Expression profiles of phases 1 and 2 metabolizing enzymes in human skin and the reconstructed skin models Episkin™ and full thickness model from Episkin™

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ABSTRACT

Background: Episkin™ and full thickness model from Episkin™ (FTM) are human skin models obtained from *in vitro* growth of keratinocytes into the five typical layers of the epidermis. FTM is a full thickness reconstructed skin model that also contains fibroblasts seeded in a collagen matrix.
Objectives: To assess whether enzymes involved in chemical detoxification are expressed in Episkin™ and FTM and how their levels compare with the human epidermis, dermis and total skin.
Methods: Quantification of the mRNA expression levels of phases 1 and 2 metabolizing enzymes in cultured Episkin™ and FTM and human epidermis, dermis and total skin using Realtime PCR.
Results: The data show that the expression profiles of 61 phases 1 and 2 metabolizing enzymes in Episkin™, FTM and epidermis are generally similar, with some exceptions. Cytochrome P450-dependent enzymes and flavin monooxygenases are expressed at low levels, while phase 2 metabolizing enzymes are expressed at much higher levels, especially, glutathione-S-transferase P1 (GSTP1) catechol-O-methyl transferase (COMT), steroid sulfotransferase (SULT2B1b), and N-acetyl transferase (NAT5). The present study also identifies the presence of many enzymes involved in cholesterol, arachidonic acid, leukotriene, prostaglandin, eicosatrienoic acids, and vitamin D3 metabolisms.
Conclusion: The present data strongly suggest that Episkin™ and FTM represent reliable and valuable *in vitro* human skin models for studying the function of phases 1 and 2 metabolizing enzymes in xenobiotic metabolisms. They could be used to replace invasive methods or laboratory animals for skin experiments.

1. Introduction

Human skin, the largest organ of the body, plays an important protective role against injury, excess water loss as well as detoxification and metabolism of chemicals from environmental pollutants, drugs or cosmetics [1]. A proper understanding of the mechanisms involved in the biotransformation of endogenous as well as exogenous chemical compounds in the skin is thus of major importance. To develop alternative methods to animal, artificial human skin models have been developped to study, *in vitro*, metabolism, efficiency or safety of chemicals. These models, including Episkin™ and FTM, are valuable tools for cosmetic industry. The major pathways of phases 1 and 2 metabolism represents approximately

1/50th of the liver biotransformation and therefore the phases 1 and 2 metabolism markers are produced at much lower level and thus more difficult to detect.

Phase 1 metabolism is catalyzed by cytochrome P450 and other oxido-reductases (esterases, epoxide hydrolases, alcohol and aldehyde dehydrogenases etc...) which transform hydrophobic chemicals into more polar hydrophilic compounds. Phase 2 metabolism is catalyzed by numerous transferases, namely UDP-glucuronosyl transferases, glutathione-S-transferases and sulfotransferases which add conjugated-groups (UDP-glucuronosyl, thiol and sulfate) to hydroxylate compounds to increase hydrophilicity and excretability, thus avoiding bioaccumulation and potential intracellular toxicity. Many cytochrome P-450-dependent enzymes are reported to be present in human skin [2,3] as well as some of the phase 2 enzymes. Knowledge of the enzymes that are specifically expressed in the skin should be of a great help for a better understanding of the mechanisms underlying chemical biotransformations in the skin.

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Episkin[TM] has been shown to be a highly valuable artificial epidermis model [4] that is being used for irritation assays [5]. Recently, an improved skin model derived from Episkin[TM] that contains fibroblasts in addition of keratinocytes, named FTM, has available become. It is thus important to characterize the full metabolic capacity of these models to assess whether they are suitable for studying the metabolism of new cosmetic and chemical compounds as replacement of the *in vivo* and *ex vivo* models. In this report, using our improved method of RealTime PCR quantification based upon detection of the log-linear phase by second derivative calculation coupled with double correction of errors [6], it is possible to obtain absolute mRNA expression levels with high sensitivity and accuracy. We have thus quantified and compared the mRNA expression levels of phases 1 and 2 metabolizing enzymes between human total skin, dermis, epidermis, Episkin[TM] and FTM. The availability of absolute mRNA expression levels of these enzymes permits to obtain a quantitative appreciation of their role in the epidermis and a more precise comparison with the *in vitro* Episkin[TM] and FTM models.

2. Material and methods

2.1. Reconstructed human skin models

Episkin[TM] and FTM are reconstructed human epidermis and full thickness reconstructed human skin models, respectively. The material was obtained from EPISKIN[TM] SNC (Lyon, France) and cultured to day 16 in a 12-well plates in medium provided by the manufacturer. The adult keratinocytes were isolated from female skin samples obtained from mammoplastic surgery cultivated on 12-well micro plates, each containing 1.1 cm^2 of reconstructed tissue [4] in medium containing Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham's (DHEM/HAMF12) (3:1) (HyClone, Logan, Utah), 10% fetal calf serum, 10 ng/ml EGF, 400 ng/ml Hydrocortisone, 10^{-6} M isoproterenol and then left to differentiate in the air-exposed culture to form a well-stratified epidermis [4].

2.2. Preparation of human epidermis

Ten human skin samples obtained from mammoplastic surgery of adult females aged from 18 to 47 years were used to prepare the epidermis. Total skin samples were treated overnight with dispase (1.8 U/ml) and trypsin (0.6%) in 10 ml of Dulbecco's Modified Eagle's Medium (DMEM) medium containing 3% (v/v) gentamycin and 3.7% (v/v) antibiotic/antimycotic. Medium was changed three times at 15 min intervals. After the last change, 20 ml of a medium containing 1.8 U/ml of dispase and 0.6% trypsin were added. After overnight incubation in dispase, dermis and epidermis were separated using forceps, washed twice in PBS and then incubated in RNA later®, a RNA stabilization solution commercialized by Ambion Inc. (Austin, TX) that helps to store tissue and cells for extended periods of time with limited RNA degradation.

2.3. RNA extraction and quantification

Total RNAs were extracted from Episkin[TM], FTM, epidermis and total skin samples using the RNA extraction kit from Qiagen Inc. (Mississauga, Ontario, Canada), and reverse transcribed into cDNA using Superscript II reverse transcriptase (Invitrogen, Burlington, Ont. Canada). Quantification of mRNA was performed on the Light-Cycler Realtime PCR system (Hoffman-La Roche Inc. Nutley, NJ) using SYBR Green detection and the second derivative calculation method as described [6]. In brief, 30 ng of total RNA were used to perform fluorescent-based Realtime PCR quantification. Reagents

obtained from the same supplier were used as described by the manufacturer. The conditions for the PCR reactions were: denaturation at 94 °C for 15 s, annealing at 55 °C for 10 s and elongation at 72 °C for 35 s. The data were normalized using the mRNA expression levels of a housekeeping gene, namely ATP5o (subunit O of ATPase) as internal standard. ATP5o has been shown to have stable expression levels from embryonic life through adulthood in various tissues [7]. The mRNA expression levels are expressed as numbers of copies/μg total RNA using a standard curve of Cp versus logarithm of the quantity ATP5o. The standard curve is established using known cDNA amounts of 0, 10^2, 10^3, 10^4, 10^5 and 10^6 copies of ATP5o and a LightCycler 3.5 program provided by the manufacturer (Roche Inc., Nutley, NJ).

2.4. Determination of phases 1 and 2 steroid metabolism in FTM and Episkin[TM]

Twenty four hour after receiving from the manufacturer, FTM and Episkin[TM] samples were punched to obtain discs of 1 cm which contains approximately the same amount of keratinocytes (information given by the manufacturer). Discs were transferred into 12 wells falcon plate containing 1 ml of fresh culture medium. 0.1 μM [^{14}C] labeled DHEA and DHT (Dupont Inc., Mississauga, Ont., Canada) were added and further incubated for 24 h at 37 °C in the cell culture incubator. After incubation, the culture medium was transferred into glass tubes and 2 ml ether was added for extraction of non conjugated metabolites (twice) as described [8]. The remaining aqueous phase was evaporated to dryness under vacuum in a speed-vac centrifuge, resuspended in 50 ul of a methanol:water (80:20) solution and separated on thin layer chromatography (TLC) Silica gel 60 plates (Merck, Darmstad, Germany), using the toluene:acetone (4:1) solvent system. Non-migrated metabolite was identified as conjugated-steroid and quantified by a Phospho-Imager Storm 860 system.

3. Results

Although protein expression levels and activity are more accurate methods to assess gene function, mRNA analysis is a more accessible, sensitive and convenient method experimentally. Moreover, it is generally observed that mRNA expression levels are in good agreement with physiological functions of the corresponding genes. Indeed, we could observe a good correlation between mRNA expression levels of genes encoding enzymes of the steroidogenic pathway with their physiological activities [9]. Using an improved method of RealTime PCR quantification [6] that allows to obtain absolute mRNA expression levels with high sensitivity and accuracy, we have compared the expression levels of phases 1 and 2 metabolizing enzymes in normal human skin, dermis, epidermis and in Episkin[TM] and FTM models.

3.1. mRNA expression levels of members of the CYP family

Among the phase 1 metabolizing enzymes, the cytochrome P450 enzymes (CYP) are involved in dealkylation or hydroxylation reactions of endogenous compounds such as steroids, retinoids, bile acids, vitamin D and xenobiotics. We have quantified 26 CYP members that are not involved in the steroidogenic pathways (Fig. 1A, B, C), while those of the steroidogenic pathways are well characterized in the literature [9,10]. The two highest expression levels are observed with CYP4B1 and CYP26B1 (Fig. 1A). CYP4B1 has been shown to be involved in arylamine N-hydroxylation and activation of carcinogenic aromatic amines [11–14] as well as ω-hydroxylation of lauric acid [15,16], while CYP26B1 is involved in retinoic acid metabolism [17,18]. It is worth noting that these two enzymes are almost equally expressed in the total skin,
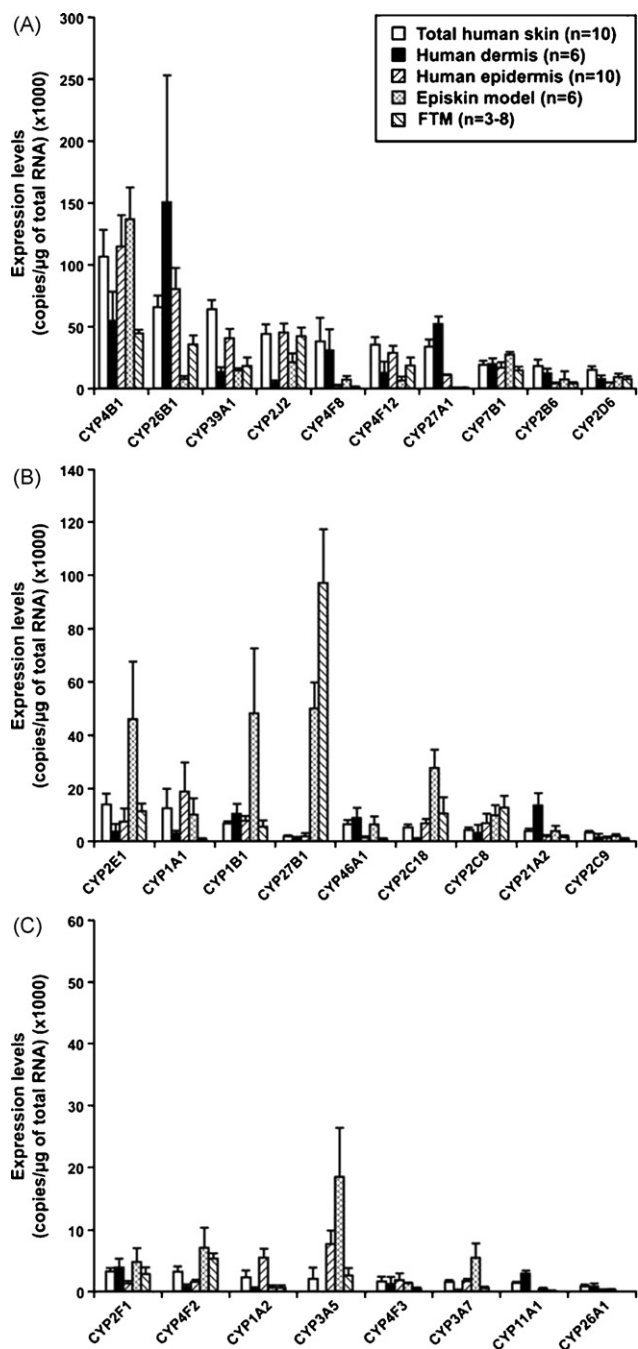
Fig. 1. mRNA expression levels of cytochrome P450-dependent phase 1 metabolizing-enzyme (CYP) in human skin, dermis, epidermis and reconstructed-skins. 30 ng of total RNA extracted from human skin biopsies, dermis epidermis and Episkin were used to quantify members of CYP family using RealTime PCR. Total RNA preparation and Realtime PCR quantification using second derivative calculations and the double correction method [6] with SYBR green fluorescence detection was performed as described in Section 2. Absolute expression levels are indicated as number of copies/ug of total RNA. The data are expressed as means ± SEM of duplicate measurements of n samples. Data in A is higher than B which is higher than C.

dermis and epidermis (Fig. 1). However, while CYP4B1 is found in Episkin™ at levels similar to the epidermis (Fig. 1A), CYP26B1 is expressed in Episkin™ at a much lower level than in the epidermis (Fig. 1A). It is noteworthy that in FTM the expression level of this enzyme is increased to the level closer to that found in epidermis.

All of the remaining P450-dependent enzymes are expressed at low range levels. Among those, CYP39A1 is the enzyme that cat-

alyzes the 7α-hydroxylation of 24-hydroxycholesterol [19] while CYP2J2 [20,21] has been found to metabolize arachidonic acid into cis-epoxyeicosatrienoic acids (EETs) and CYP4F8 catalyzes the 19-hydroxylation of prostaglandin H1 and H2 in vitro [22] on the other hand, CYP4F12 possesses 78% amino acid sequence identity with CYP4F8, and catalyzes the oxidation of arachidonic acid into 18-hydroxyarachidonic acid [23] while CYP27A1 is the enzyme that catalyzes the 27-hydroxylation of cholesterol [24]. Mutations of this gene cause cerebrotendinous xanthomatosis (CTX), the disease that is characterized by high plasma and tissue cholestanol concentrations, normal-to-low plasma cholesterol concentrations, decreased chenodeoxycholic acid increased concentrations of bile alcohols and their glycoconjugates and increased concentrations of cholestanol and apolipoprotein B in the cerebrospinal fluid [25].

CYP27A1 is expressed specifically in the dermis (Fig. 1A) and is also responsible for the 25-hydroxylation of vitamin D3 [26]. It is of particular interest to observe that CYP27B1, the enzyme that catalyzes 1α-hydroxylation of 25-hydroxy vitamin D3 to produce the active form of vitamin D (1α,25-dihydroxy vitamin D3) is more specifically expressed in Episkin™ and FTM models than in total skin (Fig. 1B). We could also observe that CYP7B1, the enzyme that catalyzes 7α-hydroxylation of 27-hydroxycholesterol and DHEA [27], CYP2B6 that catalyzes the O-deethylation of 7-ethoxy-4-trifluoromethylcoumarin and many other P450-dependent enzymes involved in xenobiotic metabolism, namely CYP2D6, CYP2E1, CYP1A1, CYP1B1, CYP2C8, CYP2C18, CYP2F1 and CYP3A5, are expressed at low levels (Fig. 1A and B). CYP2C9, CYP1A2 and CYP3A7 are expressed at a very low level in total skin, human epidermis as well as in the Episkin™ and FTM models (Fig. 1B and C).

3.2. Expression levels of non-cytochrome P450 phase I metabolizing enzymes

As illustrated in Fig. 2A, non-cytochrome P450 phase 1 metabolizing enzymes are expressed at much higher levels than cytochrome P450-containing phase 1 enzymes. Among those, ADH1B, a zinc-containing alcohol dehydrogenase suggested to be involved in the oxidation of alcohol to aldehyde [28] shows the highest expression level reaching ~3 millions copies/µg total RNA. This enzyme is also known to possess the ability to oxidize retinol into retinal [29]. The present data (Fig. 2A) indicate that ADH1B is specifically expressed in the dermis.

In the range of 90,000–880,000 copies/µg total RNA we can find DHRS8, the enzyme also known as type 11 17β-hydroxysteroid dehydrogenase. This enzyme is shown to catalyze the transformation of 5α-androstane-3α,17β-diol (3α-diol) into androsterone (ADT) [30] and it is also identified as a cutaneous T-cell lymphoma associated antigen [31]. As shown in Fig. 2A, this enzyme is selectively expressed in the dermis.

Other non cytochrome P450 phase 1 metabolizing enzymes expressed at high levels in the skin are the epoxide hydrolases EPHX1 [32,33] and EPHX2 [34,35]. These enzymes are microsomal and cytoplasmic epoxide hydrolases, respectively. Their activity consists in the hydrolysis of arene and aliphatic epoxide to less reactive and more water soluble dihydrodiol by the Trans addition of water. As shown in Fig. 2A, EPHX1 is selectively and highly expressed in the dermis while EPHX2 is more selectively expressed in the epidermis, but at a much lower level. HADH2 is another enzyme that is expressed at high levels in the skin. It plays an essential role in the mitochondrial β-oxidation of short chain fatty acids [36,37] with the highest activity toward 3-hydroxybutyryl-CoA. As illustrated in Fig. 2A, HADH2 is expressed highly in all skin components studied, namely total skin, dermis, epidermis, Episkin™ and FTM.
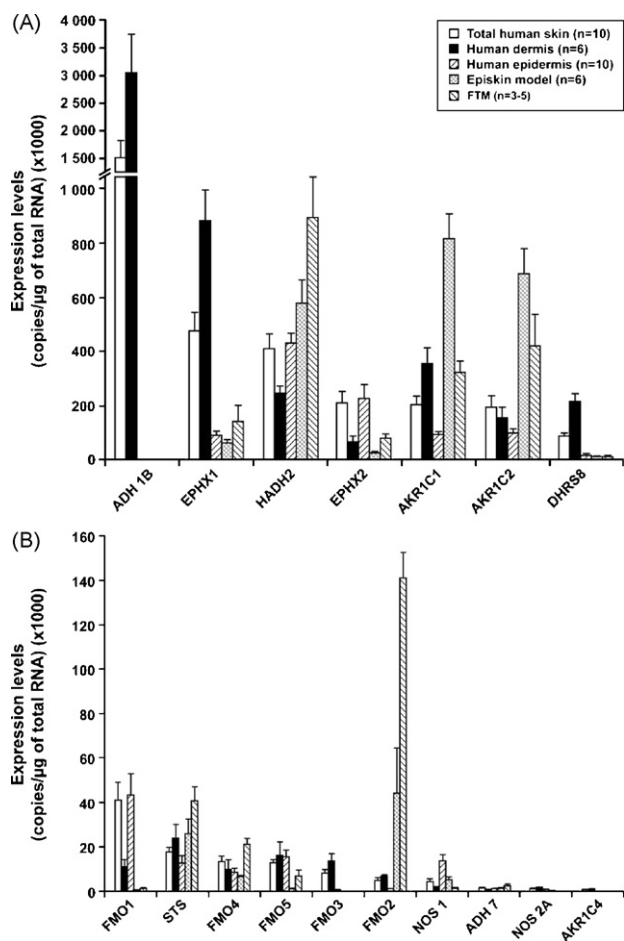
Fig. 2. mRNA expression levels of non-cytochrome P450-dependent phase 1 metabolizing-enzymes in human skin, dermis, epidermis and reconstructed-skins. Experimental procedures are as described under Fig. 1.

Fig. 3. mRNA expression levels phase 2 metabolizing-enzymes in human skin, dermis, epidermis and reconstructed-skins. Experimental procedures are as described under Fig. 1.

The enzymes type 3 3α-hydroxysteroid dehydrogenase (AKR1C2) [38,39] and 20α-HSD (AKR1C1) [40] that catalyze the transformation of dihydrotestosterone (DHT) into 3α-diol and progesterone into 20α-hydroxyprogesterone, respectively, are also highly expressed in the skin, dermis, epidermis Episkin™ and FTM, the most higher expression level being found in Episkin™ (Fig. 2A). The five members of the flavin monooxygenase (FMO1-5) family that catalyze the N-oxygenation of secondary and tertiary amines [41,42] are expressed at low levels with some selectivity in the skin (Fig. 2B). These enzymes are involved in the oxidative metabolism of a variety of xenobiotics such as drugs and pesticides and their expression levels are similar to those of cytochrome P450s. FMO1 is expressed more selectively in the epidermis while FMO2 and FMO3 are more selectively expressed in the dermis and FMO4 and FMO5 are expressed almost equally in the total skin, dermis and epidermis. Their expression profile in Episkin™ and FTM is quite different from that of the epidermis: FMO1, FMO3 and FMO5 are almost absent in Episkin™ while FMO2 is expressed at a relatively high level and FMO4 at a low level. The difference of expression level between epidermis and Episkin (FMO2 is more highly expressed in Episkin while FMO5 is more highly expressed in epidermis) could be due to a stimulating effect found in culture media or an inhibiting factor secreted by fibroblasts.

Steroid sulfatase (STS) [43] that converts the inactive cholesterol sulfate (CHOLS), DHEA sulfate (DHEAS) and estrone sulfate (E1S) into their metabolizable forms, CHOL, DHEA and E1, respec-

tively, is expressed almost equally in total skin, dermis, epidermis, Episkin™ and FTM at relatively low levels (Fig. 2B). Patients that have a mutated STS gene are found to have X-linked ichthyosis [44].

The nitric oxide synthase form 1 (NOS1) is expressed selectively in the epidermis (Fig. 2B) at a level similar to STS. Other isoforms of alcohol dehydrogenase, nitric oxide synthase and type 1 3α-HSD, namely ADH7, NOS2A and AKR1C4, respectively, are almost absent in the skin and reconstructed human skin models (Fig. 2B). It is noteworthy that the enzyme 11β-HSD1 is not significantly expressed in Episkin™ but it is highly expressed in sebocytes (data not shown).

3.3. Phase 2 metabolizing -enzymes

Among phase 2 metabolizing enzymes, GSTP1, a Pi form of glutathione transferase family that catalyzes the conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles [45] is expressed at the highest level in the skin and reconstructed human skin models (Fig. 3A) with more than 2 millions copies/ug total RNA, thus suggesting that glutathione conjugation is the major elimination process in the skin and reconstructed human skin models, Episkin™ and FTM.

The enzyme that exhibits the second highest expression level is SULT2B1b, a cholesterol and DHEA sulfonation enzyme [46,47]. It
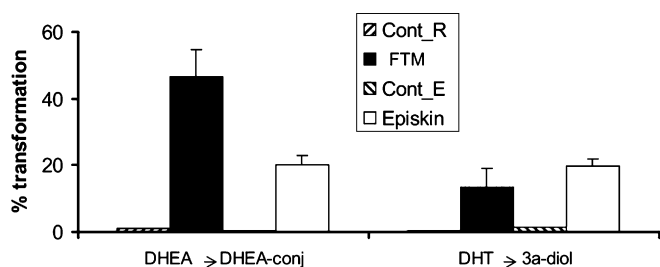
Fig. 4. Example of phases 1 and 2 steroid metabolizing enzymes in FTM and Episkin™. Discs of 1 cm diameter of FTM and Episkin™ were incubated in the presence of 0.1 μM of [14C]DHEA and [14C]DHT. Their ability to convert DHEA into conjugated-DHEA (DHEA-conj) and DHT into 3α-diol (3a-diol) which mainly represent the activity of SULT2B1b and AKR1C2, respectively, was illustrated. Experimental procedures were as described under Section 2. Data are expressed as mean ± SD from three wells.

is expressed specifically in the epidermis, Episkin™ and FTM and at much lower level in the dermis (Fig. 3A).

The high expression level of the enzyme catechol-O-methyl transferase (COMT) [48] in the skin is somehow surprising. In fact, this enzyme is well recognized to catalyze the inactivation of catecholamine neurotransmitters as well as catechol hormones, drugs and xenobiotics. It is expressed at relatively high levels in all skin fractions, total skin, dermis, epidermis, Episkin™ and FTM, and appears to be stimulated in Episkin™ and FTM.

Two additional glutathione transferases (GSTT1 [49] and GSTM5 [50]) and a phenol sulfotransferase SULT1A1 [51,52] that catalyze the sulfate conjugation of catecholamines, phenolic drugs and neurotransmitters are also expressed at relatively high levels with SULT1A1 and GSTM5 selectively expressed in the dermis. NAT5 (N-acetyltransferase protein 5) [53] possesses the highest expression level among N-acetyltransferase enzymes in all the studied preparations, namely, total skin, dermis, epidermis Episkin™ and FTM (Fig. 3A). It is also known as ARD1 (arrest-defective protein 1), and is part of the major N(alpha)-acetyltransferase complex in eukaryotes where it is responsible for alpha-acetylation of proteins and peptides [54]. Protein acetylation has been implicated in gene expression regulation and protein–protein interactions [55,56].

Estrogen sulfotransferase SULT1E1 as well as N-acetyl transferase 1 (NAT1) and uridine glucuronosyl transferases (UGT2B28 and UGT2B4) are expressed at low levels. Many other UGT isoenzymes (UGT1A1, UGT2B17 and UGT2B15) are found expressed at a very low level of less than 5000 copies/μg total mRNA, the limit of detection in our assay. Other isoforms of sulfotransferase (SULT1B1 and SULT2A1) and N-acetyl transferase 2 (NAT2) are not detected in total human skin, human epidermis, and Episkin™ and FTM models.

3.4. Determination of phases 1 and 2 steroid metabolism in FTM and Episkin™

Previously, we have shown that the use [^{14}C]labeled steroid is a very convenient manner to assess steroid metabolism [10]. Since data from mRNA analysis described above show high expression levels of AKR1C2, a phase 1 steroid metabolism, that transforms DHT into 3α-diol, and SULT2B1b, a phase 2 steroid metabolizing enzymes that transforms DHEA into DHEA-S, taking the advantage of the availability of commercial [14C]DHEA and DHT, we would like to assess how these mRNA expression levels are translated into activities. As illustrated in Fig. 4, there is a high production of conjugated-DHEA and 3α-diol. The data is in good agreement with a high expression level of SULT2B1b and AKR1C2, respectively.

4. Discussion

In the present report, we show that many of the phases 1 and 2 metabolizing enzymes are expressed in normal and reconstructed human skins. As summarized in Tables 1–3, some are more selectively expressed in the dermis, such as CYP4F8, CYP27A1, CYP46A1, CYP21A2, ADH1B, EXPH1, FMO3, SULT1A1, and GSTM5. Others are more selectively expressed in the normal human epidermis, such as CYP39A1, CYP1A1, CYP2C18, CYP3A5, EPHX2, NOS1 and SUL2B1b. With regards to the reconstructed epidermis, some genes are more selectively expressed in Episkin™ as compared with the epidermis, such as CYP39A1, CYP4F12, CYP2E1, CYP1B1, CYP46A1, CYP2C18, CYP27B1, CYP3A7, AKR1C1, AKR1C2, GSTP1, and FMO2.

It is noteworthy that many of the enzymes that are differentially expressed in Episkin and in the epidermis, such as CYP26B1, CYP2J2, CYP4F12, CYP2E1, CYP1B1, CYP2C18, CYP3A5, EPHX2, AKR1C1, FMO and GSTT1, possess in FTM an expression level closer to that of the epidermis than that of Episkin™. These enzymes are most probably modulated by signalling factors secreted by fibroblasts that are present in FTM and absent in Episkin™. The present data also suggest that FTM is a skin model that is closer to the intact epidermis than Episkin™. FTM thus represents an interesting model for studying phases 1 and 2 drug metabolizing enzymes.

Taking the advantage of the sensibility and availability of [14C]DHEA and [14C]DHT that permits to assess the activity of a phase 1 (AKR1C2) and phase 2 (SULT2B1b) metabolizing enzymes, we have shown that there is a good agreement between mRNA expression levels and enzymatic activities (Fig. 4).

Previously, Nohynek et al. [57] have shown that Episkin™ possesses N-acetyltransferase activity able to transform p-aminophenol and p-phenylenediamine into their N-acetylated derivatives. Our data suggest that this activity could be catalyzed by CYP4B1 that has been shown to possess N-acetyltransferase activity [11–14], and is highly expressed in the Episkin™ and epidermis (Fig. 1A) or by the conventional N-acetyltransferase NAT1 or NAT5 enzymes. NAT5 is highly expressed in the dermis, epidermis, Episkin™ and FTM (Fig. 3A) and is known to be involved in gene expression regulation and protein–protein interactions [55,56]. Its activity, however, toward simple chemical compounds such as p-aminophenol and p-phenylenediamine could not be ruled out. On the other hand, CYP4B1 is also reported to possess the ability to catalyze ω-hydroxylation of lauric acid and activate 2-aminofluorene (2-AF) and 4-ipomeanol that are a procarcinogen and a pulmonary toxin, respectively [16,58,59].

Enzymes that are differentially expressed between the epidermis and skin models Episkin™ and FTM, such as CYP27B1 (Fig. 1B) and FMO2 (Fig. 2B) are most probably modulated by factors found in culture medium. Indeed, GSTP1 and some cytochrome P450-dependent enzymes have been found to be modulated by retinoic acid [60] that is added to the Episkin™ and FTM culture medium. Many of these enzymes are shown to possess the ability to oxidize xenobiotics as well as endogenous compounds.

The present data are classified according to three expression levels, namely high (0.5–5 millions copies/μg total mRNA and more), intermediate (50,000–500,000 copies/μg total mRNA) and low (5000–50,000 copies/μg total mRNA), respectively. The majority of cytochrome P450-dependent enzymes are expressed at low levels. This could be due to the fact that they are generally induced by their substrates and their expression levels remain low in the absence of these substrates or inducing agents. This could also suggest that the step of hydroxylation is the limiting step, while the step of conjugation by phase 2 metabolizing-enzymes is not a limiting step which requires the presence of high levels of phase 2 metabolizing enzymes to insure rapid elimination.

Table 1
Expression levels of P450 phase 1 metabolizing enzymes.

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions	Ref.
CYP4B1	+	(+)	+	+	(+)	Arylamine N-hydroxylation, Lauric acid ω-hydroxylation	[10–13] [14,15]
CYP26B1	+	+	+	(+/−)	(+)	Retinoic acid metabolism	[16,17]
CYP39A1	+	(+)	+	(+)	(+)	24-Hydroxy cholesterol 7α-hydroxylation	[18]
CYP2J2	+	(+)	+	(+)	+	Arachidonic acid metabolism, cis-epoxy-eicosatrienoic acid	[19,20]
CYP4F8	+	+	(+/−)	(+)	(+/−)	Prostaglandin H1&2 19-hydroxylation	[21]
CYP4F12	+	(+)	+	(+)	+	Arachidonic acid oxidation to 18-hydroxy arachidonic acid	[22]
CYP27A1	+	+	(+)	(+/−)	(+/−)	Cholesterol 27-hydroxylation Vitamin D3 25-hydroxylation	[23] [25]
CYP27B1	(+/−)	(+/−)	(+/−)	(+)	+	25-Hydroxy vitamin D3 1α-hydroxylation	
CYP7B1	(+)	(+)	(+)	(+)	(+)	27-Hydroxy cholesterol & DHEA 7α-hydroxylation	[26]
CYP2B6/2D/2E1/1A1/1B1/ 2C8/2C18/2F1/3A5	(+)	(+)	(+)	(+)	(+)	7-Ethoxy-4-trifluoromethyl coumarin-O-deethylation	
CYP2C9/1A2/3A7	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)		

++++ > 1 million copies/μg total RNA.
+++ > 500,000 copies/μg total RNA.
++ > 200,000 copies/μg total RNA.
+ > 50,000 copies/μg total RNA.
(+) > 10,000 copies/μg total RNA.
(+/−) > 5000 copies/μg total RNA.

Interestingly, it has been shown that treatment with dexamethasone induced expression of CYP1A, 2B, 2E and 3A in murine skin [61]. In addition, CYP1A1 has been shown to be induced by TCDD [62] via a signal transduction pathway involving the aromatic hydrocarbon (Ah) receptor. Furthermore, Sadek and Allen-Hoffman [63] have showed that CYP1A1 mRNA and enzyme activity are induced in cultured human keratinocytes, but not in dermal fibroblasts. This is in good agreement with our data that show that CYP1A1 is more selectively expressed in the epidermis (Fig. 1A).

Retinol and retinoic acid play a key role in the development of epithelial tissues and in certain epidermal disorders [64]. Their rapid metabolism allows to have limited biological effect

[65]. The major metabolic pathway of retinoic acid consists of the hydroxylation of C-4 position leading to the production of inactive 4-hydroxyretinoic acid, which is further oxidized into 4-ketoretinoic acid and other more polar metabolites [66]. Interestingly, CYP26B1, that shows the second highest CYP expression level in the present study (Fig. 1A), is the enzyme that plays a key role in retinoic acid metabolism [18]. It is involved in the specific inactivation of all-trans-retinoic acid (RA), and is responsible for the generation of several hydroxylated forms of RA, including 4-OH-RA, 4-oxo-RA, and 18-OH-RA. The enzyme has a preferred activity toward all-trans-RA >9-cis-RA > 13-cis-RA. It has been shown that human skin transforms retinol into retinaldehyde and then into

Table 2
Expression levels of non P450 phase 1 metabolizing enzymes.

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions	Ref.
ADH1B	++++	++++	−	−	−	Alcohol oxidation into aldehyde Retinol oxidation into retinal	[27] [28]
DRHS8 or 17b-HSD 11	+	++	(+)	(+)	(+)	5a-androstane-3a, 17b-diol oxidation into Androsterone	[29]
EPHX1	++	+++	+	+	+	Arene & aliphatic epoxide hydrolysis	[31,32]
EPHX2	++	+	++	(+)	+		[33,34]
HADH2	++	++	++	+++	+++	3-Hydroxybutyryl-CoA mitochondrial b-oxidation	[35,36]
AKR1C2 or 3a-HSD	+	+	(+)	+++	++	DHT reduction into 3-diol	[37,38]
AKR1C1 or 20a-HSD	+	+	(+)	+++	++	Progesterone reduction into 20a-hydroxyprogesterone	[39]
FMO1	(+)	(+/−)	(+)	−	(+/−)	N-oxidation of secondary & tertiary amines	[40,41]
FMO2	(+)	(+)	(+/−)	(+)	+		
FMO3	(+)	(+)	(+/−)	−	−		
FMO4	(+)	(+)	(+)	(+)	(+)		
FMO5	(+)	(+)	(+)	(+/−)	(+)		
STS	(+)	(+)	(+)	(+)	(+)	Cholesterol, DHEA & estrone sulfates hydrolysis	[42,43]
NOS1	(+/−)	(+/−)	(+)	(+/−)	(+/−)		
NOS2A/ADH7/AKR1C4	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)		

++++ > 1 million copies/μg total RNA.
+++ > 500,000 copies/μg total RNA.
++ > 200,000 copies/μg total RNA.
+ > 50,000 copies/μg total RNA.
(+) > 10,000 copies/μg total RNA.
(+/−) > 5000 copies/μg total RNA.

Table 3
Expression levels of phase 2 metabolizing enzymes.

Gene codes	Total human skin	Human dermis	Human epidermis	Episkin™	FTM	Physiological functions	Ref.
GSTP1	++++	++++	++++	++++	++++	Reduced glutathione conjugation to hydropobic electrophiles	[44]
GSTT1	++	++	++	+	++		[48]
GSTM5	+	++	(+)	–	–		[49]
SULT2B1b	+++	+	++++	++++	++++	Cholesterol & DHEA sulfation	[45–46]
SULT1A1	++	++	(+)	(+)	+	Phenol & catecholamine sulfations	[50,51]
SULT1E1	(+)	(+/−)	(+)	(+)	(+)	Other sulfotransferase isoforms	
SULT1B1/2A1	–	–	–	–	–		
COMT	++	++	++	+++	++++	Catechol methylation	[47]
NAT5	+	+	+	+	+	Protein N-acetylation	[52-55]
NAT1	(+)	(+)	(+)	(+)	(+)	N-acetylation	
NAT2	–	–	–	–	–		
UGT2B28/2B4/1A1/2B17/2B15	(+/−)	(+/−)	(+/−)	(+/−)	(+/−)	Glucuronosyl conjugation	

++++ > 1 million copies/μg total RNA.
+++ > 500,000 copies/μg total RNA.
++ > 200,000 copies/μg total RNA.
+ > 50,000 copies/μg total RNA.
(+) > 10,000 copies/μg total RNA.
(+/−) > 5000 copies/μg total RNA.

retinoic acid by two enzymatic steps [67]. The enzyme is, however, almost absent in Episkin™, but is expressed in FTM, suggesting that it is modulated by factors secreted by fibroblasts.

In addition to enzymes that are involved in xenobiotic and drug metabolisms, such as CYP1A1, CYP1B1, CYP2B6, CYP2E1, CYP3A5, CYP4B1, EPHX1, FMO1-5, and NAT1, the present study also identifies the presence of many enzymes involved in the metabolism of cholesterol and sterols (CYP39A1, CYP27A1, CYP7B1, and CYP46A1) [19,24,26,27], arachidonic acid (CYP2J2) [20], leukotrienes and prostaglandins (CYP4F8 and CYP4F12) [22,23], eicosatrienoic acids (CYP2C8) [68] and vitamin D3 (CYP27A1 and CYP27B1) [26].

It is worth noting that the phase 2 metabolizing enzymes, except for UGTs, are generally expressed at much higher levels than the phase 1 metabolizing enzymes. This is in good agreement with the role of the skin as an important organ involved in detoxification and elimination of chemical compounds by sweat glands. Flamand et al. [69] have successfully developed genotoxicity test procedures using the reconstructed human skin model Episkin™. Our data showing the presence of many enzymes of phases 1 and 2 in Episkin™ and FTM are in agreement with these findings. In addition, the high similarity profiles between mRNA expression in human epidermis and those of reconstructed human skin models, Episkin™ and FTM, strongly suggest that these two models represent interesting tools for testing cytotoxicity and genotoxicity of chemical compounds as well as for studying the mechanisms of xenobiotic and drug detoxification and elimination. The high expression levels of NAT5 in these reconstructed human skin models also suggest their potential use for studying gene expression regulation and protein–protein interactions through acetylation. Our data also permit to identify specific phases 1 and 2 genes expressed in various skin compartments and provide valuable information about the potential metabolic capabilities of human skin and the strong similarity observed for the reconstructed skin models in the gene expression profiles of phase 1 and phase 2 metabolizing enzymes. The present study is the first step in the characterization of the chemical metabolism capabilities of human skin and reconstructed human skin models. Further studies at the protein expression and catalytic activity levels are necessary to confirm the functionality of drug and xenobiotic metabolizing enzymes in the skin and skin models. Since the expression levels of these enzymes in the skin are much lower than those found in the liver, much lower activities are expected, and more sensitive experimental conditions and analytical methods are required for the detection of activities in the skin. The absence of phase 1 and phase 2 metabolizing activities using conventional method for the liver could thus not be used to conclude that these activities are not present in the skin. Using reconstructed skin models that allow to have much higher cell number or tissue amount and more concentrated metabolites will greatly improve sensitivity of enzymatic assays.

The simple epidermis model Episkin™ could be preferred for mechanical investigations of the epidermal compartment in the domains of absorption, irritancy or corrosion. On the other hand, the FTM could be considered as a complete skin equivalent. It could thus represent a useful tool for obtaining biological parameters about the efficiency, toxicity, metabolism, mechanisms and molecular interactions of chemicals with the human skin without using invasive methods or using laboratory models that are prohibited by cosmetic industry.

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