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Original Article

Xenobiotic metabolism capacities of human skin in comparison with a 3D epidermis model and keratinocyte-based cell culture as *in vitro* alternatives for chemical testing: activating enzymes (Phase I)

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Abstract: Skin is important for the absorption and metabolism of exposed chemicals such as cosmetics or pharmaceuticals. The Seventh Amendment to the EU Cosmetics Directive prohibits the use of animals for cosmetic testing for certain endpoints, such as genotoxicity; therefore, there is an urgent need to understand the xenobiotic metabolizing capacities of human skin and to compare these activities with reconstructed 3D skin models developed to replace animal testing. We have measured Phase I enzyme activities of cytochrome P450 (CYP) and cyclooxygenase (COX) in ex vivo human skin, the 3D skin model EpiDerm™ (EPI-200), immortalized keratinocyte-based cell lines and primary normal human epidermal keratinocytes. Our data demonstrate that basal CYP enzyme activities are very low in whole human skin and EPI-200 as well as keratinocytes. In addition, activities in monolayer cells differed from organotypic tissues after induction. COX activity was similar in skin, EPI-200 and NHEK cells, but was significantly lower in immortalized keratinocytes. Hence, the 3D model EPI-200 might represent a more suitable model for dermatotoxicological studies. Altogether, these data help to better understand skin metabolism and expand the knowledge of *in vitro* alternatives used for dermatotoxicity testing.

Abbreviations: CYP, cytochrome P450-monooxygenase; COX, cyclooxygenase; PGE_2, prostaglandin E2; 3-MC, 3-methylcholanthrene; EPI-200, reconstituted epidermis model EpiDerm™ (MatTek); NHEK, normal human keratinocytes; HLM, human liver microsomes.

Key words: cyclooxygenase – cytochrome P450-monooxygenase – skin – xenobiotic metabolism

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Introduction

Skin is the largest organ and represents the body's protective surface as the first and outermost contact site for topically applied substances. Beside the skin's important tasks in preventing mechanical and physical harm, its potential to detoxify chemicals often remains unconsidered. However, skin plays a key role in controlling not only the penetration and distribution but also the metabolism of topically applied chemicals (1) and is thus a first-pass organ for penetrating substances. Numerous examples exist showing that skin metabolism is not only protective but also contributes to skin pathologies such as contact dermatitis or carcinogenesis (2,3). This implies a need for safety assessment for all ingredients of cosmetic products and the need to employ appropriate models to test chemical ingredients for their potential to cause skin irritation, sensitization, as well as genotoxic damage.

Animal testing was the method of choice for dermatotoxicological testing for decades. Decreasing acceptance of animal use in cosmetic testing in the public, arising from ethical concerns, forces the revision of traditional procedures and the establishment of alternative methods. In addition, animal testing for cosmetics was prohibited by the Seventh Amendment to the EU Cosmetics Directive, including skin irritation and genotoxicity. The need for adequate substituting test models is evident, but even though several alternative skin models are available rare knowledge exists about the resemblance of their catalytic activities (4) compared to native human skin.

For years, keratinocyte-derived cell lines have been used for the investigations of mechanisms of action of dermatotoxic compounds or the prevention of skin diseases (5–11) as they represent a cost- and time-effective tool to address these questions. They may also be appropriate to explore human metabolic interindividual differences (12). The most critical issues concerning immortalized keratinocyte-based monolayer models have been the lack of three-dimensional tissue (3D) properties as well as accumulation of spontaneous chromosomal aberrations. Owing to these insufficiencies, 3D human epidermal equivalents were developed over the last years (13–15). Such 3D skin models consist of primary human keratinocytes that are grown at the air–liquid interphase and differentiate into a multilayered 3D tissue. They were already examined, for example, by COLIPA for their suitability for genotoxicity testing (16–18). However, until now, a systematic assessment of xenobiotic metabolism capacities of monolayer cultures

in comparison with human skin and 3D models has never been performed.

Therefore, the aim of this study was the characterization of the xenobiotic metabolizing activities of native human skin compared with *in vitro* models such as (i) the commercially available 3D epidermal model EpiDerm™ (EPI-200; MatTek MatTek Corporation) based on human primary keratinocytes and (ii) several human skin-derived cell monolayers. The latter one including the well-known keratinocyte-based cell lines, HaCaT (19) and NCTC 2544 (20) as well as primary human epidermal keratinocytes (NHEK). For use in dermatotoxicology, it is necessary that alternative testing methods reproduce xenometabolic pathways of human skin so that the results of *in vitro* testing are meaningful. Particular concern for the evaluation of the toxicity of compounds is the activation of genotoxic compounds via CYP and also the effect of compounds to induce the activity of these enzymes. To characterize the enzyme metabolism of skin models compared with human skin in this study, cells and EPI-200 epidermal models were primarily treated with 3-methylcholanthrene (3-MC) as a prototypical arylhydrocarbon receptor agonist and CYP inducer.

Materials and methods

Chemicals and materials

All chemicals, if not otherwise specified, were purchased from Sigma-Aldrich (Taufkirchen, Germany) in highest purity available. Cell culture media were obtained from PAA (Pasching, Austria) and PromoCell (Heidelberg, Germany), the DC protein quantification kit from BioRad (Munich, Germany). Prostaglandin E2 EIA monoclonal and fluorescent COX kit was acquired from Cayman Chemical Company (Ann Arbor, MI, USA). Liver microsomes were bought from CELSIS In Vitro GmbH (Neuss, Germany).

Keratinocyte/tissue culture

HaCaT cells were cultured in DMEM, NCTC 2544 and A431 cells were cultured in MEM each containing 10% (v/v) fetal calf serum (FCS) and 1% (v/v) of antibiotic/antimycotic solution (PAA; Cat. No. P11-002). A431 were supplemented with non-essential amino acids (PAA). Primary NHEK keratinocytes from 30 to 40-year-old female donors were cultured in KGM-2 containing supplements from Promocell. For subsequent enzyme assays, media were FCS-free if not otherwise specified.

The human reconstituted epidermis EpiDerm™ (EPI-200) was purchased from MatTek Corporation, Ashland, MA, USA (donor 1188 and for one experiment 254). After delivery, the tissue recovered for 24 h at 37°C and 5% CO_2 in serum-free EPI-100-NMM-PRF media (900 μl per model). Induction experiments were carried out in EPI-100-NMM-PRF medium.

Enzyme induction

Cells and EPI-200 epidermal models were treated with 3-methylcholanthrene (3-MC), rifampicin (RIF), dexamethasone (DEX) and 6-(4-chlorophenyl:imidazo[2,1-b]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime [CITCO, (21) in DMSO (stock concentration 10, 50, 100 and 10 mM, respectively)] and cyclophosphamide (CP, 50 mM in PBS). Incubation times for induction were 24 h (CYP1), 48 h (CYP3A) and 72 h (CYP2) unless otherwise noted [time-points chosen and adjusted in accordance with hepatocyte data (21)]. Maximum solvent concentration was 0.1%.

Cell viability, cytotoxicity and protein content assessment

Assay kits for the measurement of cell viability (Cell Titer Blue and CytoTox One; Promega GmbH, Mannheim, Germany) were applied according to the manufacturer. Protein in monolayer cell culture was determined using CBQCA protein quantification kit (Molecular Probes; Invitrogen, Germany) and bovine serum albumin as reference protein.

Human *ex vivo* skin samples

Skin samples were obtained from the hospital Kaiserswerther Diakonie in Düsseldorf, Germany. The samples originated from female patients of different age and unknown pharmacological background. Patients were informed and agreed to donate removed tissue for scientific purpose. The use of *ex vivo* skin from breast reduction has been fully approved by the local Ethics Committee at the Heinrich-Heine-University of Düsseldorf. Skin samples were collected immediately after surgery and kept cold during the transport (<1 h).

Preparation of subcellular fractions from human *ex vivo* skin and EPI-200 models

Microsomal and cytosolic fractions from human skin were prepared in accordance with previously described methods (22) using a Ultra Turrax T25 homogenizer (IKA, Staufen, Germany). The number of individual microsomal preparations was $n = 8$ for EPI-200 and $n = 10$ for skin biopsies. The functionality of the preparation protocol was confirmed by comparing CYP enzyme activities of commercially available mouse liver microsomes with obtained results of in house preparation of mouse microsomes that were highly comparable (data not shown).

Cytochrome P450 enzyme activity assays

CYP1/2B activities were measured via resorufin-based substrates (Data S1). Dicoumarol (10 μM) was added to all resorufin-based CYP experiments to prevent metabolism of the reaction product resorufin by NQO1 (23) and Phase II enzymes.

The multi-CYP1/2 substrate 7-methoxy-4-trifluoromethylcoumarin was assayed according to the method described by (24).

CYP3A was assayed using 7-benzyloxyquinoline as substrate according to (25). The luminescent Luc-BE CYP3A-assay was carried out as recommended by the manufacturer (Promega; Data S1).

Cyclooxgenase enzyme activity assays

PGE_2 formation as a measure for COX-2 activity (26) was measured using a monoclonal EIA and a COX fluorescent activity assay kit (Cayman Chemical Company, Ann Arbor, MI, USA; Data S1).

Statistics

All experiments were conducted at least $n = 3$. Using the Student's unpaired t-test; $P < 0.05$ was considered significant. Limit of detection (LOD) was defined as mean of blank measurements plus three times standard deviation of the blank. LOQ was defined as the twofold LOD.

Results

Xenobiotic Phase I metabolism in human skin and EPI-200 models

Performance of all CYP assays was verified with rat, mouse, minipig and human liver microsomes (HLM). All of those displayed substantial hepatic enzyme activities for CYP1A1/1B1 (EROD), CYP1A2 (MROD), CYP2C9 (MFC *O*-dealkylase) and CYP3A (BQ-dealkylation and luminescence generated from Luc-BE), whereas no CYP2B6 activity (PROD) was seen above the limit of detection (LOD; <0.2 pmol/min/mg in resorufin dealkylase assays) in any human sample (Fig. 1a–e). In contrast to liver, human skin and EPI-200 microsomes displayed no EROD, MROD, MFC *O*-dealkylase and – in common with the human liver samples – no PROD activities above the LOD.

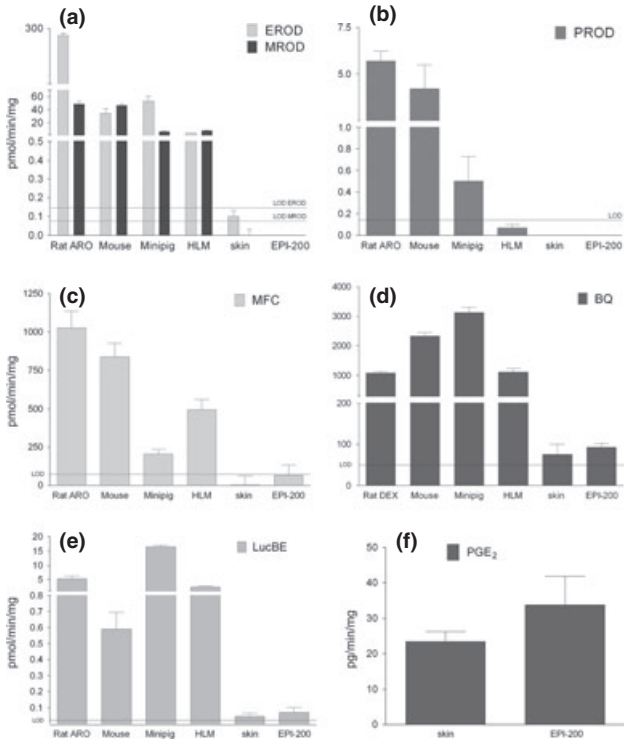

Figure 1. Specific CYP activity (pmol/min/mg) was examined in positive control microsomes from liver compared with untreated human skin (n = 10) and EPI-200 (n = 8) microsomes. Rat liver microsomes (Rat ARO) were from Aroclor 1254-induced animals, all other microsomes were non-induced samples. LOD = limit of detection. Specific PGE2 formation (pg/min/mg) was examined in untreated human skin (n = 10) and EPI-200 (n = 8) microsomes. (a) Activity of CYP1 measured by ethoxyresorufin *O*-deethylation (EROD) and methoxyresorufin *O*-demethylation (MROD) assays. (b) Activity of CYP2B measured by pentoxyresorufin *O*-depentylation (PROD). (c) Activity of CYP1 and CYP2 measured by the broad spectrum substrate MFC. (d) Activity of CYP3A measured by the substrate BQ. (e) Activity of CYP3A measured by the substrate LucBE. (f) Basal levels of PGE_2 production in skin and EPI-200 microsomes. The data are shown as pg PGE_2/min/mg.

In contrast to resorufin dealkylase activities, BQ- and Luc-BE (CYP3A) rates were 76 (±41) and 94 (±13) pmol BQ/min/mg and 0.05 (±0.03) and 0.08 (±0.05) pmol Luc-BE/min/mg in skin and EPI-200 microsomes, respectively. These values just exceeded the given LOD. In the study conducted by our co-workers (BASF), CYP3A examined via BROD also remained below their respective LOQ. These data indicate very low to undetectable basal enzyme activities in human skin as well as in the EPI-200 model.

CYP-mediated biotransformation is increased upon treatment with specific inducers. Therefore, we investigated CYP induction in intact EPI-200 models by systemic application of prototypical inducers. In intact models, basal EROD, MROD, PROD and MFC activities were below the LOD and the BQ turnover was 5.5 (±0.9) pmol/min/mg (Fig. 2b) confirming our measurements in microsomes. EROD and MROD activities, however, were inducible by 3-MC after 24 h in a concentration-dependent fashion up to 1.7 (±0.8) pmol/min/mg for EROD and 0.7 (± 0.3) pmol/min/mg for MROD (Fig. 2a). Basal and rifampicin- or CITCO-induced (72 h) PROD and MFC activities remained below the LOD. CYP3A4 activity was not inducible by rifampicin or dexamethasone after 48 h (Fig. 2b).

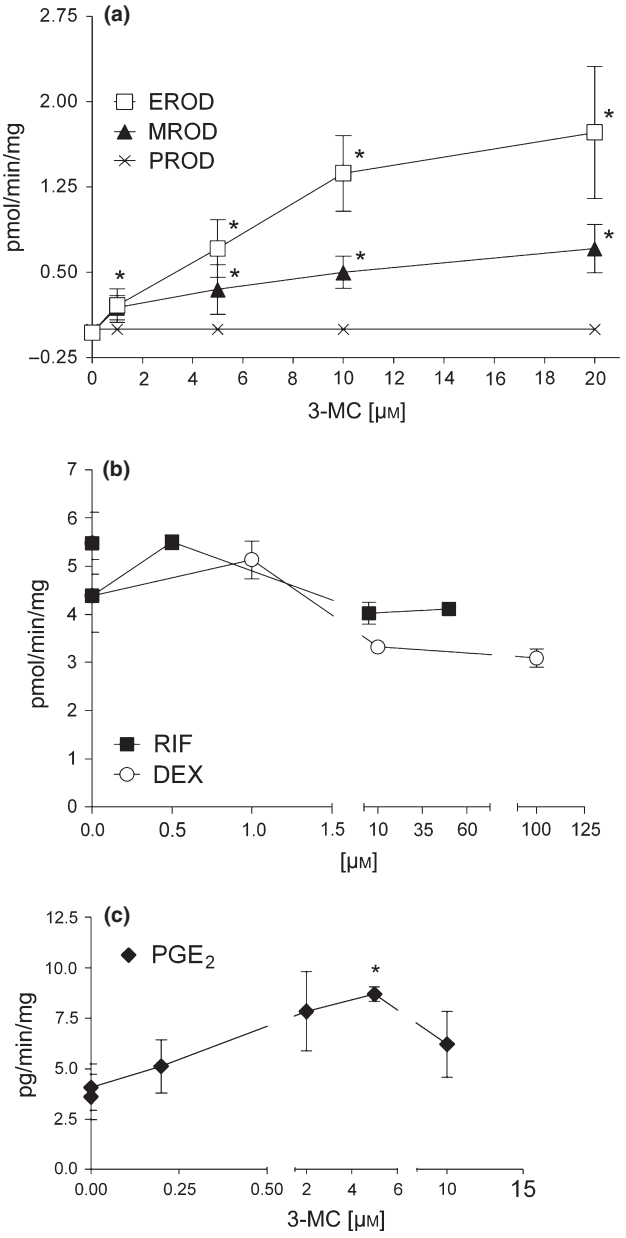

Figure 2. Induction of enzyme activity in intact EPI-200 models by the model compounds 3-MC, RIF and DEX (μM). The asterisks indicate significant difference (P = 0.05) to solvent (DMSO) control. (a) Induction of CYP1 (EROD, MROD) and CYP2B (PROD) in intact EPI-200 models induced by the model compound 3-MC (μM). PROD induction was additionally examined by specific CYP2B inducer treatment (CITCO and cyclophosphamide) that leads to the same result as depicted here. Activity is shown as pmo/min/mg. (b) Induction of CYP3A in intact EPI-200 models induced by rifampicin (RIF) and dexamethasone (DEX). Activity (pmol/min/mg) was determined via benzyloxyquinoline (BQ) assay. (c) PGE_2 production in intact EPI-200 upon 3-MC (μM) treatment. The data are shown as pg PGE_2/min/mg.

Basal COX activity in microsomes was present (23.5 ± 8.7 and 33.7 ± 19.8 pg/min/mg of PGE_2 formation; Fig. 1f). Basal PGE_2 production in intact EPI-200 tissue was about 10-fold lower (3.6 ± 1.9 pg/min/mg). After treatment with 3-MC, PGE_2 formation in intact tissues increased approximately twofold to 7.6 pg/min/mg within 24 h in a concentration-dependent manner (Fig. 2c).

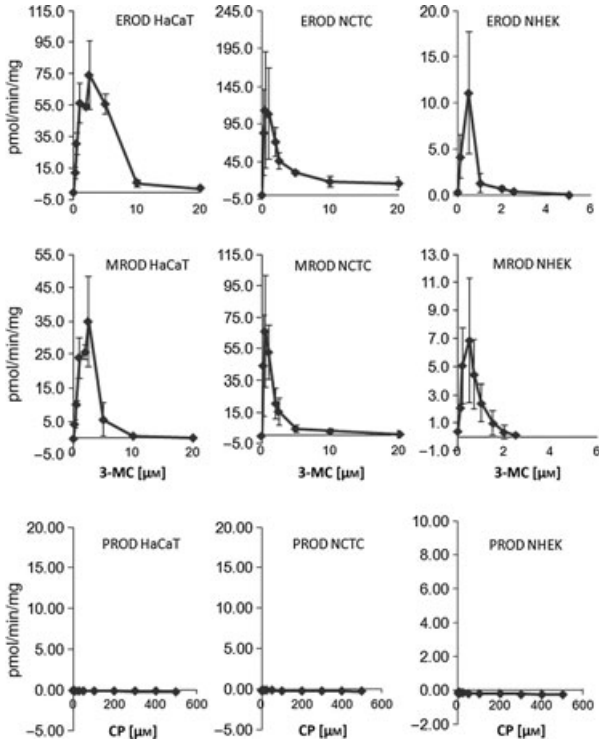

Figure 3. Induction of enzyme activity in immortalized cell lines (HaCaT, NCTC) and primary keratinocytes (NHEK) by the compounds 3-MC and CP (μM). Induction of CYP1 (EROD, MROD) activity was induced by 3-MC (μM). PROD induction was carried out using rifampicin, CITCO and cyclophosphamide (72 h). The latter compound is depicted here (CP), but the same result was obtained with the other two CYP2B inducers. Activity is shown in pmol/min/mg.

Interindividual variability and the duration of inducer treatment may impact on enzymatic activities. Therefore, basal microsomal CYP1A/1B and COX activities were measured in EPI-200 models from two different donors (donors 1188 and donor 254) after 24, 48 and 72 h upon tissue arrival. Independently from donor and timepoint after arrival, EROD activity was below the LOD. Also PGE_2 formation over time was similar under all conditions tested (Fig. S1).

Xenobiotic Phase I metabolism in cell lines

As shown in Fig. 3, basal EROD activity for HaCaT was below detection and for NCTC and NHEK cells just above the LOD at 0.2–0.3 pmol/min/mg. EROD and MROD activities were increased by 3-MC in all three cell lines (Fig. 3). At 5 μM 3-MC, the highest EROD and MROD induction was seen in NCTC 2544 with 114 ± 77 and 70 pmol/min/mg, respectively. At that concentration, HaCaT cells showed 75 pmol/min/mg for EROD and 35 pmol/min/mg for MROD. NHEKs displayed maximal EROD activity with 2.5 μM 3-MC (11.1 ± 6.6 pmol/min/mg). Similar results were obtained using MFC (data not shown). Thus, immortalized cell lines like NCTC 2544 and HaCaT are more responsive to CYP induction by 3-MC than primary keratinocytes. Moreover, inducer concentrations for maximum differ between cell types (Fig. 3).

The ability to induce CYP2B in hepatocytes decreases rapidly when cells are transferred to monolayer cultures (27,28). To find out whether this is also the case for keratinocyte cultures, we tested the inducibility of CYP2B (PROD) by rifampicin (RIF, 0–50 μM), CITCO (0–10 μM) and cyclophosphamide (CP, 0–500 μM), which was reported to induce CYP2B6 more than twofold in hepatocyte culture (29). None of these chemicals elevated PROD activity in any cell type after 24–72 h (example for cyclophosphamide shown in Fig. 3).

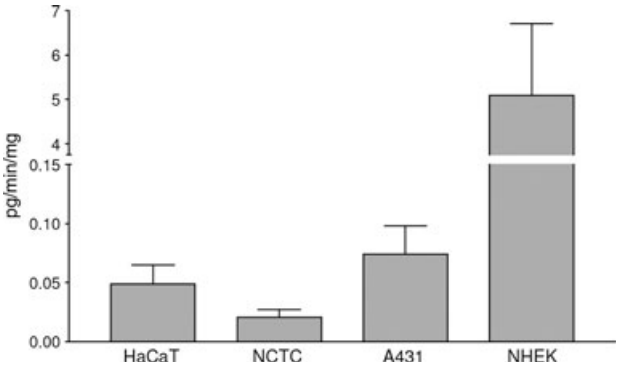

Figure 4. Basal levels of PGE_2 production in immortalized cell lines (HaCaT, NCTC), a squamous cell carcinoma line (A431) and primary keratinocytes (NHEK). The data are shown as pg PGE_2/min/mg.

Basal CYP3A activity was detectable in the immortalized HaCaT und NCTC cell lines with 38 ± 13 and 33 ± 5 pmol/min/mg, respectively, as measured by BQ as CYP3A substrate for monolayer cells. These values were comparable with turnover rates obtained for microsomal activities of human skin and EPI-200. In contrast, no BQ turnover was measured in NHEKs (Fig. S2). As serum conditions affect metabolic activities *in vitro* (30), we also assayed BQ in immortalized cells using inactivated serum. Treatment with either serum, rifampicin or dexamethasone treatment for 48 h failed to increase BQ metabolism above basal levels in HaCaT and NCTC cells. Dexamethasone decreased BQ turnover in NCTC, an effect which was similar to that seen in intact EPI-200 (Fig. S2).

PGE_2 formation as a measure of COX activities was detected in HaCaT (0.05 ± 0.03 pg/min/mg), NCTC (0.02 ± 0.01 pg/min/mg) and the epidermal squamous cell carcinoma strain A431 (0.07 ± 0.02 pg/min/mg) employed as a tumor cell line control. In contrast, primary NHEKs exhibited more than 100-fold higher PGE_2 production (5.1 ± 2.8 pg/min/mg; Fig. 4). Incubation of monolayers with 3-MC did not significantly affect COX activities (Fig. S3). The highest increase in PGE_2 production in the cell culture experiments was measured at inducer concentrations between 1 to 2 μM 3-MC, which caused an up to 1.7-, 1.5- and 1.4-fold increase, however, not significant induction of PGE2 production in HaCaT, NCTC and NHEK.

Discussion

The aim of this study is to systemically compare Phase I drug metabolizing enzyme activities of human skin, 3D epidermal models and keratinocytes. We have demonstrated that CYP1A1, 1B1, 1A2, 2B6 and 3A activity were below the limits of quantification in any of the cell models tested. However, basal CYP3A4 and COX activities were present in all human skin models except for CYP3A4 in NHEKs.

Studies from two groups determined that human skin possesses CYP-dependent microsomal enzyme activity metabolizing

polycyclic aromatic hydrocarbons or is induced by such compounds (31–33). Moreover, high CYP activities were observed in epidermal hair follicles (34). As skin is a compartmented organ, it was a major finding that the main CYP metabolism of skin takes place in the epidermal layer, that is epidermal keratinocytes, rather than in dermal fibroblasts [(35,36) and references therein]. This is why only epidermal models were chosen in this study.

A variety of researchers have studied CYP mRNA, protein and/or activity in skin and keratinocyte-derived *in vitro* models (12,18,30,37–39). As CYP mRNA expression does not always correlate well with enzyme activity (36,39), CYP mRNA expression in skin will not be discussed here, although others have made extensive investigations into this aspect (18,36,38,39). With regard to enzyme activity, previous results are ambiguous. For example, while one group identified CYP1A1 and 1B1 protein and EROD activity in human untreated epidermis (40), others, in agreement with our findings, did not (30). In 3D epidermal models, CYP activity measurements were more in harmony with our findings in that basal CYP1A1/1B1 protein expression and basal EROD activity were consistently below limits of detection (41,42). Our co-workers' study presents similar results in reconstructed epidermal (MatTek) and full thickness skin (Phenion, Henkel AG & Co.KGaA, Düsseldorf, Germany) models indicating that basal CYP-catalysed biotransformation occurs at a very low level unless it is enhanced by an external trigger (4). Basal CYP1 activity measured in some human skin microsomes may arise from lifestyle or nutrition as food constituents and contaminants are well known to induce AhR-dependent CYP expression (43) or might be due to seasonal variation as CYP expression is dependent on seasonal UV irradiation (44).

Wattenberg and Leong (45) illustrated that cutaneous arylhydrocarbon hydroxylase activity is induced by 3-MC in rats. Similar findings were reported in human skin (31,32). EROD and MROD inducibility by 3-MC in 3D skin models in agreement with Harris et al. (41) was confirmed in this study clearly demonstrating the presence of functional CYP protein (Fig. 2a).

NHEK and NCTC keratinocytes showed very low basal CYP1A/1B activity, while these activities were below the LOD in HaCaT cells, but CYP1A/1B were inducible in all three cell types by 3-MC with the lowest induction rate in NHEKs. These basal CYP1A/1B activities as well as their induction responses in HaCaT and NHEK or NCTC measured in this study support the recently reported data using B(a)P or 3-MC as inducers (46,47). It was also observed that CYP1A/1B activities decreased in all monolayer keratinocytes after maximal induction (Fig. 3). As no significant cytotoxicity of 3-MC was detected in our set-up (LDH, data not shown), the proposed mechanism for this observation is substrate inhibition, as reported for resorufin-based CYP substrates like EROD earlier (48). CYP1A1/1B induction by 3-MC was also observed in 3D models, but, in contrast to monolayers, no substrate inhibition occurred. This is possibly due to a different distribution of the compound within the 3D model where all cells are exposed via the medium. Also, the amount of cells/surface area is much higher in a 3D model. These data suggest that EpiDerm models because of their 3D structure are suitable alternative models for studying CYP1A/1B induction in skin (Fig. 2a).

CYP2B6 activitiy was not detectable in skin microsomes, EPI-200 models nor cell lines (Figs 1b and 2a and 3) and is most probably not transcribed in human skin as indicated by Hu et al. (18). PROD activity in HLM did not exceed the detection limit, while rodent liver samples showed significant PROD activity (Fig. 1b) indicating that the suitability of the PROD assay for human samples is questionable and rather suited for experiments based on rodent catalytic activity. Such species-specificities of CYP towards particular substrates have been reported previously (49).

To answer the question whether native skin and/or skin cell-derived *in vitro* models possess basal or inducible CYP2B6 activity, we employed the broad spectrum CYP substrate MFC, which is mainly metabolized by CYP2B6 (50). In contrast to HLM, human skin and EPI-200 microsomes did not metabolize MFC (Fig. 1c). Induction experiments in the cell lines of this study with model inducers for a variety of CYP enzymes suggest that induced MFC turnover is owing to enhanced CYP1A/1B activity because selective inducers of CYP2B6, 2E1 and 3A did not significantly alter MFC metabolism (Table S1). Lack of basal MFC turnover in skin and epidermal models/cell lines also indicates that there is no measureable basal MFC turnover via CYP1A1, 1B1, 1A2, 2B6, 2C19 and 2E1. These data are in agreement with Rolsted et al. (30) who reported that two keratinocyte cell lines lack basal CYP2B6 activity. However, these authors did find CYP2C9 (a minor pathway in MFC metabolism) and CYP2E1 enzyme activities in human skin microsomes. Lack of MFC turnover in our studies does not necessarily contradict these reported activities for CYP2C9 and 2E1 as the MFC substrate might have lower sensitivity and specificity towards these enzymes.

The CYP3A family is of particular importance for xenobiotic detoxification in skin as many drugs employed in dermatology are metabolized by these enzymes. Members of the CYP3A family have been reported to be present in rodent (51) as well as in human skin, where CYP3A5 appears to be the key enzyme at the basal level without inducer (35,52). Both CYP3A substrates utilized in this study indicate low basal CYP3A activity in skin, which is in agreement with Rolsted et al. (30). For the first time, this study shows that CYP3A activity in EPI-200 models and human skin is very similar (Fig. 1d,e). In contrast to Rolsted et al. (30), basal CYP3A activities were detected in HaCaT cells. This might be due to different HaCaT subclones because this cell line has been existing for a long time (19). Differences in HaCaT subclones were observed recently also for *N*-acetylation activities (47). NCTC cells also exhibited basal CYP3A activity. In any of these *in vitro* models, CYP3A activity was not inducible above a factor of 1.2 under the conditions tested (Figs 2b and Fig S2). CYP3A activity was lacking in NHEKs as was also seen in the Rolsted et al. (30) study. This was not owing to lack of FCS in the culture medium of primary cells which, in contrast to immortalized cell lines, are cultured without serum because (i) Rolsted et al. (30) tested this hypothesis and (ii) EPI-200 expressed basal CYP3A activity in serum-free medium (Figs 1 and 2). These data suggest that the choice of cell line, subclone and culture conditions may be crucial for the experimental outcome.

A very different picture is obtained for COX activities. COX is not only involved in inflammatory reactions (53), but also thought to play a major role in activation of chemicals during the COX catalysed peroxidase reaction (54). COX enzyme activities were measured at well-detectable levels in all models tested. In EPI-200, duration of incubation (24–72 h) or donor (Fig. S1) did not affect COX activities. The largest difference in COX activities is seen

between immortalized and non-immortalized cellular systems: PGE_2 production is clearly lower in immortalized than in primary cells or native skin (Figs 1f and 4). Therefore, for example, EPI-200 and HaCaT PGE_2 production was found to be very similar to COX metabolism rates reported by others (55,56). One possibility causing differences in COX activity – as already discussed for CYP3A – was the presence/absence of serum. We ruled out this option by cultivating NHEK in serum-containing media (data not shown). We also included a squamous cell carcinoma line (A431) in our studies because COX-2 is known to be a major player in inflammation and tumorigenesis (57,58). However, these cells produced the same low amount of PGE_2 as the other immortalized lines (Fig. 4). Therefore, a further possibility might be loss of cellular PGE_2 production during the cellular immortalization process. This hypothesis is supported by the observation that immortalization of breast cancer cell lines (hMEC) causes a dramatic decrease in COX-2 expression by an unknown mechanism (59).

We have recently shown that AhR activation causes COX-2 induction in mouse skin and HaCaT cells (5). Here, PGE_2 formation was induced via the AhR ligand 3-MC in HaCaT, NHEK and EPI-200 (Fig. 2c, and Fig. S3). However, induction was only statistically significant in EPI-200. These data indicate that *in vitro* models based on primary cells are the method of choice and that EPI-200 models are the most suitable for experiments involving COX induction.

In conclusion, basal CYP activities are very low, while COX activities are substantial in reconstructed epidermis and human skin microsomes. Contrastingly, basal and inducible biotransforming capacities from monolayer cells differed from organotypic tissues and most interestingly also from each other. This is in accordance with a recent report on key differences in dermal papilla cells cultured in 2D compared with 3D cultures or *in vivo* follicles (60). Hence, reconstructed tissues such as EPI-200 should rather be considered for dermatotoxicological studies as they maintain Phase I native-like metabolic competence. For some chemical testing queries, usage of simple monolayer cultures might also be plausible but they have to be chosen with care.

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Conflict of interest

The authors state no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Material and methods.

Figure S1. COX activity (nmol/min/mg) was measured by the fluorescent ADHP assay (Cayman Corp.) in microsomes prepared from EPI-200 after different cultivation durations (24–72 h).

Figure S2. Induction of CYP3A activity rifampicin (RIF) and dexamethasone (DEX) for 48 h. The assay was BQ.

Figure S3. PGE_2 production in keratinocytes upon 3-MC treatment.

Table S1. MFC turnover (pmol/min/mg) 24 h after treatment with respective inducers ($n = 3$).

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