

N-acetylation of aromatic amines: Implication for skin and immune cells

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1. ABSTRACT

Frequently, aromatic amine (AA) contact to the skin occurs via occupational or 'life style' exposure to hair dye intermediates and couplers, usually monocyclic p-phenylenediamines and meta-substituted aminophenols. The transport of AA from the outer surface to the systemic circulation predominantly follows the intracellular route. Skin tends to have relatively higher phase II compared to phase I xenobiotic metabolizing enzyme capacity, and levels are generally regarded to be lower than those in liver are. Inside skin cells AA are primarily N-acetylated and detoxified by N-acetyltransferase 1. AA activation via hydroxylation or chemical oxidation competes with acetylation and is only of importance under circumstances when N-acetylation capacities are limited. The reactive AA derivatives are able to elicit effects by virtue of their modifications of skin proteins resulting in irritant or allergic contact dermatitis. Overall, the effective acetylation of topically applied AA in skin cells emphasizes a protective role of cutaneous acetylation mediating a classical "first-pass" effect, which attenuates systemic exposure.

2. SKIN EXPOSURE AND DERMAL ABSORPTION OF AROMATIC AMINES

2.1. Human skin organization

The skin is a complex organ, which serves as defensive shield of the human body against environmental influences. It mediates a broad set of protective barrier functions including the protection against pathogens and chemicals (1, 2) using a specialized set of immune cells such as epidermal dendritic cells (Langerhans cells) (3, 4) and at least 2 types of dermal dendritic cells (5-7). Protecting functions of the skin also comprise barrier functions as defense against the entry of chemicals due to the conformation of corneocytes and lipids in the outer layer of the epidermis (8).

The skin is composed of two different layers, the dermis and the epidermis. The dermal part of the skin consists of two stratified lineages of dermal fibroblasts with adipocytes as major cell types in the lower part, pervaded by blood and lymph vessels (9). The basal membrane connects the dermal with the outer epidermal layers, which represents the dynamic part of the skin composed mainly of renewing

keratinocytes and, besides others, Langerhans cells. Keratinocytes migrate from the basal layer to the apical surface thereby passing through a regulated differentiation program. Proliferating keratinocytes from the basal layer arrest in the cell cycle after a few division and undergo differentiation in order to terminally form non-viable corneocytes, which build together with the extracellular lipid matrix the dense network of the stratum corneum (10).

2.2. Exposure and dermal absorption

Exposure of the skin towards aromatic amines (AA) occurs frequently in both occupational as well as “life style” exposure settings. AA are widely used as industrial chemicals in the production of pesticides (11), pharmaceuticals (12), dyes (13) and rubber materials (14, 15), where workers can be exposed as a result of direct cutaneous contact but also from vapors, aerosols and particles. Personal or “life style” exposures can occur due to the use of consumer products, including hair dyes (13), tattoo pigments (16), henna tattoos (17) or cosmetic colorants (18).

In general, chemicals can enter the skin via the intercellular route, the transcellular route as well as via diffusion along hair follicles and sweat glands. The transport of a chemical from the outer surface to the systemic circulation is known as dermal or percutaneous absorption according to the “OECD guidelines for dermal absorption” (19, 20) and the “OECD guiding document for the conduct of dermal absorption studies” (21). This global term is often divided into penetration (entry of a chemical into a particular skin layer), permeation (passage through a particular layer and penetration into another layer) and resorption (uptake of a chemical into the skin lymph and vascular system, which mostly leads to the entry into systemic circulation) according to (22).

Dermal absorption of AA has been reviewed in the present issue with view on both, occupational (23) as well as “life style” (24) exposures. Especially with regard to a group of aromatic amines with occupational exposure several factors influencing absorption have been elaborated including intrinsic chemical properties like water solubility or lipophilicity, applied dose as well as vehicle ingredients (23). Thus, depending on the compound, dose and vehicle, absorption data range between 0 and $2.962 \pm 125 \mu\text{g}/\text{cm}^2$ (25). A compilation (based on Scientific Committee on Consumer Safety opinions) of dermal absorption data for aromatic amine hair dye ingredients, generated under consumer exposure conditions, reports a range

between $0.2 \mu\text{g}/\text{cm}^2$ for *p*-aminophenol (PAP) and $82.9 \mu\text{g}/\text{cm}^2$ for *p*-toluenediamine (PTD) sulfate (24).

Altogether, human skin is frequently exposed to AA, which are to a variable extent but readily absorbed. In the living epidermis, AA absorption occurs via the transcellular route as well as via the intercellular route. This can be drawn from studies that quantified N-acetylated metabolites formed during skin passage, since N-acetylation requires cellular uptake (26).

3. N-ACETYLATION AS PREDOMINANT BIOTRANSFORMATION OF AROMATIC AMINES IN THE SKIN

3.1. Xenobiotic metabolism in the skin

The ability of skin to transform chemicals by xenobiotic metabolizing enzymes (XMEs) is often considered as further barrier function, which is of particular importance, since the skin underlies extensive exposure to the external environment and represents a frequent route of uptake for chemicals.

Until recently, researchers have predominantly characterized metabolic profiles of human skin using mRNA expression analysis (e.g. (27-29)) and little has been published on the capacity of skin to metabolize xenobiotics. Overall skin tends to have relatively higher phase II than phase I XME capacity (30, 31), and skin levels are generally regarded to be lower than those in liver are. This was lately confirmed by a study comparing enzyme levels in whole human skin and liver samples using a broad comprehensive proteomic approach (liquid chromatography tandem mass spectrometry). They detected 36 XMEs in the skin, which had mostly 4-10 fold lower protein levels compared to liver. Although 46 enzymes were exclusively detected in liver, some XMEs like glutathione S-transferase pi (2-fold) or membrane primary amine oxidase (6.7.-fold) had even higher protein levels in skin tissue (32).

Specifically, basal mRNA and protein expression of the major phase I XMEs cytochrome p450 (CYP1) family 1-3 have been reported by us and others (33, 34). However, using the proteomic technique mentioned above CYP1A1, CYP2E1, CYP3A4, and CYP3A5 were below the limit of detection ($0.1\text{-}0.2 \text{ pmol}/\text{mg}$ microsomal protein) (32). Basal CYP enzyme activities like CYP1A/1B (35) as well as CYP2C9, CYP2E1 and CYP3A4 (36) were detectable in some skin samples, whereas other studies found no basal CYP family 1-3

activities (31). However, using cultured keratinocytes as a model, CYP1 activity was found to be inducible by classical inducers like 3-methylcholanthrene (31) and benzo[a]pyrene (37).

Besides CYPs, several other phase I XMEs have been found to be active in human whole skin samples as well as in cultured keratinocytes including flavin-dependent monooxygenases (38) and cyclooxygenases (31, 39).

Major phase II enzymes of the skin include glutathione S-transferases (GST), uridine diphosphate-glucuronosyltransferases (UGT), sulfotransferases (SULT) as well as N-acetyltransferases (NAT). GST mRNA and protein are expressed in human skin (32, 40) with GST pi as the most abundant isoform (29, 32, 41). 1,3-Dinitro-4-chlorobenzene conversion confirms GST enzyme activity in human skin (31, 42). Among UGTs, UGT1 is the major isoform found in human skin (43) and glucuronidation of 4-methylumbelliferone by skin cytosols has been demonstrated (44). Sulfation of dopamine, p-nitrophenol and minoxidil (45) by skin homogenates proved the presence of active SULT in human skin.

3.2. N-acetylation of aromatic amines in the skin

Regarding the biotransformation of aromatic amines, NATs are of particular importance. NATs catalyze the transfer of an acetyl moiety from the co-substrate acetyl-coenzyme A to either the nitrogen of aromatic amines (N-acetylation) or to the oxygen of N-hydroxyl-arylamines (O-acetylation). Mostly, N-acetylation mediates aromatic amine detoxification whereas O-acetylation is associated with activation of bicyclic aromatic amines (46). NAT1 is ubiquitously expressed in all human tissues analyzed so far (e.g. intestine (47), breast tissue (48), ureter (49), bladder (50), mammary gland (51), blood cells (52, 53) or skin (54)) but activities and likely protein levels vary extensively between different cell types (see e.g. (55)). NAT1 protein belongs to the group of low abundance proteins. It was calculated to constitute only 0.002% of soluble protein in HeLa cells (56), which is comparable to the estimated NAT2 level in liver (57). Very high levels (0.005-0.01% of cytosolic protein) were reported for the breast cancer cell line MCF-7. For human skin, NAT1 protein was not detectable using proteomics and therefore, based on the detection of this approach for this protein, calculated to be below 0.01% (32). In contrast to the protein level, NAT1 activity is fairly detectable in whole skin cytosols.

For the substrate p-toluidine values between 0.5 and 3 nmol/mg/min (44) and for p-phenylenediamine (PPD) our group reported 0.4 to 3.7 nmol/mg/min (54). These values are based on NAT1 in the epidermis (54), although *NAT2 mRNA* was found in low abundance in normal primary keratinocytes (54) as well as dermal fibroblasts (58).

3.3. N-acetyltransferase 1 mediated acetylation of aromatic amines during dermal absorption

During the passage of aromatic amines through the skin, they are likely taken up by cells and converted to their acetylated derivatives. For aromatic hair dye ingredients quantitative data exist for whole human skin samples and models consisting of reconstructed epidermis (for summary see Table 1).

In order to simulate hair dye relevant exposure times, the aromatic amines 2-hydroxy-4-amino-toluene (AHT) (59), p-phenylenediamine (PPD), p-aminophenol (PAP) (55), 2-methoxymethyl-p-phenylenediamine (ME-PPD) (60) and 2-nitro-p-phenylenediamine (2NPPD) (61) were topically applied to the models for 0.5 or 1h, afterwards the formulation was removed with water, and overall absorption including their N-acetylated derivatives was determined after 3h and 24h. The chemical structures of parent compounds and detected metabolites are given in Table 2.

Independent of whether they studied whole skin or solely epidermis between 0.4 and 15% of the applied dose were taken up, and between 19.4 and 100% of that portion were metabolized. In the case of AHT, PPD, PAP and ME-PPD only acetylated metabolites were found. In addition, skin exposure to 2NPPD (61) resulted in the generation of 1,2,4-triaminobenzene (7 and 29% depending on the vehicle) due to reduction of the nitro group, which paralleled 2NPPD acetylation. Only N4-acetyl-2NPPD was detected, suggesting that the second amino group in the case of 2NPPD and the other two amino groups of 1-,2-,4-triaminobenzene were not accessible for acetylation. Limited accessibility was also assumed for the amino group in position 2 from ME-PPD (60), since diacetylated ME-PPD was only found in 1 out of 4 donors as a minor metabolite (1% of the absorbed dose).

Interestingly, the extent of acetylation of 2NPPD (not absorption) clearly depended on the vehicle used. Whereas 90% of the penetrated

Tabel 1. ???

Compound	2-nitro-p-phenylenediamine (2NPPD)	2-nitro-p-phenylenediamine (2NPPD)	2-nitro-p-phenylenediamine (2NPPD)	2-hydroxy-4-aminotoluene (AHT)	2-methoxy-methyl-p-phenylenediamine (ME-PPD)	p-phenylene-diamine (PPD)	p-aminophenol (PAP)	p-phenylene-diamine (PPD)	p-aminophenol (PAP)	p-phenylene-diamine (PPD)	p-aminophenol (PAP)
Characteristics of application											
Vehicle	Ethanol	Hair dye formulation	Hair dye formulation	Hair dye formulation	Hair dye formulation	Culture medium	Culture medium	Culture medium	Culture medium	Culture medium	Culture medium
Test skin	Skin disc	Skin disc	Skin disc	Skin disc	Skin disc	Epiderm®	Epiderm®	Epiderm®	Epiderm®	Episkin®	Episkin®
Preparation of test skin	n.d.	n.d.	n.d.	200-400 µm thickness	500 µm thickness	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Number of donors	2	2	2	3	4	1	1	1	1	1	1
Type of application	Topically	Topically	Topically	Topically	Topically	Topically	Topically	Culture medium	Topically	Culture medium	Topically
Applied dose [µg/cm ²]	4.7	4.7	4.7	1500	1863.7	1.5	1.5	2.2	1.5	3.9	1.5
Time of application [h]	0.5	0.5	0.5	1	1	0.5	0.5	24	24	24	24
Mean overall recovery of applied dose [%]	73	64	96	96	100	> 85	> 85	> 85	> 85	94	91
Determination of skin absorption											
Recovery time after application [h]	24	24	23	23	23	23.5	23.5	0	0	0	0
Mean absorbed dose [%]	9.2	9.5	1.7	1.7	0.4	15	10	78.8 ¹	100 ¹	74.7 ¹	85 ¹

(Contd....)

Tabel 1. (Contd....)

Compound	2-nitro-p-phenylenediamine (2NPPD)	2-nitro-p-phenylenediamine (2NPPD)	2-hydroxy-4-aminotoluene (AHT)	2-methoxy-methyl-p-phenylenediamine (ME-PPD)	p-phenylene-diamine (PPD)	p-aminophenol (PAP)	p-phenylene-diamine (PPD)	p-aminophenol (PAP)	p-phenylene-diamine (PPD)	p-aminophenol (PAP)
Absorbed dose [$\mu\text{g}/\text{cm}^2$]	0.4	0.4	25.6	6.9	0.2	0.2	1.7	1.5	2.9	1.3
Determination of skin metabolites										
Recovery time after application [h]	3	3	23.5	23.5	23.5	23.5	0	0	0	0
Absolute dose acetylated [$\mu\text{g}/\text{cm}^2$]	0.4	0.1	5.0	3.3	0.2	0.2	1.7	1.5	1.7	0.8
Percentage of absorbed dose										
- Metabolized	97	60	19.4	48	100	100	100	100	60	80.3
- Acetylated	90	30	19.4	48	100	100	100	100	60	80.3
- Found as other metabolite	7 (triaminobenzene)	29 (triaminobenzene)	0	0	0	0	n.q. (4-aminophenyl-formamide), n.q. (N-[4 (formylamine)-phenyl]acetamid)	0	0	0
- Found as protein bound	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	14	17
- Found as parent compound	3	40	80.6	52	0	0	21.1	0	20.4	3.5
Reference	(42)	(42)	(39)	(41)	(43)	(43)	(43)	(43)	(40)	(40)

Table 2. Chemical structures of aromatic amines and acetylated metabolites published in skin acetylation studies

Compound	2-nitro-p-phenylenediamine (2NPPD), 2-nitro-benzene-1, 4-diamine	2-hydroxy-4-amino-toluene (AHT), 5-amino-2-methylphenol	2-methoxymethyl-p-phenylenediamine (ME-PPD), 2-methoxybenzene-1,4-diamine	p-phenylenediamine (PPD), benzene-1,4-diamine	p-aminophenol (PAP) 4-aminophenol
Structure					
Acetylated metabolite(s)	N-(4-amino-3-nitrophenyl)-acetamide (N4-acetyl-2NPPD)	N-(3-hydroxy-4-methylphenyl)-acetamide (acetyl-AHT)	N,N'-(2-methoxy-benzene-1,4-diy)-diacetamide, (di-acetyl-ME-PPD)	N-(4-aminophenyl)-acetamide, mono-acetyl-PPD (MAPPD)	N-(4-hydroxyphenyl)-acetamide, (ACPAP) acetaminophen
Structure					

Table 3. Summary of dose-response studies for human skin acetylation of cell culture and topically applied aromatic amines using reconstructed epidermal models

Compound	p-phenylenediamine (PPD)									p-aminophenol (PAP)	
	Via culture medium below the model skin						Topically on the outer layer of the model skin				
Applied doses [$\mu\text{g}/\text{cm}^2$]	3.9			2.2			1.5			1.5	
	19.5			11			7.3			7.5	
	48.8			55			14.7			15	
	97.5			110			36.7			18.8	
	195										
Time of application [h]	24						0.5				
Recovery time after application [h]	0						23.5				
Percentage of applied dose	res. PPD	MAPPD	DAPPD	res. PPD	MAPPD	DAPPD	res. PPD	MAPPD	DAPPD	res. PAP	AcPAP
After application and recovery time	n.d.	10.5	50.3	21.1	16.1	62.7	0 ¹	0.5 ¹	7.7 ¹	0 ¹	9 ¹
Found as parent compound or	n.d.	18 ¹	12 ¹	53.6	11.2	35.2	0.8 ¹	0.5 ¹	8.5 ¹	0 ¹	9 ¹
Acetylated derivative	n.d.	9 ¹	5 ¹	77.7	11.8	10.6	1 ¹	0.5 ¹	8 ¹	0 ¹	8 ¹
	n.d.	5 ¹	0.2 ¹	89.7	8.6	5.1	5.5 ¹	1.5 ¹	5.9 ¹	0 ¹	7.5 ¹
	n.d.	2 ¹	0.2 ¹								
Reference	(40)			(43)			(43)			(43)	

res.: residual; n.d.: no data; MAPPD: monoacetyl PPD; DAPPD: diacetyl PPD, AcPAP: acetylated PAP; ¹values not given, estimated from figures

dose was acetylated using ethanol, only 30% was acetylated when 2NPPD was applied in a semi-permanent hair dye formulation (Table 1). The difference may be due to other NAT substrates in that formulation. Indeed the formulation contained 2-nitro-N-(2-hydroxyethyl)aniline (HC Yellow No. 2) in unknown concentrations. Although the latter does not contain a primary amino group, reduction of the nitro group might generate another NAT1 substrate, which could impair 2NPPD acetylation (61).

Exposure to PPD or PAP for 24h resulted in 74.4-100% absorption of the applied dose as well as extensive acetylation of the absorbed fraction. Acetylated metabolites (60-100%), protein bound compound (14% for PPD, 16.7% for PAP) as well as trace amounts of a formamide derivative were detected using this experimental setting (Table 1) (55, 62).

Dose-response data (see Table 3) for PPD indicate that percentages of acetylated PPD decrease with increasing doses applied. This decrease was accompanied by enhanced levels of residual parent compound. In addition, at least

for 24h exposures (55, 62), the preference for monoacetyl-PPD (MAPPD) formation increased to the expense of diacetyl-PPD (DAPPD) formation. In contrast, no dose response relationship was reported for PAP, meaning quantitative acetylation of the absorbed dose and no residual parent compound was detected irrespectively of the applied dose (62).

These studies with special emphasis on aromatic amine hair dye ingredients confirm that these compounds are readily absorbed and acetylated by skin cells in a very effective and in part even quantitative way.

3.4. Implications for systemic exposure to aromatic amines

As reported for aromatic hair dye ingredients discussed above, a large fraction of the absorbed dose is N-acetylated in the skin. To what extent the cutaneous acetylation impairs systemic exposure to the aromatic amine AHT was elucidated by Goebel and colleagues (59) by studying the *in vivo* metabolism of AHT in Wistar rats. AHT metabolites were detected as glucuronide, sulfate and acetyl derivatives in urine after topical, oral and intravenous

application. Depending on the application route, no qualitative change of the metabolite profile was observed with AHT-sulfate as the major metabolite after intravenous and oral application. In contrast, topical applications resulted in a quantitative difference. Under these circumstances, 66% of the dose was transformed into acetyl-AHT compared to 32% after oral AHT treatment, and 37% after intravenous AHT administration. These results confirm the effective acetylation of AHT in rat skin and emphasizes the protective role of cutaneous acetylation mediating a classical “first-pass” effect, which attenuates systemic exposure (59).

3.5. N-acetyltransferase 1 activity of different skin cells

Effective N-acetylation in human skin is mainly mediated by keratinocytes, on the one hand due to their abundance in the epidermis and on the other hand due to the high activity of NAT1 in those cells. NAT1 activities of primary keratinocytes vary between 0.1 (54), 3 (63) and 8 nmol/mg/min (64) depending on the donors and substrates. NAT1 activity of the keratinocyte cell line HaCaT varied between different shipments/subclones (range 12.0-44.5 nmol/mg/min) and was in average 3.4-fold higher compared to primary cells (64).

Kawakubo and colleagues detected NAT1 activities in cytosolic fractions of human skin (9 donors) for mono- and diacetylation of PPD, 0.4 to 3.7 nmol/mg/min and 0.7 to 3.3 nmol/mg/min respectively, which were only slightly lower than those found in primary keratinocytes (7 donors), namely 0.1 to 4.3 nmol/mg/min and 1.3 to 6.6 nmol/mg/min, respectively (54). These data indicate that skin cells other than keratinocytes contribute only marginally to overall cutaneous N-acetylation capacity.

Regarding other primary epidermal cells, NAT1 activity has not been reported yet. However, the acetylation of 2-aminofluorene to its acetylated metabolite by a malignant melanoma cell line (65) may point to NAT1 activity also in melanocytes. Monocyte-derived dendritic cells, which are often used as a model for dendritic cells, have been found to convert PPD as well as p-aminobenzoic acid (PABA) to their acetylated derivatives (66) indicating NAT1 activity for dendritic cells as well.

Data about the contribution of the dermal part of the skin to cutaneous aromatic amine acetylation are rare. Up to now, results of dermal fibroblasts from only one single donor have been

reported (58). Furthermore, the dermis is pervaded by a network of blood vessels, and blood cells migrate during inflammatory responses to the dermis and epidermis. Therefore, blood cells may also contribute to the overall N-acetylation capacity of human skin. Data for NAT1 activities of peripheral blood mononuclear cells vary between 15 and 20 nmol/mg/min for PABA (66-69). Risch and colleagues reported that NAT1 activity in erythrocytes is likely to be 100-fold greater than the NAT1 activity associated with leucocytes, whereas the average value of the pool activity was 7.6 nmol/min/pmol haemoglobin for the substrate PABA (70). For dermal dendritic cells, NAT1 activity data are, to the best of our knowledge, not available at the moment.

Since tattoo colorants are directly applied into the dermal layer, those colorants could provide a unique opportunity to estimate dermal biotransformation reactions. For analysis of dermal N-acetylation, degradation products of tattoo colorants with aromatic amine structure might be useful. Several tattoo pigments can undergo degradation by sunlight (71, 72) or due to removal by laser treatment (73). *In vivo* studies using mouse skin (74) confirmed degradation of the dye Pigment Red 22 to aromatic amines like 2-methyl-5-nitroaniline and 2,5-dichloroaniline. The extent of dermal acetylation of those aromatic amines may point to the contribution of the dermis to the overall acetylation capacity of the skin.

Together, available data indicate that skin cells other than keratinocytes contribute only marginally to overall cutaneous N-acetylation capacity, whereas blood cell might have particular impact under certain conditions.

4. IMPLICATIONS FOR SKIN

4.1. Skin related effects

An important skin related effect of aromatic amines is the induction of skin sensitization, elicitation of allergic and irritant contact dermatitis. The latter reflects an innate inflammatory response to direct skin injury with an immediate reaction of the skin. Allergic contact dermatitis additionally involves an inflammatory response mediated by compound-specific T cells at the exposed area. It is often referred to as systemic toxicity, because sensitization occurs outside the skin in the draining lymph nodes. PPD exposure can cause both, allergic and irritant contact dermatitis (75, 76).

Beside PPD, several other oxidative hair dye ingredients are known to cause allergic contact dermatitis. Regarding skin sensitization and allergic contact dermatitis, N-acetylation is considered as deactivating process. Amongst others, this perspective is supported by us demonstrating for instance that MAPPD and DAPPD have only very limited capacities to reactivate T cells from PPD allergic subjects by patch testing (77) and *in vitro* studies using T cells of PPD-allergic individuals (78, 79). Additionally, Aeby and colleagues found that N-acetylated PPD derivatives did not activate naive T cells in the murine Local Lymph Node Assay (80).

The prevalence-estimate for allergic contact dermatitis induced by the model compound PPD in the general population is only about 0.11% based on positive patch test reactions causally related to consumer hair dye use (81). This indicates that the acetylation mediated protection of the skin against contact sensitization is impaired only under certain conditions. Reduced NAT1 activity of keratinocytes might increase the aromatic amine load encountering skin residing antigen presenting cells (APCs). Despite their own ability to N-acetylate, which has been shown for monocyte-derived dendritic cells as a model for dendritic cells (66), they might not counterbalance the acetylation of surrounding keratinocytes due to their comparatively low abundance in the skin.

4.2. Influence of N-acetyltransferase 1 modulation

Although modulation of NAT1 is not well understood in terms of *in vivo* relevance yet, *in vitro* data show that aromatic amines, like PPD and PABA can cause saturation (82) and enzyme inhibition in keratinocytes (64). This is at least in part caused by a substrate-dependent NAT1 protein degradation (64). Furthermore, NAT1 activity of recombinant NAT1 protein (83), bronchial (84) and lens (85) epithelial cells and a breast cancer line (86) can be reduced by oxidants like hydrogen peroxide and peroxyntirite as well as UV irradiation, which cause oxidative stress leading to oxidative modification in the active center of the NAT1. In addition, also cysteine reactive chemicals have been shown to inhibit NAT1 activity by forming adducts with the active center cysteine (87, 88). Co-exposure of skin cells to NAT1 inhibitors during dermal absorption of AA may lead to reduced NAT1 activities in keratinocytes as well as APCs. The latter may be of certain relevance for the activation of the immune system by these chemicals. Consequently, we could speculate that sensitization

occurs when N-acetylation capacities are limited or reduced by inhibition.

4.3. Potentials for metabolic activation in the skin

Hydroxylation is a common pathway for metabolic activation of aromatic amines (for review see (89) or (90)). Overall, available studies indicate relevant species differences (82, 91). For several aromatic amine hair dye ingredients, oxidative metabolism has been studied using pooled human liver microsomes and/or hepatocytes. 2-methyl-5-hydroxyethylaminophenol (MHEAP) and AHT were found to generate oxidative metabolites, which were however not protein reactive. PTD, PAP, m-aminophenol, p-methylaminophenol, N,N'-bis(2-hydroxyethyl)-p-phenylenediamine formed no oxidative metabolites, which was also the case for PTD, MHEAP and AHT. Furthermore, hydroxylation of PTD was analyzed using recombinant human CYP1A1, 1A2, 1B1, 2C9, 2C19, 2D6 and 3A4, and again no hydroxylation was detected (92). However, hydroxylated PTD has readily been detected using mouse hepatocytes (93) as well as rat hepatic microsomes (94). The latter study found a doubling of hydroxylation activity towards the substrate PTD using microsomes from Aroclor 1254-treated rats. These differences support the known interspecies differences regarding xenobiotic metabolism and might point to murine hepatocytes or rat hepatic microsomes to be the most sensitive systems to study the potential of PTD hydroxylation.

Metabolic activation of aromatic amines is not likely to occur in human skin, since basal expression of oxidative enzymes is low or even not detectable (32). However, PPD is able to induce CYP1A1 in keratinocytes (95) and PTD was found to bind to the aryl hydrocarbon receptor and induce CYP1A activity (94), which was also the case for acetylated aminofluorene (96). The relevance of these findings is unclear but may lead to increased capacities towards other CYP1 substrates.

5. SUMMARY

Human skin exposure to arylamines might take place by skin contact via hair dyes, rubber products and azo colorants in various consumer products. The dermal uptake of aromatic amines results in variable but significant N-acetylation in human skin. For the bifunctional aromatic amine PPD *in vitro* and *in vivo* data demonstrated that monoacetylation is sufficient to abolish its

sensitization potential. However, up to now it is not known whether this result can be transferred to other arylamines. Moreover, N-acetylation capacities may be saturated or inhibited under certain circumstances. Available data on NAT1 inhibition by aromatic amines and biochemical enzyme saturation is indicative for a threshold for dermally applied NAT substrates up to which the skin is able to completely deactivate the parent compound. However, to clearly elucidate the conditions leading to insufficient detoxification within skin and subsequent initiation of an adaptive immune response clearly needs further research.

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SULT: sulfotransferases; NAT: N-acetyltransferase; AHT: 2-hydroxy-4-amino-toluene; PPD: p-phenylenediamine; PAP: p-aminophenol; ME-PPD: 2-methoxymethyl-p-phenylenediamine; 2NPPD: 2-nitro-p-phenylenediamine; MAPPD: monoacetyl-PPD; DAPPD: diacetyl-PPD; APC: antigen presenting cells; PABA: p-aminobenzoic acid; MHEAP: 2-methyl-5-hydroxyethylaminophenol

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Abbreviations: AA: aromatic amines; OECD: organisation for economic co-operation and development; XME: xenobiotic metabolizing enzymes; CYP: cytochrome p450; GST: glutathione S-transferase; UGT: uridine diphosphate –glucuronosyltransferases;