Arch Toxicol (2013) 87:1683–1696
DOI 10.1007/s00204-013-1090-9

TOXICOKINETICS AND METABOLISM

Xenobiotic metabolizing enzyme activities in cells used for testing skin sensitization in vitro

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Received: 18 April 2013 / Accepted: 27 June 2013 / Published online: 20 August 2013
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Abstract For ethical and regulatory reasons, in vitro tests for scoring potential toxicities of cosmetics are essential. A test strategy for investigating potential skin sensitization using two human keratinocytic and two human dendritic cell lines has been developed (Mehling et al. Arch Toxicol 86:1273–1295, 2012). Since prohaptens may be metabolically activated in the skin, information on xenobiotic metabolizing enzyme (XME) activities in these cell lines is of high interest. In this study, XME activity assays, monitoring metabolite or cofactor, showed the following: all three passages of keratinocytic (KeratinoSens® and LuSens) and dendritic (U937 und THP-1) cells displayed *N*-acetyltransferase 1 (NAT1) activities (about 6–60 nmol/min/mg S9-protein for acetylation of *para*-aminobenzoic acid). This is relevant since reactive species of many cosmetics are metabolically controlled by cutaneous NAT1. Esterase activities of about 1–4 nmol fluorescein diacetate/min/mg S9-protein were observed in all passages of investigated keratinocytic and about 1 nmol fluorescein diacetate/min/mg S9-protein in dendritic cell lines. This is also of practical relevance since many esters and amides are detoxified and others activated by cutaneous esterases. In both keratinocytic cell lines, activities of aldehyde dehydrogenase (ALDH) were observed (5–17 nmol product/min/mg cytosolic protein). ALDH is relevant for the detoxication of reactive aldehydes. Activities of several other XME were below detection, namely the investigated cytochrome P450-dependent alkylresorufin *O*-dealkylases 7-ethylresorufin *O*-deethylase, 7-benzylresorufin *O*-debenzylase and 7-pentylresorufin *O*-depentylase (while NADPH cytochrome *c* reductase activities were much above the limit of quantification), the flavin-containing monooxygenase, the alcohol dehydrogenase as well as the UDP glucuronosyl transferase activities.

Keywords Xenobiotic metabolizing enzymes · Human dendritic cell lines · Human keratinocytic cell lines

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Introduction

Numerous chemical compounds have been implicated as skin sensitizers resulting in allergic contact dermatitis, a common manifestation of immunotoxicity in humans (McCall et al. 2005). Traditionally, the skin sensitization potential of substances is assessed in Guinea pigs (Magnusson and Kligman 1969 and OECD TG 406, 1992). Ethical considerations demand the application of the 3R principle (refine, reduce and replace animal studies; Russell and Burch 1959) in particular for dermatotoxicity testing. For instance, the local lymph node assay in mice has been implemented as a refined testing method (OECD TG 429) to investigate sensitizing potential of chemicals. In the last decade, great efforts were made to fully replace animal studies for skin sensitization by new in vitro methods as well as *in chemico* and *in silico* methods (Adler et al. 2011; Nukada et al. 2011; Basketter et al. 2012; Mehling et al. 2012; Teunis et al. 2012; Kimber et al. 2013). The

European Union introduced the new chemical legislation (REACh, Regulation No. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals) explicitly supporting the 3R principle and defining animal testing only as a last resort. Furthermore, the 7th amendment (1223/2009EEC) to the EU regulation of cosmetic products (76/768/EEC) has banned testing of skin sensitization of cosmetic ingredients in animal studies and will ban marketing of the respective products from March 2013 on. Both regulations fortify the necessity to develop and use animal-free methods for skin sensitization testing. Since skin sensitization is a complex process, it is not expected to be adequately predicted by a single in vitro assay. Instead, a battery of several assays addressing different steps of the adverse outcome pathway should be combined. Hence, a test battery, consisting of *in chemico* peptide reactivity assay and keratinocytic cell lines and dendritic cell line assays, has been developed (Bauch et al. 2011, 2012), reflecting the early events leading to skin sensitization: protein reactivity, keratinocyte and dendritic cell activation. The suggested keratinocyte assays are the KeratinoSens® (Natsch 2010) and the LuSens (Bauch et al. 2012). The suggested dendritic cell assays are the hCLAT (human Cell Line Activation Test) (Sakaguchi et al. 2006) and the (m)MUSST [(modified) myeloid U937 dendritic-cell-activation-based skin sensitization test] (Ade et al. 2006; Python et al. 2007).

Low molecular weight substances (haptens) are not directly allergenic, but need to interact with proteins to form complete antigens. Some haptens are only formed by enzymatic transformation of a pro-hapten in the skin (Jäckh et al. 2012), and some haptens may be detoxified by skin metabolism. Hence, information on the xenobiotic metabolism competence of the cells used in the test battery is of critical interest and has been investigated in the present study. Proof of metabolic competence represents an important step into the direction of proper validation of in vitro methods. This is of major interest as there is an urgent demand to develop and to improve existing in vitro alternatives, such as for skin sensitization testing.

Materials and methods

Chemicals, reagents and stock solutions

If not otherwise stated, all chemicals of p.a. quality were purchased from Sigma-Aldrich. Stock solutions were prepared for 7-ethylresorufin (0.2 mM in DMSO), 7-benzylresorufin (0.5 mM in DMSO), 7-pentylresorufin (1 mM in DMSO), resorufin (1 mM in DMSO), benzydamine (BA, 100 mM in aqua bidest.) and benzydamine-*N*-oxide (BA-Nox, 10 mM in 100 mM Tris–HCl pH 8.5), ethanol (100 mM in 66.6 mM pyrophosphate buffer pH 9), propanal (50 mM in 88.9 mM pyrophosphate buffer pH 8.8), fluorescein diacetate (6 mM in acetone), fluorescein (78 µM in acetone/DMSO (1+1/v+v), *para*-aminobenzoic acid (PABA, 1.25 M in DMSO), N-acetylated *para*-aminobenzoic acid (AcPABA, 1 mM in DMSO), 4-methylumbelliferone (MUF, 5 mM in DMSO), MUF-glucuronide (1 mM in DMSO), 4-hydroxybiphenyl (50 mM in DMSO), dithiothreitol (DTT, 100 mM in aqua bidest.), and UDP-glucuronic acid (UDP-GA, 30 mM in DMSO). Stock solutions were stored at −20 °C, with the exception of the stock solution of ethanol that was prepared always freshly before the experiment. Cofactors as well as additives such as the following were freshly prepared: NADPH (20 mM in 100 mM Tris–HCl pH 8.5); NAD^+ for ADH determination: 28 mM in ADH buffer (4.43 g pyrophosphate, 1.88 g semicarbazide, 282 mg glutathione, 211 mg glycine, pH 9, per 500 mL); NAD^+ for ALDH determination: 10 mM in 88.9 mM pyrophosphate buffer pH 8.8; NADH for ADH determination: 1 mM in ADH buffer (4.43 g pyrophosphate, 1.88 g semicarbazide, 282 mg glutathione, 211 mg glycine, pH 9, per 500 mL); NADH for ALDH determination: 1 mM in 88.9 mM pyrophosphate buffer pH 8.8; $MgCl_2$ 0.2 M (for CYP determination), 50 mM (for UGT determination) in 0.1 M Tris–HCl pH 7.5; 4-methylpyrazole (7.5 mM in 88.9 mM pyrophosphate buffer pH 8.8); acetyl-CoA (4 mg/mL 50 mM Tris–HCl pH 7.5); EDTA (100 mM, for FMO determination in aqua bidest., for NAT1 determination in 50 mM Tris–HCl pH 7.5); dithiothreitol (DTT): for preparation of subcellular fractions 10 mM in aqua bidest., for FMO determination 100 mM in aqua bidest., for NAT1 determination 100 mM in 50 mM Tris–HCl pH 7.5; dicumarol (1 mM in 0.1 M NaOH/100 mM Tris–HCl pH 7.5); Brij58 [0.5 % (w/v) in aqua bidest].

Cell lines

Two keratinocytic cell lines derived from HaCat cells (Boukamp et al. 1988), an immortal, but non-tumorigenic human keratinocytic cell line, were used: KeratinoSens® donated by Givaudan and LuSens developed by BASF SE. The KeratinoSens® cells possess a luciferase gene under control of the human aldo keto reductase AKR1 C2 antioxidant response element (ARE); the LuSens cells possess a luciferase gene under control of the rat NADH/NADPH quinone reductase NQO1 ARE (most contact allergens are themselves—or are metabolized to—electrophiles and activate the antioxidant response element ARE: Bauch et al. 2011).

Two dendritic cell lines, U937 und THP-1, both of them human monocytic leukemia cell lines were received from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH [German Collection of

Microorganisms and CellCultures]), Braunschweig. U937 is a cell line established from a diffuse histiocytic lymphoma displaying many monocytic characteristics (Galvão dos Santos et al. 2009). THP-1 is a cell line derived from the blood of a patient with acute monocytic leukemia (Qin 2012).

Culture conditions were as published (Bauch et al. 2011, 2012).

Subcellular fractions

The cytosolic fraction, the post-mitochondrial S9 supernatant (S9) fraction and microsomes were derived from cells of the above-described lines and as a positive control from the liver of 300–350 g body weight male Wistar rats from Charles River (Sulzfeld, Germany), which had been pretreated intraperitoneally with 500 mg Aroclor 1254 per kg body weight for 5 days.

The post-mitochondrial S9 supernatant (S9) fraction was prepared as follows. The cells or the rat liver were homogenized ice-cooled in 3 volumes of homogenization buffer consisting of 0.25 M saccharose, 1 mM ethylenediamine tetra-acetic acid (EDTA) or 0.05 M Tris–HCl pH 7.5 and 10 mM D,L-dithiothreitol (DTT), respectively, the cells twice for five seconds using a Labsonic 2000 (Labotec, Wiesbaden, Germany), the rat liver in a Potter (Julab, Braun, Melsungen, Germany). The homogenate was centrifuged at 9,000*g* for 15 min at 4 °C.

Microsomes and cytosolic fraction were prepared by centrifuging the S9 fraction at 100,000*g* for 1 h at 4 °C. The resulting supernatant was used as cytosolic fraction; the pellet was resuspended in 2 mL wash medium (0.15 M KCl) and centrifuged again under the same conditions. The resulting pellet was suspended in 1 mL resuspension buffer (1 mM glutathione, 1 mM EDTA, 4 mM $MgCl_2$, 0.1 M KH_2PO_4 pH 7.5 and 200 mL/L of 20 % aqueous glycerol) per 1 g initial weight of cells or liver tissue, respectively. The fractions were stored at −80 °C.

Determination of enzyme activities

Cytochrome P450-dependent monooxygenase activities were determined in triplicates in the microsomal fraction using the alkyl-resorufin dealkylase assay essentially according to Burke et al. (1994) and specifically under the following conditions: 2 μM 7-*O*-ethylresorufin or 10 μM 7-*O*-pentylresorufin or 5 μM 7-*O*-benzylresorufin were incubated for 15 min at 37 °C with 10 μg microsomal protein in 100 mM Tris–HCl pH 7.5 including a NADPH generating system (0.1 mM NADPH, 5 mM glucose-6-phosphate, 10 U glucose-6-phosphate dehydrogenase) as well as 10 mM $MgCl_2$ and 2 mM 5′-AMP. A total of 100 μM dicumarol was included to prevent further resorufin turnover by NADH/NADPH quinone oxidoreductase (NQO; DT-diaphorase). A total of 100 μL of the sample was transferred into a black 96-well plate (Greiner, Frickenhausen, Germany) for quantitation of the enzymatic product resorufin by monitoring its fluorescence using an Infinite 200 spectrophotometer (Tecan, λ_{ex} 550 nm, λ_{em} 585 nm). Enzymatic activities were calculated by subtracting the fluorescence at zero time from the value at incubation time intervals as long as the reaction proceeded linearly with respect to incubation time. As positive control of the methodology, the model substrates were incubated with rat liver microsomes (see "Subcellular fractions").

Flavin-containing monooxygenase (FMO) activities were determined in the microsomal fraction with benzydamine (BA) as substrate essentially according to Yeung and Rettie (2006), specifically under the following conditions: 5 mM BA was incubated in duplicates with 10 μg microsomal protein in 0.1 M Tris–HCl (pH 8.5) including 10 mM NADPH, 1 mM DTT and 1 mM EDTA for 30 min at 37 °C. The reaction was stopped by addition of 1 volume ice-cold acetone followed by centrifugation for 5 min at 10,000*g* and 4 °C. In the supernatant fraction, the enzymatic product monooxygenated BA (BA-Nox) was analyzed by RP-HPLC (Hewlett Packard 1100; Hypersil CN; 250 × 4.5 mm, pore size 5 μm) with fluorescence detection (JascoFP1520, λ_{ex} = 305 nm, λ_{em} = 375 nm, gain 10 (Ubeaud et al. 1999). The mobile phase was acetonitrile/methanol/0.01 M KH_2PO_4 (pH 7) in a ratio of 4:1:2.7 (v/v/v). The run was performed under isocratic condition at a flow of 1.5 mL/min. BA-Nox eluted at a retention time (Rt) of 3.6 min and BA eluted at a Rt of 7–10 min. The BA-Nox peak area obtained with heat-denatured microsomes (95 °C for 10 min) as a negative control was subtracted from the BA-Nox peak area of the active incubation. As positive control of the methodology, BA was incubated with rat liver microsomes (see "Subcellular fractions").

The alcohol dehydrogenase (ADH) activities were determined in the cytosolic fraction according to Kawashima et al. (2011), specifically by incubating in triplicates 10 mM ethanol in the presence of 80 mM semicarbazide, 12 mM glutathione and 2.8 mM NAD^+ in 60 mM glycine pyrophosphate buffer pH 9.0 with 25 μg cytosolic protein for at least 10 min at 37 °C. The rate of formation of the reduced cofactor, NADH, was determined at 340 nm in a Tecan Infinite 200 spectrophotometer by subtracting first the zero-time value from the value obtained in the active incubation at each time point and then subtracting the increment obtained in absence of substrate from the value of the active incubation. As a positive control for the methodology, cytosol of rat liver was used (see "Subcellular fractions").

Aldehyde dehydrogenase activities were determined in the cytosolic fraction with propanal as substrate according

to Jones and Lubet (1992) except using 37 °C instead of 25 °C, specifically incubating in triplicates 5 mM propanal, 1 mM NAD^+, 25 μg of cytosolic protein in 80 mM pyrophosphate buffer pH 8.8, containing 0.75 mM 4-methylpyrazole. The increase in absorbance at 340 nm due to the production of the reduced cofactor, NADH, was determined in an Infinite 200 Tecan spectrophotometer by subtracting first the zero-time value from the value obtained in the active incubation at each time point and then subtracting the increment obtained in the absence of substrate from the value of the active incubation. As a positive control for the methodology, cytosol of rat liver was used (see "Subcellular fractions").

Esterase activities were determined in triplicates in the S9 fraction with fluorescein diacetate as substrate. A total of 6 mM fluorescein diacetate stock solution in acetone was diluted 1:100 by 100 mM Tris–HCl buffer, pH 8, shortly before starting the reaction. A total of 170 μL of this diluted fluorescein diacetate solution was added to 30 μL S9 fraction containing 30 μg protein and incubated up to 16 min at 37 °C. The formation of the product fluorescein was determined at an excitation wavelength of 490 nm and an emission wavelength of 514 nm at 60-s intervals in a Tecan Infinite 200 spectrophotometer. Enzymatic product formation was determined by subtracting first the fluorescence value of the active incubation from that obtain with heat-denatured (10 min at 99 °C) S9 fraction and then subtracting the zero-time value from the value of the active incubation as long as the reaction proceeded linearly with respect to incubation time. As a positive control for the methodology, S9 fraction of rat liver was used (see "Subcellular fractions").

UDP glucuronosyltransferase activities were determined in duplicates in the microsomal fraction with 4-methylumbelliferone as planar model substrate and with 4-hydroxybiphenyl as non-planar model substrate. A total of 0.5 mM substrate was prewarmed together with 25 μg microsomal protein in 50 mM Tris–HCl (pH 7.5)/5 mM $MgCl_2$/0.05 % (w/v) Brij 58 in a final volume of 225 μL for 3 min at 37 °C; 25 μL of a UDP-GA solution in DMSO was then added to the reaction mixture (final concentration 3 mM) and incubated for 10 min. The reaction was stopped by addition of 1 volume of 0.5 M perchloric acid. The sample was centrifuged for 5 min at about 4,600*g*. To the supernatant, 2.4 volumes of chloroform were added; the mixture was vortexed for 5 min and recentrifuged for 10 min at about 4,600*g*. A total of 100 μL of the aqueous phase was then pipetted in triplicate into a black 96-well plate (Greiner, Frickenhausen, Germany). One volume of glycine–NaOH (1.6 M; pH 10.3) was added, and after shaking for 4 min, the fluorescence of the product was measured using an Infinite 200 spectrophotometer (Tecan) (glucuronosylated 4-methylumbelliferone λ_{ex} 315 nm, λ_{em} 365 nm; glucuronosylated 4-hydroxybiphenyl λ_{ex} 278 nm, λ_{em} 327 nm). The enzyme activities were calculated by subtracting from the resulting fluorescence values of the active incubations those of the corresponding zero-time incubations where the chloroform extractions were performed immediately after adding all components of the active incubations (i.e., immediately after adding UDP-GA). As positive control of the methodology, the substrates were incubated with rat liver microsomes (see "Subcellular fractions").

NAT-1 activities were determined in duplicates in the S9 fraction on the day of passaging the cells (because of the sensitivity of the enzyme and storage instability) using *para*-amino-benzoic acid (PABA) as model substrate. PABA (final concentration 0.5 mM) was incubated with 25 μg of S9 protein in 45 mM Tris–HCl (pH 7.5) including 0.4 mg/mL acetyl-CoA, 1 mM DTT and 1 mM EDTA in a final volume of 250 μL for 30 min at 37 °C with gentle shaking (450 rpm). The reaction was stopped by addition of 1 volume of 0.5 M perchloric acid. Precipitated protein was pelleted at 4 °C for 5 min at 10,000*g*. The supernatant was stored at −20 °C until analysis. Separation of N-acetylated product was performed by HPLC (Agilent 1100, Nucleosil 120-5-C18, 250 × 4 mm, pore size 5 μm) with UV detection at 263 nm. The mobile phase consisted of 1 volume acidified acetonitrile (1 mL formic acid/L) and 4 volumes aqua bidest (also acidified with 1 mL formic acid/L) and was run under isocratic condition at a flow of 1.2 mL/min. N-acetylated PABA was detected at a retention time of 4.3 min, and PABA was detected at a retention time of 3.3 min. In negative controls lacking protein but with substrate and also in heat-deactivated negative controls (protein denatured for 10 min at 95 °C), no signal was detected at the retention time of the product. As positive control of the methodology, PABA was incubated with rat liver S9 fractions (see "Subcellular fractions").

NADPH–cytochrome *c* reductase activities were measured in the microsomal fraction essentially according to Masters et al. (1967), specifically by incubating 0.1 mM NADPH and 85.5 μg cytochrome *c* [prepared from horse heart (information by Sigma)] per 220 μL final assay volume (i.e., ~32 μM) in duplicates for 3 min at 25 °C with 10 μL microsomal preparation. Formation of the oxidized cofactor, $NADP^+$, was followed at 550 nm using a Tecan Infinite 200 spectrophotometer. Heat-denatured microsomes (pool of 50 μL microsomal fraction per passage of the respective cell line heated to 95 °C for 15 min, centrifuged at 10,000*g*, the resulting supernatant used) served as negative controls. The absorption at 550 nm generated by the negative controls was subtracted from the absorption generated by the active microsomal fractions. The Sigma ready-to-use assay buffer, enzyme dilution buffer and positive control from rat liver (see "Subcellular fractions") and rabbit liver (provided ready to use within the commercial kit) were employed.

Protein concentrations were measured according to Bradford (1976).

Determination of levels of detection (LOD) and levels of quantitation (LOQ)

Data, if not otherwise specified, are means ± standard deviations (SD). For CYP, FMO, UGT, esterase and ALDH activity, LODs were determined by adding 3 SDs to the mean results of 10 incubations following the described methods in presence of substrate but without protein (mean + 3 SD). For ADH, the LOD was determined by adding 3 SD to the mean results of 10 incubations following the described method with the individual cytosolic fractions but without substrate. For NAT1 activity, LOD was determined via HPLC analysis by the peak to noise ratio for the acetylated metabolite of PABA. The LOQ was determined by multiplying the LOD by 2 (Gottwald 2000).

Results and discussion

Cytochrome P450 (CYP)-dependent alkylresorufin dealkylase activities

Determination of alkylresorufin *O*-dealkylase activities toward resorufins with alkyl substituents of various lengths and characteristics gives a remarkably good measure of the activities of various major CYP isoenzymes in a well comparable way (Burke et al. 1994; Nims et al. 1997; Raleigh et al. 1998). 7-Ethylresorufin *O*-deethylase (EROD) activity is preferentially due to the CYP1 family in human (and in the rat) (Burke et al. 1994), 7-pentylresorufin *O*-dependentylase (PROD) activity is preferentially due to the CYP2B family in human (and in the rat) (Burke et al. 1994), and 7-benzylresorufin *O*-debenzylase (BROD) is preferentially due to the CYP3A family in the mouse (Hagemeyer et al. 2010) and preferentially due to both the CYP2B family and CYP3A family in human (Niwa et al. 2003) and in the rat (Stresser et al. 2002).

All investigated alkylresorufin *O*-dealkylase activities (EROD, PROD and BROD) investigated in the microsomal fractions were below detection (<LOD) in all investigated passages of all four cell lines (three passages of each cell line) although the technical performance of the assays allowed for sensitive LODs and LOQs in the low pmol product/min/mg protein range (Table 1).

In basic agreement with these negative findings on the two keratinocytic cell lines, in a gene expression study by quantitative RT-PCR in primary human keratinocytes in culture, no mRNA expression of CYP2A6, CYP2B6 and CYP3A4 and only weak expression of CYP1A1 were observed (Table 5). Only CYP1B1 was moderately expressed (Henkler et al. 2011).

These results on gene expression are in predominant agreement and partial disagreement with earlier studies (Baron et al. 2001) reminding the necessity to take biological variability into account. Thus, Baron et al. (2001) had observed in primary human keratinocytes the expression of CYP1A1, 1B1, 2B6, 2E1 and 3A5 (no expression of CYP3A4) mRNA as well as the presence of the CYP1A1, 2B6, 2E1 and 3A protein. With respect to in vitro models of the whole skin and not just keratinocytes, in a gene expression study by quantitative RT-PCR in four organotypic skin models (all of them "full thickness", i.e., epidermis and dermis), no RNA expression of CYP1A2 and CYP2B6 was observed in three models and only weak expression in a fourth model, while CYP3A4 was only weakly expressed in

Table 1 Cytochrome P450 (CYP), flavin-containing monooxygenase (FMO) and UDP glucuronosyltransferase (UGT) activity determinations in the microsomal fractions

	Cytochrome P450[a]			FMO[a,b]	UGT[a]	
	EROD[b]	PROD[b]	BROD[b]		UGT-1[b]	UGT-2[b]
Cell lines[c]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Rat liver	1,200 ± 72	241 ± 7	436 ± 70	11.02 ± 1.46	12.6 ± 0.2	251,000 ± 32,000
LOD[d]	2.36	7.00	0.954	0.420	0.173	2,510
LOQ[e]	4.72	14.0	1.91	0.840	0.346	5,020

[a] Mean ± SD; CYP: pmol/min/mg protein; FMO and UGT-1: nmol/min/mg protein; UGT-2: FU/min/mg protein; CYP triplicate incubations, FMO and UGT duplicate incubations

[b] EROD, 7-ethylresorufin *O*-deethylase, PROD; 7-pentylresorufin *O*-dependentylase; BROD, 7-benzylresorufin *O*-debenzylase; FMO activities determined with benzydamine as substrate; UGT-1, UGT activity determined with the planar substrate 4-methylumbelliferone; UGT-2, UGT activity determined with the non-planar substrate 4-hydroxybiphenyl

[c] KeratinoSens® passages 5,7,8; LuSens passages 5,6,7; U937 passages 7,8,9; THP-1 passages 7,8,10

[d] LOD, level of detection, determined by adding 3 SDs to the mean results of 10 incubations in the presence of substrate but without protein

[e] LOQ, level of quantification, determined by multiplying the LOD by 2

all four models (Neis et al. 2010). However, CYP1A1 and CYP1B1 (as well as CYPs 2E1, 2J1, 3A5 and 4B1) were clearly expressed in all four organotypic skin models. Saeki et al. (2002) reported CYP mRNA expression for CYP1A1, 1B1 and 2E1 in cultured human Langerhans cells, keratinocytes, fibroblasts and melanocytes, while CYP2A6, 2C, 2D6, 3A5, 3A7 and 4B1 mRNA were expressed in a cell-type- and/or individual-specific manner and CYP1A2, 2A7, 2B6 and 3A4 mRNA were not detected.

If in the keratinocytic cell lines investigated in the present study CYPs of the 1A, 2B and/or 3A subfamilies were expressed, no or enzymatically inactive or too little CYP1A, CYP2B and CYP3A protein may have been formed for detecting EROD, PROD or BROD activities. Götz et al. (2010) also reported that they did not detect CYP isoenzymes in HaCaT keratinocyte cells, the cells of origin of the two keratinocytic cell lines used in the present study, and Götz et al. (2012a) reported that microsomes of human skin and the 3D human skin model EpiDerm (EPI-200) displayed no EROD, no 7-methylresorufin *O*-demethylase (MROD) (CYP1A2 substrate), no 7-methoxy-4-trifluoromethylcoumarin (MFC) *O*-dealkylase (CYP1/2 substrate) and no PROD activities above the LOD, while very low activities were observed using 7-benzyloxyquinoline (BQ) (CYP3A substrate) (76 ± 41 and 94 ± 13 pmol BQ/min/mg protein in skin and EPI-200 microsomes, respectively), values just exceeding the LOD. Also, EROD activity for HaCaT was below detection and using the primary human keratinocytes NHEK and keratinocytic cell line NCTC 2544 just above the LOD at 0.2–0.3 pmol/min/mg protein. CYP3A activity measured by BQ was detectable in the HaCaT and NCTC cell lines (38 ± 13 and 33 ± 5 pmol/min/mg protein, respectively), while no BQ activity was observed in NHEK. Baron et al. (2001) had observed low activities of EROD and PROD (10.7 and 1.43 pmol product/min/mg protein, respectively), and Hirel et al. (1995; 1996) low EROD activity (0.1–0.7 pmol/min/mg protein) in cultured primary human keratinocytes, and also Harris et al. (2002) had observed detectable EROD activity (about 79 pmol product/hour/106 cells) in cultured human keratinocytes. Hu et al. (2010) reported low (and interindividually similar) EROD activities in the 3D human skin model EpiDerm (EPI-200) cultures from four different donors, and Pham et al. had reported in 1990 low but measurable activities of EROD, PROD and BROD (0.55 ± 0.07, 0.63 ± 0.23 and 1.47 ± 0.28 pmol product/min/mg protein, respectively) in reconstituted human epidermis.

Determination of the alkylresorufin *O*-dealkylase activities (EROD, PROD and BROD) in the present study in rat liver microsomes as a positive control of the methodology showed that activities were detectable and quantifiable under the conditions used and that all three alkylresorufin *O*-dealkylase activities were found and quantifiable (Table 1). Their levels (EROD 1,200 ± 72 pmol/min/mg protein, PROD 241 ± 7 pmol/min/mg protein, BROD 436 ± 70 pmol/min/mg protein) were typical values for Aroclor 1254 pretreated male Wistar rats and are higher than the standard values in our laboratory (EROD 40.2 pmol/min/mg protein, PROD 29.3 pmol/min/mg protein, BROD 141.3 pmol/min/mg protein) in agreement with the Aroclor 1254 (500 mg/kg bw, 5 days) pretreatment of the rats used in this study in contrast to the untreated rats used for obtaining standard values.

Flavin-containing monooxygenase (FMO) activities

FMO is often responsible for the metabolism at soft nucleophilic centers (e.g., nitrogen or sulfur) of heteroatom-containing organic molecules (Oesch-Bartlomowicz and Oesch 2007). FMO activity was scored in the present study using benzydamine as substrate since it can be quantified with high sensitivity and is metabolized by several of the few FMO isoenzymes, with minimal contributions from CYPs, and therefore represents a useful in vitro probe for FMO activity in a wide range of tissues and cell types (Yeung and Rettie 2006). Benzydamine represents an especially good substrate for FMO1 and FMO3 (Stormer et al. 2000), the latter being expressed in the skin of the majority of human individuals (Janmohamed et al. 2001) [and being the major form of the human liver (Stormer et al. 2000)].

FMO activity for this model substrate benzydamine investigated in the microsomal fractions was below detection (<LOD) in all three passages of the tested four cell lines (Table 1).

In basic agreement with these negative findings, gene expression data by quantitative RT-PCR also showed that in human primary keratinocytes monolayer cultures FMO1 and FMO3 were not expressed (Table 5).

Determination of the benzydamine-*N*-oxide-forming activity in rat liver microsomes used as a positive control in the present study showed that FMO activity was easily detectable and quantifiable under the conditions used (Table 1). The level in rat liver microsomes (11.02 ± 1.46 nmol/min/mg protein) was much higher than the determined LOD (0.420 nmol product/min/mg protein) and LOQ (0.840 nmol product/min/mg protein), albeit lower than the values reported by Kawaji et al. (1993) in the literature (37 ± 3 nmol/min/mg protein). The lower levels observed in the present study are in agreement with the Aroclor pretreatment of the rats used in this investigation in contrast to the untreated rats used for obtaining the values reported by Kawaji et al. (1993) since in the experience of our laboratory, pretreatment of rats with Aroclor may lead to a decrease in FMO levels in the rat liver (König, unpublished observations).

Alcohol dehydrogenase (ADH) activities

ADH activities were determined in the cytosolic fraction using ethanol as substrate in the presence of semicarbazide in order to prevent the further reaction of the product by ALDH and the back reaction of the product by ADH.

The ADH activity was below the level of quantification (LOQ) in all three passages of the tested four cell lines (with one single outlier: numerically 30.1 nmol product/min/mg protein in passage 7 of the THP-1 cell line), in the majority of passages (7/12) even below detection (<LOD) (Table 2). The determined LODs 3.77–23.2 nmol product/min/mg protein and LOQs 7.55–46.5 nmol/min/mg protein in the various cell lines (Table 2) showed that the assays were performed under conditions of reasonable sensitivity. In contrast to all other enzyme activities investigated in this study, the LODs and LOQs for ADH were determined based on incubations with the cytosolic fractions of the individual cell lines but without substrate. Hence, the LODs and LOQs are different in the individual cell lines. The reason for this approach, which differs from that used for other enzyme activities in this study, is that for ADH, the incubations without protein, but with substrate resulted in decreasing absorption at 340 nm with time, possibly due to oxidation of NADH present in the sample.

In basic agreement with the predominantly negative findings of the present study, gene expression data by quantitative RT-PCR also showed that in human keratinocytes, primary cultures ADH1A and ADH1C were not expressed and ADH1B only weakly and only in the cultures derived from one of the two individuals investigated (Table 5).

Determination of ADH activity in rat liver cytosol as a positive control of the methodology showed that ADH activity was detectable and quantifiable under the conditions used (Table 2). The level in rat liver cytosol (13.5 ± 2.1 nmol/min/mg protein) was somewhat lower than the values reported in the literature by Kawashima et al. (2011) (27–33 nmol/min/mg protein), probably since in contrast to the study by Kawashima et al. in the present study, the rat liver was not homogenized in a medium containing Triton X 100.

Aldehyde dehydrogenase (ALDH) activities

ALDH, responsible for the detoxication of reactive aldehydes (Oesch-Bartlomowicz and Oesch 2007), was determined in the cytosolic fraction with propanal as substrate in the presence of 4-methylpyrazol in order to inhibit the reduction in propanal by ADH and in presence of an excess propanal in order to inhibit the back reaction of the product propionic acid to propanal.

ALDH activities, determined in the cytosolic fraction, were clearly detectable and quantifiable in all three passages of the two keratinocyte cell lines with twofold–threefold variation between passages (with no continuous trend from earlier to later passages), but quite similar activities in the two cell lines (4.77–16.4 nmol product/min/mg protein in the KeratinoSens® cells compared with 8.04–17.3 nmol product/min/mg protein in the LuSens cells, Fig. 1). The activities were above LOQ (3.91 nmol product/min/mg protein).

In basic agreement with the positive findings in the two keratinocytic cell lines, gene expression data by quantitative RT-PCR also showed that in human keratinocytes, primary cultures ALDH1A1 and ALDH2 mRNAs were expressed (Table 5).

In the two dendritic cell lines, however, the ALDH activity was in all passages below the LOQ, in the majority of passages (4/6) even below LOD (Table 3). The observation that in two passages of the cell line THP-1, the apparent

Table 2 Alcohol dehydrogenase activities in the cytosolic fractions

Cell line	Passage	Activities[a] (nmol/min/mg protein)	LOD[b] (nmol/min/mg protein)	LOQ[c] (nmol/min/mg protein)
KeratinoSens®	5	<LOD	16.1	32.3
	7	<LOD		
	8	<LOD		
LuSens	5	<LOQ	6.40	12.8
	6	<LOQ		
	7	<LOQ		
U937	7	<LOD	23.3	46.5
	8	<LOD		
	9	<LOD		
THP-1	7	30.1 ± 8.4	3.77	7.55
	8	<LOD		
	10	<LOQ		
Rat liver		13.5 ± 2.1	1.73	3.45

[a] Mean ± SD of triplicate incubations

[b] LOD, level of detection, determined by adding 3 SDs to the mean results of 10 incubations with the cytosolic fractions (pooled from the three passages), but without substrate

[c] LOQ, level of quantification, determined by multiplying the LOD by 2

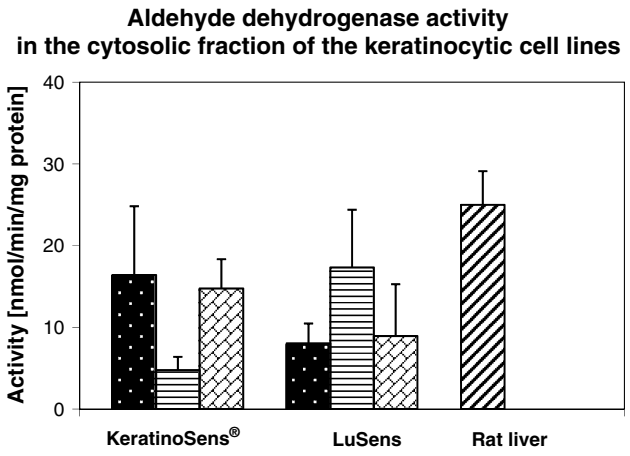

Fig. 1 Aldehyde dehydrogenase activity in the cytosolic fraction of two keratinocytic cell lines and Aroclor-induced male Wistar rat liver as a positive control. *KeratinoSens®*: *First bar* 5th passage, *second bar* 7th passage, *third bar* 8th passage. *LuSens*: *First bar* 5th passage, *second bar* 6th passage, *third bar* 7th passage. Values represent means ± SDs of triplicate incubations. Level of detection (LOD, determined by adding 3 SDs to the mean results of 10 incubations in the presence of substrate but without protein): 1.96 nmol product/min/mg protein; level of quantification (LOQ, determined by multiplying the LOD by 2): 3.91 nmol product/min/mg protein

Table 3 Aldehyde dehydrogenase activities in the cytosolic fractions of the dendritic cell lines

Cell line	Passage	Activities[a] (nmol/min/mg protein)
U937	7	<LOD
	8	<LOD
	9	<LOD
THP-1	7	<LOQ
	8	<LOD
	10	<LOQ
Rat liver		25.0 ± 4.0
LOD[b]		1.96
LOQ[c]		3.91

[a] Mean ± SD of triplicate incubations

[b] LOD, level of detection, determined by adding 3 SDs to the mean of 10 incubations in the presence of substrate but without protein

[c] LOQ, level of quantification, determined by multiplying the LOD by 2

activities were below LOQ, but above the LOD may indicate the presence of a borderline ALDH activity in this cell line, but verification of this would require further investigations beyond the scope of the present study. The fact that the determined LOD (1.96 nmol product/min/mg protein) and LOQ (3.91 nmol product/min/mg protein) were much below the activity which was determined in the rat liver cytosolic fraction (25.0 ± 4.0 nmol product/min/mg protein, Table 3) showed that the measurements were executed under conditions of reasonable sensitivity.

Esterase activities

Esterases are relevant cutaneous xenobiotic metabolizing enzymes. Predominantly esterases are catalyzing detoxication reactions (Oesch-Bartlomowicz and Oesch 2007), but they also are able to hydrolytically activate esters and amides shown in the skin or preparations derived from skin for 3-alkyl esters of naltrexone and other opioid antagonists, steroid-derived esters, and other prodrug esters such as ethylnicotinate, retinyl palmitate, vitamin E acetate, esters of temozolomide, methyl parahydroxybenzoate, 1-alkylazacycloalkan-2-one prodrug esters of ketoprofen, [Arg8]-vasopressin, Fluazifop-butyl and phenylacetate (Oesch et al. 2007; Wulferink et al. 2001; Barker and Clothier 1997; McCracken et al. 1993). Also, Bätz et al. (2013) have demonstrated esteratic cleavage of the prednisolone diester prednicarbate and of fluorescein diacetate in reconstructed human skin and reconstructed human epidermis as well as in excised human skin and monolayer cultures of normal and immortalized human keratinocytes and of fibroblasts.

Esterase activity was monitored in the S9 fractions by determining the hydrolysis of fluorescein diacetate. All four cell lines tested clearly had esterase activity (Fig. 2), all of them way above the determined LOD (22.7 pmol

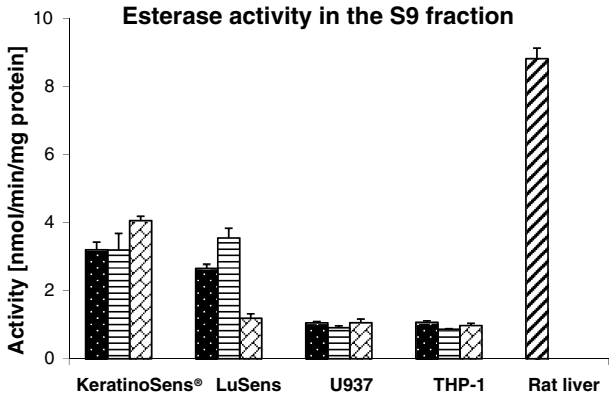

Fig. 2 Esterase activity in the S9-fraction of two keratinocytic cell lines (*KeratinoSens®* and *LuSens*), two dendritic cell lines (*THP-1* and *U937*) and Aroclor-induced male Wistar rat liver as a positive control. *KeratinoSens®*: *first bar* 5th passage, *second bar* 7th passage, *third bar* 8th passage. *LuSens*: *first bar* 5th passage, *second bar* 6th passage, *third bar* 7th passage. *THP-1*: *first bar* 7th passage, *second bar* 8th passage, *third bar* 10th passage. *U937*: *first bar* 7th passage, *second bar* 8th passage, *third bar* 9th passage. Values represent means ± SDs of triplicate incubations. Level of detection (LOD, determined by adding 3 SDs to the mean results of 10 incubations in the presence of substrate but without protein): 22.7 pmol product/min/mg protein; level of quantification (LOQ, determined by multiplying the LOD by 2): 45.4 pmol product/min/mg protein

product/min/mg protein) and LOQ (45.4 pmol product/min/mg protein). The keratinocytic cell lines had with 1.19–4.06 nmol product/min/mg protein a somewhat higher activity than the dendritic cell lines with 0.869–1.07 nmol product/min/mg protein (Fig. 2). The keratinocytic cell line KeratinoSens® appeared to have a slightly higher esterase activity (3.20–4.06 nmol product/min/mg protein) compared with the LuSens keratinocytic cell line (1.19–3.55 nmol product/min/mg protein), while no difference in the esterase activities was apparent between the two dendritic cell lines THP-1 and U937 (0.869–1.07 nmol product/min/mg protein, Fig. 2).

All cell lines tested in this study had remarkable esterase activities, all of them within about one order of magnitude compared with the activity observed in Aroclor 1254-induced rat liver S9 as a positive control (0.869–4.06 nmol product/min/mg protein in the tested cell lines compared with 8.82 ± 0.31 nmol product/min/mg protein in rat liver S9).

UDP glucuronosyltransferase (UGT) activities

UGT activities were determined in the microsomal fractions using the planar 4-methylumbelliferone as a preferential substrate for the UGT family 1, the predominantly expressed UGT family in the skin (Hu et al. 2010), and the non-planar 4-hydroxybiphenyl as a preferential substrate for the UGT family 2 (for UGTs only these two families exist [Arand and Oesch 2004]).

The UGT1 activity was below detection (<LOD) in all three passages of the tested four cell lines (Table 1). The determined LOD (0.173 nmol product/min/mg protein) and LOQ (0.346 nmol product/min/mg protein) showed that the assays were able to detect and to quantify product in the subnanomol product/min/mg protein range (Table 1).

In contrast to the undetected enzymatic activity, gene expression data by quantitative RT- PCR showed that in human keratinocytes primary cultures, UGT1A10 was highly expressed (Table 5). Moreover, Götz et al. (2010) reported that they were able to determine a UGT1 activity in HaCaT cells, the precursor cell line of the two keratinocytic cell lines used in this study, and Götz et al. (2012b) reported that UGT activity determined with 4-methylumbelliferone as substrate was measurable in microsomes of ex vivo human skin (1.3 ± 0.2 nmol/min/mg protein) and in the 3D human skin model EpiDerm (EPI-200) (1.8 ± 0.2 nmol/min/mg protein). Hu et al. (2010) reported "significant" (about 35–50 % conversion) UGT activity with 4-methylumbelliferone as a substrate in EpiDerm (EPI-200) cultures from three donors.

In the present investigation, determination of UGT1 activity in rat liver microsomes as a positive control of the methodology showed that the UGT1 activity (12.6 ± 0.2 nmol product/min/mg protein) was easily detectable and quantifiable under the conditions used (two orders of magnitude above the LOD; Table 1). This level in rat liver microsomes (12.6 ± 0.2 nmol product/min/mg protein) was in the same order of magnitude as reported previously from our laboratory (8.47 ± 0.87 nmol product/min/mg protein: Jäckh et al. 2011), but lower than the values reported in the literature by Okamura et al. (2006) (95 nmol product/min/mg protein), probably since in contrast to the methodology used in our investigations in the study by Okamura et al., a preincubation (of 30 min) was used and UGT is known to be a latent enzyme (i.e., an enzyme the in vitro activity of which is increased by various means including preincubation) (Burchell et al. 1976).

The UGT2 activity also was below detection (<LOD) in all three passages of all four cell lines tested (Table 1). Determination of UGT2 activity in rat liver microsomes as a positive control showed that UGT2 activities were easily detectable and quantifiable under the conditions used: the determined UGT2 activity in rat liver microsomes (251,000 ± 32,000 fluorescence units/min/mg protein) was much higher than the determined LOD (2,510 fluorescence units/min/mg protein) and LOQ (5,020 fluorescence units/min/mg protein) (Table 1).

N-acetyl transferase 1 (NAT1) activities

NAT is expressed in human skin and represents a contributor to the direct detoxication in the human skin of aromatic amines including hair dye ingredients (Garrigue et al. 2006; Kawakubo et al. 2000; Nohynek et al. 2005). Human NAT1 is practically ubiquitously expressed, while NAT2 is primarily localized to the liver (with some expression in the intestine) (Husain et al. 2007). Accordingly, NAT1 but not NAT2 mRNA was found in both neonatal and adult human epidermal keratinocytes and these cells were able to N-acetylate dapsone and sulfamethoxazole, a detoxication reaction against the delayed-type hypersensitivity caused by these drugs (Reilly et al. 2000). Therefore, only NAT1, but not NAT2, was determined in the present study.

Determination of NAT1 was performed in the S9 fractions with *para*-aminobenzoic acid (PABA) as selective substrate of human NAT1 (Hein et al. 1993; Butcher et al. 2000). All three passages of all four investigated cell lines had observable and quantifiable NAT1 activity, which with 6.00–60.2 nmol product/min/mg protein (Fig. 3) was in all cases much higher than the LOD of 0.667 pmol product/min/mg protein and the LOQ of 1.33 pmol product/min/mg protein.

Götz et al. (2012b) also had reported NAT activities with *para*-toluidine, a substrate of human NAT1 and NAT2 with preference for NAT1 (Liu et al. 2007) in the living monolayer keratinocyte cell line cultures of HaCaT, the cells of

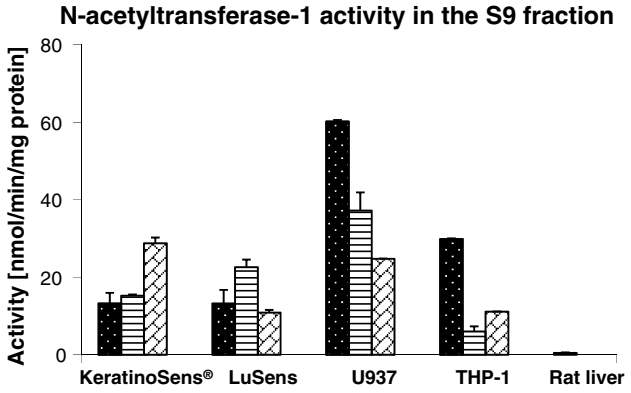

Fig. 3 *N*-acetyltransferase activity in the S9-fraction of two keratinocytic cell lines (*KeratinoSens*® and *LuSens*), two dendritic cell lines (*THP-1* and *U937*) and Aroclor-induced male Wistar rat liver as a positive control. *KeratinoSens*®: *first bar* 5th passage, *second bar* 7th passage, *third bar* 8th passage. *LuSens*: *first bar* 5th passage, *second bar* 6th passage, *third bar* 7th passage. *THP-1*: *first bar* 7th passage, *second bar* 8th passage, *third bar* 10th passage. *U937*: *first bar* 7th passage, *second bar* 8th passage, *third bar* 9th passage. Values represent means ± SDs of duplicate incubations. Level of detection (LOD, determined by the noise to peak ratio of the acetylated PABA): 0.667 pmol product/min/mg protein; level of quantification (LOQ, determined by multiplying the LOD by 2): 1.33 pmol product/min/mg protein

origin of the two keratinocytic cell lines used in the present study (0.65 ± 0.37 nmol/min/mg protein) and in the keratinocytic cell line NCTC (0.35 ± 0.22 nmol/min/mg protein) as well as the primary human keratinocyte NHEK cells in culture (0.16 ± 0.08 nmol/min/mg protein) [and also in the cytosolic fraction of ex vivo human skin and in the 3D human skin model EpiDerm (EPI-200) (0.6–1.4 nmol product/min/mg protein)].

In the present study, the activity in the rat liver S9 fraction was lower (0.466 ± 0.152 nmol product/min/mg protein) than in any of the three passages of any of the four cell lines tested (Fig. 3). The activity in the rat liver S9 fraction is in line with low activities of NAT1 reported in the literature for rat liver cytosol (0.5–1.5 nmol product/min/mg protein) (Hein et al. 2008).

The relatively high NAT1 activities observed in all passages of all cell lines investigated are of practical relevance, since cutaneous NAT1 is involved in the metabolic control of many sensitization reactions (Schnuch et al. 2011; Lichter et al. 2008; Oesch et al. 2007).

In the dendritic cell line U937, NAT1 activities seemingly decreased with increasing passages (Fig. 3), but this is likely an artifact due to the fact that in contrast to all other NAT1 activity measurements in this study in the last two passages of the U937 cell line, the notoriously labile NAT1 activity was not determined on the day of the S9 preparation. On the other hand, the NAT1 activities seemingly increased with increasing passages in the KeratinoSens® keratinocytic cell line (Fig. 3). Whether the apparent decreases in NAT1 activities in the U937 cells and/or increases in the KeratinoSens® cells are causally related to the increasing number of passages would require further examination, which is beyond the scope of the present study. The fact that in the LuSens keratinocyte cell line and in the THP-1 dendritic cell line no continuous trend of increasing or decreasing NAT1 activity with increasing passages was observed (Fig. 3) indicates that the apparent trends of NAT1 changes with increasing passages in the KeratinoSens® and U937 cells may not reflect a causal relationship.

NADPH cytochrome *c* reductase activities

Since in all four cell lines studied the investigated microsomal CYP, FMO and UGT enzyme activities were below detection, the activity of the ubiquitous microsomal enzyme NADPH cytochrome *c* reductase (Shen and Kasper 1993) was determined as a quality control for the microsomal fractions. The NADPH cytochrome *c* reductase was clearly active in all used passages of all four cell lines (Table 4).

Table 4 NADPH cytochrome *c* reductase activities in the microsomal fractions

Cell line	Passage	Activity (units/mL microsomal preparation)[a]	
		Individual passage	Average[b]
KeratinoSens®	5	5.63×10^{-3}	
	7	3.70×10^{-3}	
	8	2.71×10^{-3}	$4.01 \pm 1.48 \times 10^{-3}$
LuSens	5	2.43×10^{-3}	
	6	7.45×10^{-3}	
	7	4.62×10^{-3}	$4.83 \pm 2.51 \times 10^{-3}$
U937	7	1.60×10^{-2}	
	8	1.05×10^{-2}	
	9	3.18×10^{-2}	$1.94 \pm 1.10 \times 10^{-2}$
THP-1	7	1.22×10^{-2}	
	8	5.06×10^{-3}	
	10	1.52×10^{-2}	$1.08 \pm 0.52 \times 10^{-2}$
Rat liver		4.11×10^{-2}	
Rabbit liver		1.33×10^{-2}	
LOD[c]		3.02×10^{-4}	
LOQ[d]		6.04×10^{-4}	

[a] Units defined as the amount of protein required to reduce 1 μmol oxidized cytochrome *c* per min at 25 °C in the presence of 100 μM NADPH

[b] Mean ± SD of duplicate incubations

[c] LOD, level of detection, determined by adding 3 SDs to the mean of 6 incubations in presence of substrate but without protein

[d] LOQ, level of quantification, determined by multiplying the LOD by 2

Table 5 Expression of various xenobiotic enzyme genes in human keratinocytes primary cultures

MOs	mRNA expression[a]	Other XMEs	mRNA expression[a]
CYP1A1	+	ADH1A	−/−
CYP1B1	++	ADH1B	−/+
CYP2A6	−	ADH1C	−/−
CYP2B6	−	ALDH1A1	+/+
CYP2E1	+	ALDH2	+/+
CYP3A4	−	NAT1	+/++
FMO1	−/−	NAT2	−/−
FMO3	−/−	GST p1	++/++
		UGT1A10	++/++

Henkler et al. (2011), details to be published elsewhere

−: Not detected; +: weak expression (ΔCt > 10); ++: moderate expression (ΔCt 5–10); +++: strong expression (ΔCt < 5); data were obtained from monolayer cultures of keratinocytes, when the keratinocytes were derived from two donors: donor 1/donor 2

MO monooxygenase, *CYP* cytochrome P450, *FMO* flavin-containing monooxygenase, *XME* xenobiotic metabolizing enzyme, *ADH* alcohol dehydrogenase, *ALDH* aldehyde dehydrogenase, *NAT* *N*-acetyltransferase, *GST* glutathione S-transferase, *UGT* UDP glucuronosyltransferase

[a] mRNA expression quantified by quantitative real-time PCR (qPCR) using TaqMan DNA probes (Applied Biosystems, Darmstadt, Germany), the Ct (cycle threshold) of the gene of interest subtracted from the Ct β-actin = ΔCt

The activities (2.43×10^{-3}–3.18×10^{-2} units/mL microsomal preparation) were unambiguously above the LOD (3.02×10^{-4} units/mL microsomal preparation) and above the LOQ (6.04×10^{-4} units/mL microsomal preparation) and similar to the positive controls, rat and rabbit liver, which had activities of 4.11×10^{-2} and 1.33×10^{-2} units/mL microsomal preparation, respectively (Table 4), confirming that the microsomal preparations used were valid, enzymatically active in vitro preparations.

Conclusion

In line with the well-known fact that during dedifferentiation of primary cells to cell lines differentiated functions of xenobiotic metabolism in many cases decrease or are lost (Glatt et al. 1987, 1990), the activities of several xenobiotic metabolizing enzymes were below detection in the keratinocytic and dendritic cell lines investigated in the present study. This may, in many cases, just be the price to be paid for the improved culturability of cell lines compared with their parent primary cells.

However, quite remarkably, several xenobiotic enzyme activities were clearly observable and quantifiable in the cell lines investigated. NAT1, which in living mammalian organisms is quite ubiquitously distributed (including human skin: Oesch et al. 2007), was well conserved and highly active (in the higher nmol product/min/mg protein range) in all investigated passages of keratinocytic and dendritic cell lines studied. This is of high practical relevance, since cutaneous NAT1 is involved in the metabolic control of many sensitization reactions (Schnuch et al. 2011; Lichter et al. 2008; Oesch et al. 2007). Also, the esterase activity was well conserved and relatively high (in the nmol product/min/mg protein range) in all investigated passages of keratinocytic and dendritic cell lines studied. Also this is of high practical relevance, since many esters and amides are detoxified while others are activated by cutaneous esterases (Oesch et al. 2007; Wulferink et al. 2001). In the keratinocytic cell lines, ALDH was quantifiable and present with relatively high activities (in the nmol product/min/mg protein range). ALDH plays a major role for the detoxication of reactive aldehydes (Oesch et al. 2007).

Several xenobiotic metabolizing enzyme activities were not detected. This was true for the investigated CYP, FMO, ADH and UGT activities.

The xenobiotic metabolizing enzyme activities in the two keratinocytic cell lines (Tables 1, 2, 3; Figs. 1, 2, 3) were in predominant agreement with the observations in gene expression studies in primary keratinocytes (Table 5), which indicates that the loss of xenobiotic metabolism in the cell lines compared with their parent primary cells was rather moderate. As described in more details above under "Results and discussion", overall similarities between expression in primary keratinocytes and enzyme activities in the two keratinocytic cell lines were observed for FMO, ADH, ALDH and NAT1, predominant similarities, but also some differences between expression in primary keratinocytes and enzyme activities in the two keratinocytic cell lines were observed for CYP and quite basic differences for UGT. The investigated CYP activities were below detection in the two keratinocytic cell lines. In some studies in primary keratinocytes reported in the literature, some CYP activities were detected, but in some other studies in primary keratinocytes, CYP activities were below detection (discussed in detail in the "Results and discussion" section) (see especially Götz et al. 2012a). UGT1A10, known to catalyze glucuronidation of a wide range of structurally diverse chemicals including 4-methylumbelliferone (Xiong et al. 2006), one of the two substrates used in the present investigation, was clearly expressed on the mRNA level in primary keratinocytes (Table 5), while the enzymatic UGT activities were below detection in the keratinocytic cell lines investigated in this study (Table 1).

With respect to the xenobiotic metabolizing enzymes in the dendritic cell lines investigated in the present study, to the best of our knowledge, there are no data in corresponding primary cells with which a comparison could be attempted.

All in all, the similarities of xenobiotic metabolizing enzymes in the investigated keratinocytic cell lines compared with primary keratinocytes are quite remarkable and the dendritic cell lines investigated also possess a remarkable repertoire of xenobiotic metabolizing enzymes. In the keratinocytic and dendritic cell lines investigated, some relevant enzymes were, however, missing, more so in the dendritic cell lines compared with the keratinocytic cell lines. Dendritic cells may inherently possess less xenobiotic metabolizing competence compared with keratinocytes.

The information gained in the present study is of evident importance for a better interpretation of results obtained with the investigated cell lines. Moreover, for possible attempts to optimize an in vitro test battery for skin sensitization potential, the results will obviously be helpful.

Conflict of interest Some of the authors were involved in the development of skin sensitization in vitro assays.

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