Genotoxicity Testing and Metabolism Studies using the Phenion® Full Thickness Skin Model

Phenion
Competence in Skin Physiology

Henkel
A Brand like a Friend

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Introduction

- Existing in vitro genotoxicity tests exhibit a high sensitivity yet a very low specificity. One reason seems to be the „in vitro artificial metabolic activity of the liver S9 mix“ (Ku et al., 2006, 4th IWTG).
- We introduced the Phenion® Full Thickness Skin Model in genotoxicity testing as an equivalent for human skin, which is the first site of contact of many cosmetic ingredients, thus being exposed to relatively high concentrations.
- In order to check resemblance to native skin, we analysed and compared the tissue- and species-specific xenobiotic metabolism of the native skin with the Phenion® Full Thickness Skin Model and an Epidermis model.
- The Micronucleus-Test (MNT) is a basic genotoxicity test which is suited to detect clastogenic and aneugenic effects as mutagenicity endpoints.
- An OECD guideline is in preparation for an in vitro MNT with cell lines which is already recommended as part of several testing strategies.
- Here we show first results of MNT analyses with the Phenion® Full Thickness Skin Model which mirrors the physiological situation of the human skin.

Conclusions

The Phenion® Full Thickness Skin Model

- displays metabolic expression patterns similar to native human skin.
- functions as a useful skin surrogate.
- was used to establish the first genotoxicity test system comprising dermis as well as epidermis.
- is a promising tool to complement existing in vitro genotoxicity test batteries in order to obtain more biological relevant data.

References

Ku W.W. et al.; Strategy of genotoxicity testing – Metabolic considerations. Mut Res. (2007); 627:59-77.

Mewes K.R., et al.; Elastin expression in a newly developed Full Thick. Skin Equivalent Skin Pharmacol Physiol. (2007); 20:85-95

Poumay, et al.; A simple reconstructed human epidermis: preparation of the culture model and utilization in in vitro studies. Arch Dermatol Res. (2004); 296: 203-211.

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Metabolism

Results

Measurement of basal gene expression of Phase I and Phase II enzymes in skin models by Real-Time PCR

	Phenion® Full Thickness Model		Epidermis Model	Native Skin (Foreskin)	
	Dermis	Epidermis		Dermis	Epidermis
CYP1A1	(+)	+	+(+)	+	++
CYP1B1	+++	+	++(+)	+++	++
CYP2E1	+	++	+	++	++
CYP2S1	+	+	++	++	++
CYP3A4	+	+	+	+	+
FMO1	++	+	-/+	++	++
FMO3	+	++	-	++	-
FMO5	+	++	+(+)	++	++(+)
GSTp1	++	+++	+++	++	+(+)

Fig.1 Comparison of basal gene expression levels between tissues, – (not detected), + (ΔCT to beta-actin control > 10), ++ (ΔCT = 5-10),+++ (ΔCT < 5), Full Thickness Model data are averaged from 3 donors; Epidermis Model and native human skin data averaged from 2 donors.

- Phase I (CYP, Cytochrome P_{450}) and Phase II (GSTp1, Glutathione S-transferase pi) enzymes are expressed.
- Dermis and epidermis have distinct metabolic characteristics.
- FMO3 (Flavonoid-dependent mono-oxygenase3) is not expressed in epidermal models but in the epidermis of native human skin and the Phenion® Full Thickness Skin Model.
- FMO1 is only slightly expressed in the Epidermis model in contrast to native human skin and the Phenion® Full Thickness Skin Model.

Material

- The commercially available Phenion® Full Thickness Skin Models were constructed as described in Mewes et al., (2007).
- Epidermis Models (produced for in house research only): Human foreskin keratinocytes were cultivated according to the protocol published by Poumay et al., (2004).

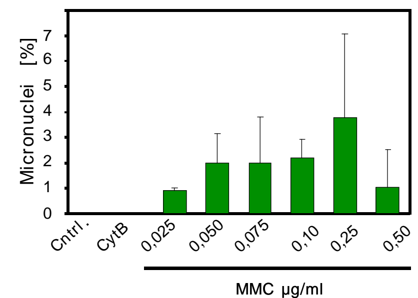
Methods

- Compartment separation: To separate the dermis from the epidermal part Phenion® Full Thickness Skin Models or human foreskin were incubated with dispase for 3h at 4°C.
- Quantitative Real-Time PCR: 2µg of total extracted RNA were transcribed to cDNA with Omniscript®-RT Kit (Qiagen). Finally, 1µl cDNA was amplified using the TaqMan gene expression assays (Applied Biosystems). All data were normalized to beta-actin.

Micronucleus-Assay

Results

A. Induced Micronuclei by MMC



B. Impact of MMC on cytotoxicity

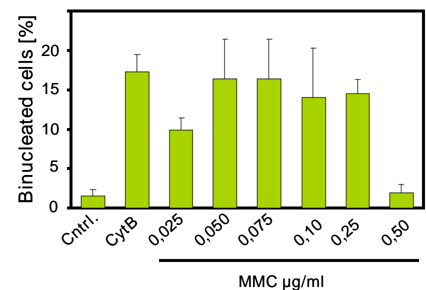


Fig.2 Systemic application of 5µg/ml Cyt B regarding the standard assay design (Fig.3) in parallel to varying concentrations of Mitomycin C (MMC) as indicated. 1000 cells per skin model were counted for measuring the rate of binucleated cells (Fig 2B), and 1000 binucleated cells were analysed for micronuclei (Fig 2A). For each concentration two experiments were performed each comprising 2 skin models.

Protocol

Cell preparation: Dermis and epidermis were separated by dispase (3h at 4°C). Cell isolation was mediated by PBS (w/o Ca/Mg, 10min, RT), 0.2% EDTA (10min, 37°C), and 0.25% trypsin (15min, 37°C). Cell preparation was performed with warm 75mM KCl followed by cell fixation with cold methanol/acetic acid (4:1).

Standard Assay Design

For the cytokinesis-blocked variant of the MNT 5µg/ml Cytochalasin B (Cyt B) were shown to induce the highest amount of binucleated cells in the shortest period of time (72h). The derived standard assay design is shown in Fig.3.

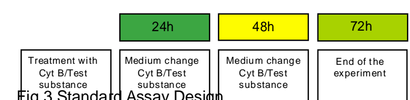


Fig.3 Standard Assay Design