In vitro Differentiation of Skin Sensitizers by Cell Signaling Pathways

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Introduction
Animal testing causes ethical problems and in view of EU regulations (e.g., EU-Guideline 76/768/EEC, Feb. 2001) and REACH the development of in vitro assays has become even more important. In this study, we 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 specific immune reactions (skin irritation) and skin sensitization was of major importance. Pathways were chosen that have a known function in transducing immune responses, which were the MAP-kinases p38, ERK1/2 and JNK1/2 as well as STAT1 and PLC. 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) for our investigations.

Materials and Methods
Animals: Hairless mice C57BL/6nHnu-fem, 10-12 weeks old, Taconic MoB, Skovlunde, Denmark
Chemo: SDS (sodium dodecyl sulfate), Trition-X100, Oxazolone (4-ethyl-methyl-3-phenyl oxazolone-5-sulfonic acid), DNBC (1-chloro-2,4-dinitrobenzene), DNFB (1-bromo-2,4-dinitrobenzene), Sigma Chemicals, Deisenhofen, Germany. Phorbol ester (Phorbol 12-myristate 13-acetate), GEF (Gefii, Karlsruhe, Germany) on PG (1,2-propylene), KinaseChrom 1000 and AST-2000 (CellSystems, St. Katharinen, Germany). Human skin explants: skin explants were prepared from the back skin of female hairless C57Bl/6nHnu mice. Human skin explants were obtained from mammoplasty surgeries on female patients younger than 44 years at the Florence-Nightingale-Krankenhaus, Kaiserswerther Diakonie, Düsseldorf, Germany.

Culture conditions and skin explantation: AST-2000 and EST-1000 were cultured as described by the manufacturer (CellSystems, St. Katharinen). Skin explants were cultured on mesh inserts in the air-liquid-interface after removal of fatty and subdermal tissue. All skin models were incubated at 37°C, 5% CO2 and 95% humidity.

Exposure to irritant and sensitizing compounds: The compounds were reconstituted in or applied to the MTT assay buffer to determine the concentrations of the lowest observed effect level (LOEL). The LOELs were determined as the concentration resulting in a decrease in viability of about 10% after 24h of exposure. The skin models were exposed to the appropriate compound and skin model. The concentrations were determined by titration of compound skin explants reconstituted skin models (LOEL). *: p < 0.1 vs. vehicle-treated control, **: p < 0.05 vs. vehicle-treated control. Measurements of samples treated for 1h and 3h were performed in triplicate.

Results
Determination of lowest observed effect levels
- both irritant and sensitizing compounds have irritant properties leading to a dose-dependent decrease in viability
- to keep irritation of all compounds equal the lowest observed effect levels (LOELs) were determined for each compound and skin model
- the LOEL was defined as the concentration resulting in a decrease in viability of about 10% after 24h of exposure
- skin models were exposed to the appropriate concentrations for the analyses of cell signaling pathways

Materials
- Human skin explants
- Mouse skin explants
- Skin model AST-2000
- Skin model EST-1000

Methods
- Culture conditions
- Exposure to compounds

Results
- Determination of lowest observed effect levels
- Analysis of cell signaling pathways

Conclusion
- analysis of MAP kinase activation provides a promising tool to identify sensitizing compounds in vitro
- skin explants, either murine or human, seem to have the best capability for identifying sensitizing compounds since complex interactions leading to an activation of all three MAP kinases can be measured
- disadvantages of skin explants are high inter- and individual variabilities
- especially for human skin explants, the availability is limited and patients need to be of about equal age
- using the AST-2000 a specific activation of p38 and JNK was obtained after exposure to sensitizing compounds
- the EST-1000 showed high induction levels of p38 specific for exposure to sensitizing compounds compared to those found for skin explants
- 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