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Suitability of the in vitro Caco-2 assay to predict the oral absorption of aromatic amine hair dyes

Cindy Obringer [a,*], John Manwaring [a], Carsten Goebel [b], Nicola J. Hewitt [c], Helga Rothe [b]

[a] The Procter & Gamble Co., Cincinnati, OH, USA
[b] Procter & Gamble Service GmbH, Schwalbach, Germany
[c] Scientific Writing Services, Erzhausen, Germany

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ABSTRACT

Oral absorption is a key element for safety assessments of cosmetic ingredients, including hair dye molecules. Reliable in vitro methods are needed since the European Union has banned the use of animals for the testing of cosmetic ingredients. Caco-2 cells were used to measure the intestinal permeability characteristics (P_{app}) of 14 aromatic amine hair dye molecules with varying chemical structures, and the data were compared with historical in vivo oral absorption rat data. The majority of the hair dyes exhibited P_{app} values that indicated good in vivo absorption. The moderate to high oral absorption findings, i.e. ≥60%, were confirmed in in vivo rat studies. Moreover, the compound with a very low P_{app} value (APB: 3-((9,10-dihydro-9,10-dioxo-4-(methylamino)-1-anthracenyl)amino)-N,N-dimethyl-N-propyl-1-propanaminium) was poorly absorbed in vivo as well (5% of the dose). This data set suggests that the Caco-2 cell model is a reliable in vitro tool for the determination of the intestinal absorption of aromatic amines with diverse chemical structures. When used in combination with other in vitro assays for metabolism and skin penetration, the Caco-2 model can contribute to the prediction and mechanistic interpretation of the absorption, metabolism and elimination properties of cosmetic ingredients without the use of animals.

1. Introduction

The safety assessment of hair dyes naturally involves the measurement of dermal exposure since this route is the most relevant for consumer exposure: a hair color formulation containing the dye molecules is applied to the hair with access to the scalp for approximately 30 min and then washed off. However, knowledge of oral exposure is also important because the oral route is a key requirement in safety assessment in order to estimate systemic exposure with no effect levels (i.e., NOELs). The traditional in vivo model to analyze systemic exposure involved oral, intravenous (i.v.) and dermal application of a compound to the shaved skin of a rat, followed by measurement of plasma concentrations and excretion in urine and feces of the parent compound and its metabolites (OECD TG427, 2004). As part of the 7th Amendment to the Cosmetics Directive of the European Union, these in vivo rodent toxicokinetics studies were banned as of March, 2013 (EU, 2003). Therefore, reliable in vitro methods are key tools helping to provide information on absorption, metabolism and elimination needed to conduct a safety assessment. We have integrated a toolbox of in vitro models into our safety assessment strategy in order to predict skin absorption, as well as skin and hepatic metabolism properties of hair dye ingredients (Manwaring et al., 2015). Our findings showed that the combined use of in vitro absorption and metabolism assays using ex vivo skin, keratinocytes and hepatocytes have correctly predicted the toxicokinetic properties of a number of aromatic amine hair dye ingredients. Carrying on from this work, we describe here how the in vitro Caco-2 model can be applied to predict the intestinal absorption of aromatic amine hair dyes following oral exposure. When used in combination, in vitro assays predicting dermal and oral absorption as well as dermal and hepatic metabolism can provide a reliable basis to estimate the systemic exposure of the parent compound. Furthermore, the data can be particularly useful for read-across scenarios from a data-rich compound to one with limited data.

Caco-2 cells originate from a human colon carcinoma and can differentiate spontaneously into cells resembling mature small intestinal

Abbreviations: AEP, 2-amino-5-ethylphenol; CEN, 2-chloro-6-ethylamino-4-nitrophenol; HAP, 4-hydroxypropylamino-3-nitrophenol; APB, HC Blue 16; HCR, HC Red no 13; HCY, HC Yellow no 13; HDAP, 1-hydroxyethyl-4,5-diamino pyrazole; ACP, 2-amino-6-chloro-4-nitrophenol; AHT, 4-amino-2-hydroxytoluene; AMC, 4-amino-m-cresol; AMP, 6-amino-m-cresol; AME, absorption, metabolism and elimination; AUC, area under the curve; DMSO, dimethyl sulfoxide; HBSS, Hank's Balanced Salt Solution; HMA, hydroxyethyl-3,4-methylenedioxyaniline; HPD, hydroxyethyl-p-phenylenediamine; NOAEL, no adverse effect level; NOEL, no effect level; OECD, Organization for Economic Co-operation and Development; P-gp, P-glycoprotein; SCCS, Scientific Committee on Consumer Safety; TDA, toluene-2,5-diamine; TEER, transepithelial electrical resistance.

* Corresponding author.
E-mail address: obringer.cm@pg.com (C. Obringer).

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enterocytes. These cells express carrier proteins similar to the small intestine and, moreover, the TC-7 sub-clone has been shown to express the apically-located efflux pump, P-glycoprotein (P-gp) (Raeissi et al., 1999). This model is widely used to predict the absorption across the intestinal barrier (Sambuy et al., 2005; Skolnik et al., 2010), and a good correlation between oral drug absorption in humans and the apparent drug permeability (P_{app}) across the in vitro Caco-2 cell barrier has been shown (Artursson and Karlsson, 1991; Grès et al., 1998). This in vitro model was also included in the report of the 46th ECVAM workshop, identifying it as a suitable model for drug absorption (Le Ferrec et al., 2001).

The validity and suitability of such assays are typically confirmed by reference compounds for high and low absorption. In these particular studies, propranolol was used as a high permeability reference since 90% of the dose is absorbed in humans (Grès et al., 1998); and ranitidine as a low permeability reference of which only 50% of the dose is absorbed in humans. In the majority of the assays, a third reference compound, namely vinblastine, was also included as a second low absorption reference compound since its absorption is low and influenced by the presence of P-gp (Ogihara et al., 2006).

In this paper, we assess the applicability of the in vitro Caco-2 assay for cosmetics ingredients by predicting the permeability characteristics of 14 aromatic amine hair dye molecules related to phenylenediamine, aminophenol and anthraquinone based on their comparison with historical oral absorption data from rats (Fig. 1). The compounds chosen were all hair dye ingredients for which historical in vivo data were available.

2. Materials and methods

2.1. Chemicals

The hair dyes tested were TDA (toluene 2,5-diamine, CAS number 615-50-9); AMC (4-amino-m-cresol, CAS number 2835-99-6); AMP (6-amino-m-cresol, CAS number 2835-98-5); HPD (hydroxyethyl-p-phenylenediamine, CAS number 93841-25-9); HMA (hydroxyethyl-

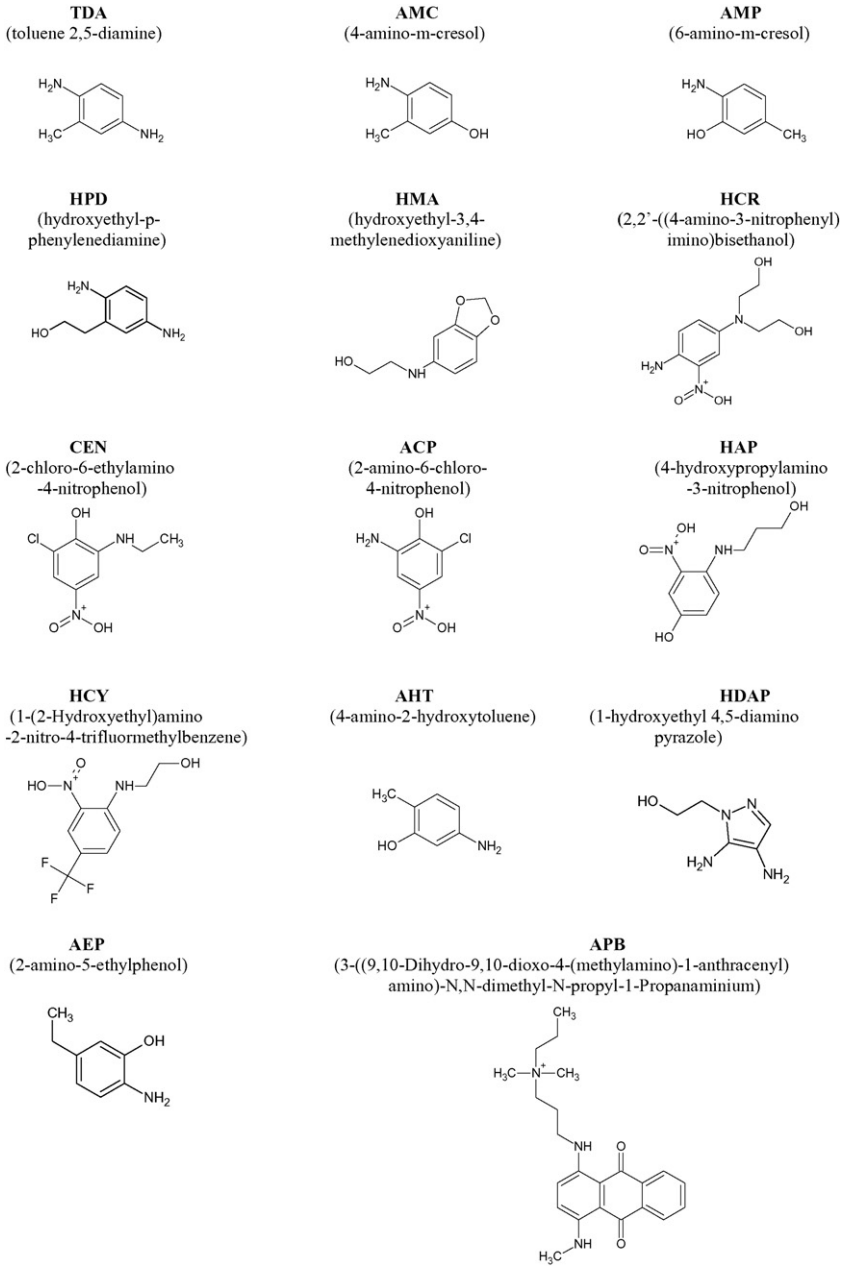

Fig. 1. Chemical structure of aromatic amine hair dye molecules similar to phenylenediamine, aminophenol and anthraquinone. All structures are shown as the free base form.