Dermal xenobiotic metabolism: A comparison between human native skin, four *in vitro* test systems, and a liver system

K. Reisinger[1], H.F. Merk[2], C. Wiegand.[1]
[1] Henkel AG & Co. KGaA, Düsseldorf, Germany; [2] Department of Dermatology, University Hospital, RWTH Aachen, Aachen, Germany
Kerstin.Reisinger@henkel.com

Introduction

The dermal xenobiotic metabolism contributes to potential toxicity of substances by converting penetrated compounds into harmless or toxic metabolites. Consequently, growing efforts are put into species and organ-specific safety assessment of dermal applied compounds: corrosion, irritation, genotoxicity, and sensitization. However, in contrast to the liver less is known about xenobiotic metabolism of skin and appropriate *in vitro* systems.

To select an *in vitro* system which is most similar to the *in vivo* situation the following dermal test system prepared from • cells from the same biopsies were compared regarding gene expression and enzyme activity:

- Human native skin
- Phenion® Full-Thickness Skin Model (Phenion® FT)
- Open Source - Reconstructed Epidermis (OS-REp)
- Monolayer cultures of fibroblasts or keratinocytes.

HepG2 cells grown in monolayer or as spheroids were selected as a liver test system. Gene expression and activity of representative enzymes related to the xenobiotic metabolism were compared between the dermal and the liver cells grown in 3D test systems.

Conclusion

- **Decreased complexity of dermal test systems leads to reduced basal gene expression and enzyme activity**

  Native skin > Phenion® FT/OS-REp > > Monolayers
- **Distinct basal gene expression and enzyme activity in dermis and epidermis of human native skin and Phenion® FT**
- **HepG2 grown in 3D spheroid culture express higher concentrations of liver-specific markers compared to monolayer culture**
- **A 3D liver test system shows prominent expression of phase I enzymes whereas a dermal 3D system display a clearly higher basal gene expression and activity of selected phase II enzymes**

* M. J. Gomez-Lechon *et al.*; Isolation, Culture and Use of Human Hepatocytes in Drug Research in *in vitro* Methods in Pharmaceutical Research, Academic Press, 1997
* Korff T *et al.*; Three-dimensional spheroidal culture of cytotrophoblast cells mimics the phenotype and differentiation of cytotrophoblasts from normal and preeclamptic pregnancies. Exp Cell Res. 2004; 297:415–423.
* Mewes KR *et al.*; Elastin Expression in a Newly Developed Full-Thickness Skin Equivalent. Skin Pharmacol Physiol 2007; 20:85–95.
* Poumay Y *et al.*,; A simple reconstructed human epidermis: preparation of the culture model and utilization in in vitro studies. Arch Dermatol Res. 2004; 296:203-11.

Results

Gene expression of 4 dermal test systems from the same donor

	Native human skin		Phenion® FT		OS-REp	Monolayer	
	Dermis	Epidermis	Dermis	Epidermis	(Epidermal model)	Fibroblasts	NHEK
CYP1A1	+/-/+	++/-/++	-/-/-	-/-/-	++/++/++	+/+/+	+/+/+
CYP1B1	++/+++/+++	++/++/++	++/++/++	+/+/+	+++/+++/++	++/++/++	++/++/+
CYP2A6	-/-/+	-/-/++	-/+/+	-/+/-	+/+/+	-/-/+	-/-/-
CYP2B6	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
CYP2D6	-/-/-	-/-/-	-/-/-	-/+/-	+/+/+	-/-/-	-/+/+
CYP2E1	++/++/++	++/+++/+++	+/+/+	+/++/+	++/++/++	+/+/+	+/+/+
CYP2S1	++/++/++	++/+++/+++	+/+/+	++/+/++	+++/++/++	+/+/+	++/+/++
CYP3A4	-/+/-	++/++/++	-/-/+	-/+/-	++/+/+	-/-/-	-/-/-
FMO1	++/++/++	++/+++/+++	++/++/++	+/+/+	++/++/++	+/-/+	-/-/-
FMO3	+/++/++	-/-/-	+/+/+	-/-/-	-/-/-	+/+/+	-/-/-
FMO5	++/++/++	+++/+++/+++	+/+/+	++/+/+	+++/+++/++	+/+/+	+/+/+
NAT1	++/++/++	++/++/+++	+/+/+	++/+/+	+++/++/++	+/+/+	+/++/++
UGT1A10	++/+++/++	+++/+++/+++	++/+/+	++/++/++	+++/+++/+++	-/-/-	+/++/++
GSTp1	+/++/++	++/+++/+++	++/++/++	+++/++/+++	+++/++/+++	++/++/++	+/++/++

Basal gene expression of different test systems obtained from the same donor (three donors in total). 2µg of total extracted RNA were transcribed to cDNA with Omniscript®-RT Kit (Qiagen). 1µl cDNA was amplified using the TaqMan gene expression assays (Applied Biosystems). Data of Real time-RT PCR are displayed as: - (not detected), + (ΔC_T to β-actin control > 10), ++ (ΔC_T= 5-10), +++ (ΔC_T < 5). (Donor 1/donor 2/donor 3)

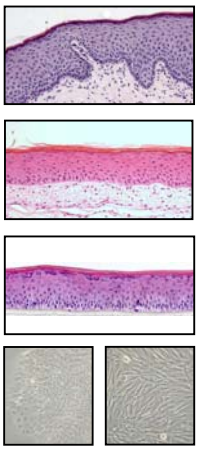
Dermal test systems

Human Native Skin:
The biopsies (foreskins) were taken from young male patients. The explants were used within three hours after surgery.

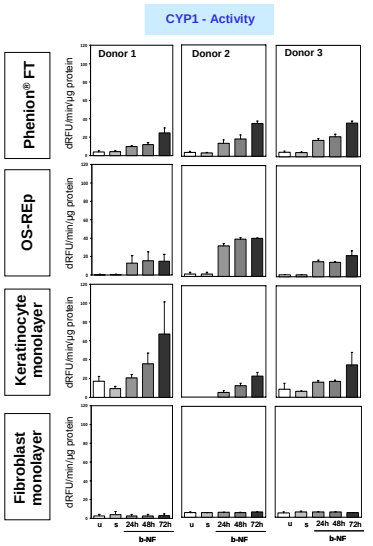
Phenion® Full Thickness Skin Model:
The skin equivalents consisted of a collagen matrix and fibroblasts and keratinocytes isolated from human foreskin tissue. After 3 weeks of cultivation under submersed conditions the skin models were cultivated at the air-liquid interface (ALI) for another 2 weeks [Mewes *et al.*, 2007].

Open Source - Reconstructed Epidermis (OS-REp):
Human foreskin keratinocytes were cultivated according to the protocol published by Poumay *et al.*, 2004

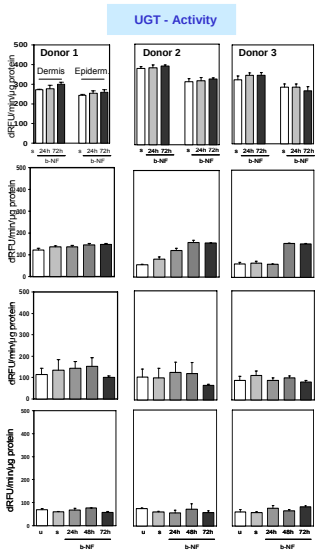
Keratinocyte and fibroblast monolayer:
Human fibroblasts and keratinocytes were isolated from foreskin tissues by first separating dermis and epidermis by thermolysin treatment overnight. The epidermal tissue was then incubated in a trypsin solution until it was completely dissociated into single cells which were cultivated in EpiLife medium (Cascade). The connective tissue was dissociated by incubation in a collagenase solution. Fibroblasts were cultivated in DMEM supplemented with FCS.



Enzyme activity of 4 dermal test systems from the same donor



Induction of CYP1 activity in different test systems obtained from the same donor (three donors in total). Cells and 3D models were treated by 25µM β-naphthoflavone for 24h-72h. The ethoxyresorufin-O-deethylase (EROD) activity was measured in living cells. Cultures were incubated with 1µM substrate and 10µM dicumarol in medium. Resorufin fluorescence was measured at 535nm excitation and 590nm emission.

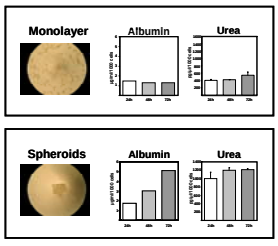


UGT-activity was determined as described by Gomez-Lechon *et al.* (1997). 100µM 4-methylumbelliferone was incubated with cultures for 1 hour in the medium. Culture medium samples were diluted 1:20 in 10mM NaOH and the remaining 4-methylumbelliferone was quantified fluorometrically at 376nm excitation and 460nm emission.

Liver test system

HepG2 monolayer:
HepG2 were maintained in DMEM supplemented with Glutamax and 10% FCS. They were seeded in monolayer cultures at 2000 cells in standard 96-well plates.

HepG2 spheroids
After tripsinizing HepG2 monolayer the cells were suspended in 10ml methyl cellulose containing medium (20% methyl cellulose stock solution and 80% phenolred-free DMEM, Korff *et al.*, 2004) and were seeded in 100µl methyl cellulose and phenolred-free DMEM (Glutamax with 10% fetal calf serum) at 200 and 400 cells per well in a 96-well non-adhesive round bottom tissue culture plate.
Supernatant was subjected to commercially available Urea Assay Kit (Biochain) or Albumin ELISA Kit (Assay Pro) to determine the release of liver specific markers into the medium.



Comparison of Skin and Liver test systems

Gene expression	CYP1A1	GSTp1	UGT1A10
Skin model	-/+	++	++
Liver model	++	-	++

Enzyme activity	EROD [dRFU/min/µgProt.]	GST [nmol/min/mg protein]	UGT [µM 4-MU/mg prot.]
Skin model	5	75	160
Liver model	50	30	15