow cytometry, as previously described (6).

Results

In our model (cocultured activation test, COCAT), all molecules upregulated CD86 and CD54 on the cocultured THP-1 cells in a concentration-dependent manner (Fig. 1). However, we observed a clear difference in the concentrations needed for upregulating CD86

and/or CD54, that is, the potency *in vitro* (designated as ECΔ, estimated via linear interpolation). In detail, much higher concentrations were needed for ME-PPD (27-fold and 14-fold) than for PPD and PTD, respectively, whereas very low concentrations (7 μM) were sufficient for BB, reflecting the differences observed in the LLNA, as shown in Table 1.

Discussion

Alternative *in vitro* methods that are able to predict the skin sensitization potencies of chemicals are crucially needed for proper safety assessment. A limitation of potency assessment with *in vitro* models is that the interaction of the chemical with keratinocytes in the viable epidermis is not considered. Using Raman spectroscopy, we recently showed that the epidermal residence time for PPD is substantial (8). Using our established coculture model consisting of human keratinocytes and DCs, we found clear concentration-dependent DC activation by BB, PPD, PTD, and ME-PPD. Ranking of these molecules according to the concentrations needed for cell activation reflects their potency rank order in the LLNA. Therefore, our model provides a valuable prediction of the sensitizing potencies of hair dye molecules. Within an integrated testing strategy, it can contribute to a better approach for the quantitative risk assessment for hair dye molecules.

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