DETERMINATION OF PHOTOTOXICITY PROPERTIES OF DIFFERENT COMPOUNDS USING A FULL THICKNESS SKIN MODEL (AST-2000)

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INTRODUCTION

AST-2000
- Organotypical coculture
- Human fibroblasts in dermis equivalent
- Human keratinocytes in differentiated epidermis
- Coculture on filter membrane, pore diameter 0.2 µm
- Surface of the skin equivalent: 0.253 inch²

Applications
- Differentiation studies
- Survey of barrier function
- Irritation studies
- Migration and wound healing studies
- Toxicity / gene-toxicity studies

A B C

Advantages
- Autonomy from donor material
- Constant conditions
- Reproducible results
- Provides physiological studies
- Similar to the in-vivo-skin

References:

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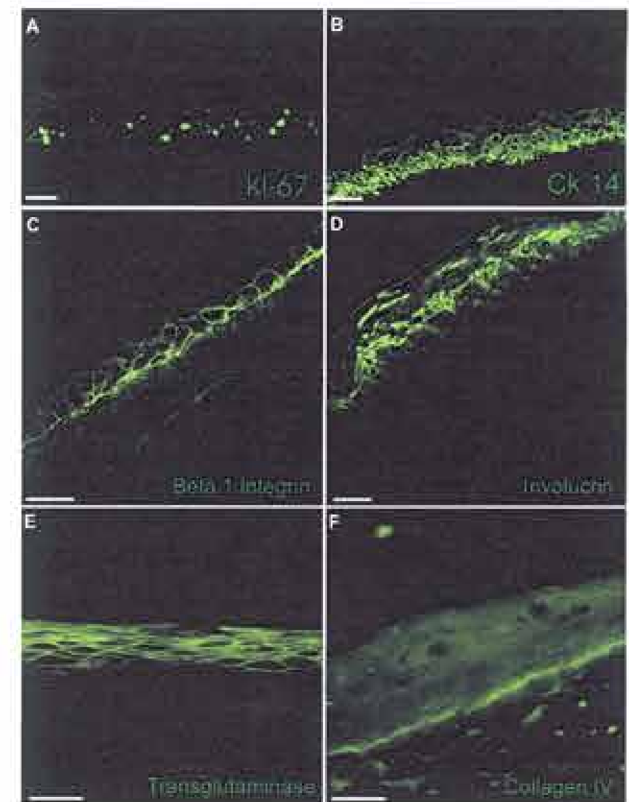
Fig. 1: AST-2000:
Comparison of native human (A) skin and AST-2000 (B). Paraffin embedding followed by H&E staining. Bar 10 µm
AST-2000 is cultured in inserts on a polycarbonate membran (C). At the end of the culture period the skin equivalent is about 0,63 cm² in size.

Fig. 2: Detection of specific markers by immunofluorescence: ------------------------------>>>
Cryosections of AST-2000 treated with antibodies directed against KI-67 (A), cytokeratin 14 (B), integrin ß1 (C), involucrin (D), transglutaminase (E) and collagen IV (F) Confocal images; Bar: 20 µm.

EXPRESSION OF SPECIFIC MARKERS



EFFECT OF SDS

VIABILITY

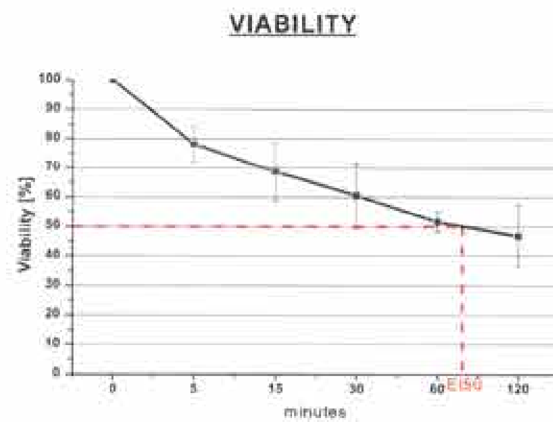


Fig. 3: Determination of Et50 (1 % SDS)
5 different lots of AST-2000 were treated for 0; 5; 15; 30; 60 and 120 minutes with 1 % SDS (n=5/Lot). Cell viability was determined using a standard MTT- assay leading to an Et50 value (meaning the time after SDS application when cell viability is reduced to 50 %) of about 80 minutes.

CYTOKINE RELEASE

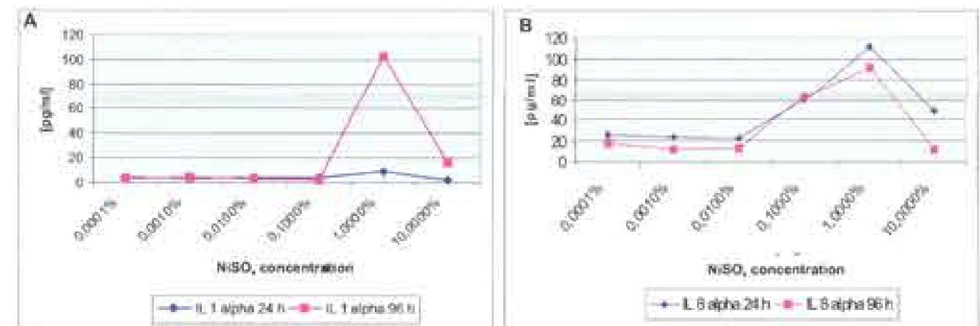


Fig: 4: Induction of cytokine release:
AST-2000 was treated with $NiSO_4$ for 24 and 96 hours. The concentration of IL 1 alpha (A) and IL 8 (B) in the culture medium was quantitated by ELISA. Notice that $NiSO_4$ provoked a specific dose- and time-dependent induction of cell inflammation mediators.

DETERMINATION OF PHOTOTOXICITY

Chlorpromazine

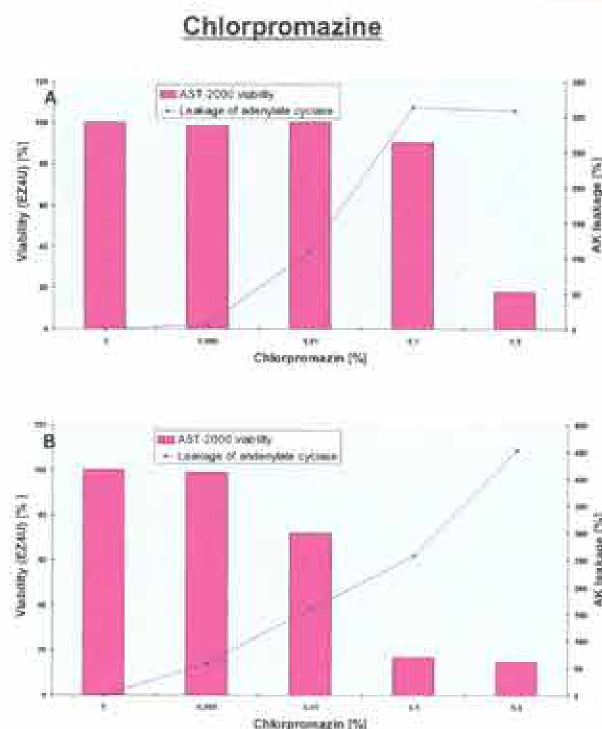


Fig. 5: Determination of phototoxic properties of chlorpromazine using AST-2000:
Different concentrations of chlorpromazine were applied topically on the epidermis of AST-2000 followed by UVA radiation (4J/cm²) after 20 minutes. After incubation over night the cell viability was determined using the EZ4U Test (Biozol) accompanied by messuring the leakage of adenylate cyclase (AK). Note that cell damage and AK leakage were significant higher in UVA treated AST-2000 as compared to unradiated control inserts (A).

Bay 3118

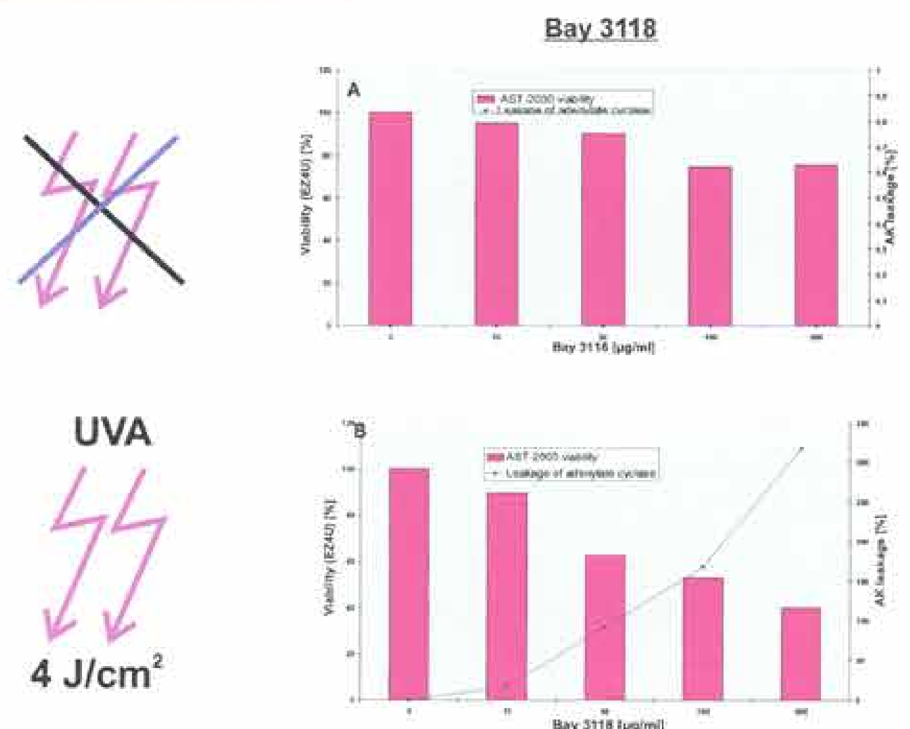


Fig. 6: Determination of phototoxic properties of Bay 3118 using AST-2000:
AST-2000 was cultured under normal conditions with medium supplemented with different concentrations of Bay 3118 for 4 hours followed by UVA radiation (4J/cm²). After incubation for additional 20 hours the cell viability was determined using the EZ4U Test (Biozol) accompanied by messuring the leakage of adenylate cyclase (AK). Note that cell damage and AK leakage were significant higher in UVA treated AST-2000 as compared to unradiated control inserts (A).

We conclude from these data, that the reconstructed skin AST-2000 is a usefull tool for investigations in the field of pharmacotoxicology and dermatology. Due to the strong similarities to native human skin paired with a consistent quality AST-2000 can be used for multiple applications such as toxicity testings and irritation studies leading to reproducible results independent from human donor material and laboratory animals.

WWW.AST-2000.COM