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

J. J. Hoffmann, E. Heisler, P. Peters, S. Karpinski, H.-W. Vohr

CellSystems Biotechnologie Vertrieb GmbH, St. Katharinen, Germany, Bayer Health Care, Institute of Genetic & Molecular Toxicology, Wuppertal, Germany
Tel.: +49 (0)2241-854420 Fax: +49 (0)2241-854421 E-Mail: jens.hoffmann@cellsystems.de

INTRODUCTION

- AST-2000
  - Organically intact human skin
  - cSUMM: 150 cells/mm²

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

- Advantages
  - Autonomy from donor material
  - Consistent conditions
  - Reproducible results
  - Phases of physiological studies
  - Similar to the in vivo skin

Fig. 1: AST-2000
Comparison of skin from human (A) and AST-2000 (B). The reference bedding was followed by H&E staining. Bar 1 μm.
AST-2000 is cultured on inserts in a polycarbonate membrane in (C). At the end of the culture period the skin-equivalent culture was harvested. In vivo skin.

Fig. 2: Detection of specific markers by immunofluorescence
Cytoskeletons of AST-2000 labeled with antibodies directed against: α1 (A), α1 (B), integrin (C, D), involucrin (C, D), von Willebrand factor (E, F). Confluent cultures. Bar 1 μm.

VIABILITY

- Fig. 3: Determination of viability (1 % SDS)
  5 different batches of AST-2000 were incubated for 1 h. 10, 20, 30, 40, and 60% of the cells were incubated with 1 % SDS (100 μL). Cell viability was determined using a spectrophotometer. A colorimetric test, and an SDS precipitation test. Change in time after SDS application when cell viability is reduced to 50 % of above.

EFFECT OF SDS

- Fig. 4: Induction of cytokine release
  AST-2000 was incubated with SDS for 24 and 48 hours. The concentration of IL-1 alpha (A) and IL-8 (B) in the cell supernatants was determined by ELISA. Notice that IL-1 and IL-8 are specific for different cells.

CYTOKINE RELEASE

- Fig. 5: Determination of phototoxic properties of chlorpromazine (A, B)
  Different concentrations of chlorpromazine were applied to the epidermis of AST-2000, followed by UV-A radiation (A) or UV-B radiation (B). After incubation, the viability of the cells was measured using BrdU incorporation. Notice that cell damage in UV-A radiation was significant higher in the increased AST-2000 vs. compared control without xenobiotics.

DETERMINATION OF PHOTOTOXICITY

- 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 24 hours followed by UV-A radiation (1 J/cm²). After incubation for additional 24 hours, cell viability was determined using the ELISA test. Notice that Bay 3118 increased the toxicity of xenobiotics higher in UV-A treated AST-2000 as compared to untreated control.

We conclude from these data, that the reconstructed skin AST-2000 is a useful tool for investigations in the field of pharmacotoxicology and dermato-logy. 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 testing and irritation studies leading to reproducible results independent from human donor material and laboratory animals.