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

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Introduction

The skin is the first site of contact with maximum exposure of external stimuli and protects the body in several ways. Its barrier function determines the local and systemic bioavailability of dermal applied substances. In addition, 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 human native skin was compared to several *in vitro* models of different physiological complexities:
(1) Phenion® Full Thickness Skin Model comprising dermis and epidermis,
(2) an Epidermal Model, and
(3 + 4) monolayer cultures of fibroblasts or keratinocytes.
To exclude donor variability the four *in vitro* models were cultivated with cells of biopsies from the same three donors.
First, the basal gene expression of phase I and phase II enzymes of three different donors were investigated by quantitative RT-PCR.
Second, CYP1A1 enzyme activity (EROD) were compared between the *in vitro* systems.

Conclusion

- **Dermis and epidermis show distinct gene expression 60% of the analyzed gene are differentially expressed**
- **Donor dependent variability in gene expression**
- **Decreased complexity leads to reduced gene expression**

  Monolayer < < Epidermal Model/Full Thickness Model < Native Skin
- **Every gene detected in native skin could be shown in 3D tissues**

Results

Basal gene expression profile of models constructed with cells from the same donor (3 donors on total):

	Phenion® Full Thickness Skin Model		Phenion	Native Skin		Monolayer	
	Dermis	Epidermis	Epidermal Model	Dermis	Epidermis	Fibroblasts	Keratinocytes
CYP1A1	-/-/-	+/-/-	++/++/++	+/-/+	++/-/++	+/+/+	+/+/+
CYP1B1	+++/+++/+++	++/+/+	+++/+++/++	+++/+++/+++	++/++/++	++/++/++	++/++/+
CYP2A6	+/+/+	+/+/+	+/+/+	-/-/+	-/-/++	-/-/+	-/-/-
CYP2B6	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
CYP2D6	-/-/-	-/-/-	+/+/+	-/-/-	-/-/-	+/+/-	+/+/+
CYP2E1	+/+/+	+/++/++	++/++/++	++/++/++	++/+++/+++	+/+/+	+/+/+
CYP2S1	+/+/+	+/++/++	+++/++/++	++/++/++	+++/+++/+++	+/+/+	++/++/++
CYP3A4	+/+/-	+/+/+	++/+/+	+/+/-	++/++/++	-/-/-	-/-/-
FMO1	++/++/++	+/+/+	++/++/++	++/++/++	++/+++/+++	+/+/+	-/-/-
FMO3	+/+/+	-/-/-	-/-/-	++/++/++	-/-/-	+/-/+	-/-/-
FMO5	+/+/+	++/++/++	+++/+++/++	++/+++/++	+++/+++/+++	+/+/+	+/+/+
NAT1	++/++/++	++/++/++	+++/++/++	++/++/++	++/++/+++	+/+/+	+/++/++
UGT	++/+/++	++/++/+++	+++/+++/+++	+++/+++/++	+++/+++/+++	-/-/-	++/++/++
GSTp1	++/++/++	++/+++/++	+++/++/+++	++/++/++	++/+++/+++	++/++/++	++/++/++

Fig. 1: Gene expression of different test systems obtained from the same donor (three donors in total). Data of Realtime-PCR are displayed as: - (not detected), + (ΔC_T to beta-actin control > 10), ++ (ΔC_T= 5-10), +++ (ΔC_T < 5).
(Donor 1/donor 2/donor 3)

EROD - acitivity

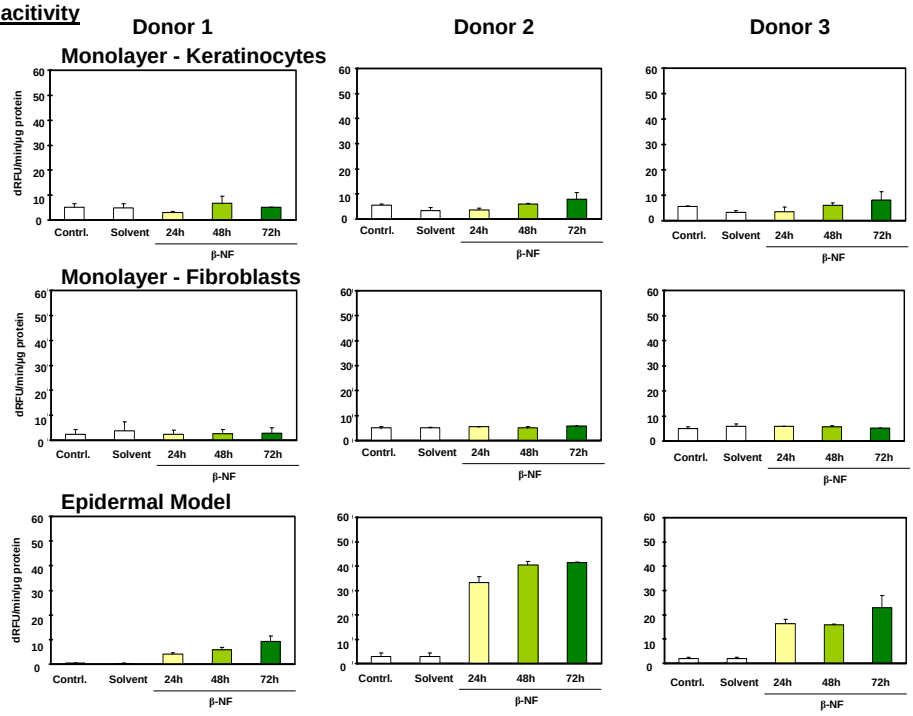

Fig. 2: Induction of CYP1 activity in different test systems obtained from the same donor (three donors in total). Cells and epidermal models were treated by 25µM β-naphthoflavone for 24h - 72h.

Methods

Production of the 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 [1].

Production of the Phenion Epidermal Model:
Human foreskin keratinocytes were cultivated according to the protocol published by Poumay [2].

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

Treatment:
For inducing, 25µM β-naphthoflavone (β-NF) was added to the medium for 24h - 72h.

Realtime PCR:
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). All data were normalized to beta-actin.

CYP1-Activity:
The ethoxyresorufin-O-deethylase (EROD) activity were 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.

Literature

[1] Mewes *et al.*, Skin Pharmacol Physiol, 20, 2007
[2] Poumay *et al.*, Arch Dermatol Res., 8, 2004