

Poster: skin models as alternatives to animal testing

***In vitro* differentiation of skin sensitizers by cell signaling pathways**

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Introduction: The EU-Guideline (76/768/EEC, Feb. 2003) prohibits the use of animal testing for the assessment of toxicological data for cosmetic ingredients as of 2009/2013. In contrast, the resolution of the REACH-program will increase the number of animals needed since approximately 30,000 chemicals have to be evaluated. For some test areas adequate *in vitro* tests to replace animal testing are missing. In this study, we focused on the test area of skin sensitization and investigated whether analyses of cell signaling pathways can provide a methodology for the detection of sensitizing compounds *in vitro*. For this purpose a differentiation between non-specific immune reactions (skin irritation) and skin sensitization was of major importance. For the induction of a local immune reaction an intact skin barrier plays a key role, since compounds need to be able to penetrate this natural barrier before reaching living immune competent cells. To mimic this situation best, human and murine skin explants were chosen and compared with the reconstituted skin models EST-1000 and AST-2000 (CellSystems, St. Katharinen, Germany).

Methods: Murine and human skin explants as well as reconstituted skin models (epidermal model EST-1000 and full-thickness model AST-2000) were exposed to different concentrations of sensitizing (Oxazolone and DNFB) or irritant compounds (SDS and TritonX-100). The lowest observed effect level (LOEL), defined as the concentration resulting in a decrease in viability of about 10% after 24h of exposure, was determined for each compound and skin model by using the MTT viability assay. Each skin model was then exposed to the appropriate concentrations of the LOEL for 1h or 3h. Phosphorylation of MAP-kinases (p38, ERK1/2 and JNK1/2), STAT1 and PLC γ were determined by cytometric bead array (CBA).

Results: In skin explants all three MAP-kinases were exclusively activated after exposure to sensitizing compounds. Differences were obtained regarding the time points of activation. Whereas in murine skin explants phosphorylation was generally detected after exposures for 3h, cell signaling proteins in human skin explants were already activated after 1h. Regarding the reconstituted skin models, exclusive phosphorylations of p38 and JNK1/2 were obtained after 3h stimulations with allergens. In contrast to skin explants, treatments with irritant compounds lead to an ERK1/2 activation after 1h exposure in the EST-1000 and AST-2000. Inductions of STAT1 and PLC γ were not detected in any of the skin models analyzed.

Discussion: In our study, MAP-kinase activation was shown to provide a promising *in vitro* tool for the discrimination between sensitizing and irritant compounds. The reconstituted skin models AST-2000 and especially the EST-1000 showed high induction levels of phospho-p38. The inductions were comparable to those found in skin explants, i.e. complex immune competent tissues, and specific for an exposure to sensitizing compounds. With respect to availability, variability and simplicity in handling, the EST-1000 turned out to be the model of choice for further analyses of compounds.

Keywords: skin explants, reconstituted skin models, irritant compounds, sensitizing compounds, cell signaling pathways