AN ALTERNATIVE METHOD FOR EVALUATING SKIN SENSITIZING POTENTIAL OF CHEMICALS USING FORWARD SCATTER

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At the basis of skin sensitization there is an adverse effect triggered by a sequence of biological mechanisms called human allergic contact dermatitis (hACD). Substances able to induce these responses following skin contact are known as skin sensitizers. The assessment of skin sensitization potency of these chemicals, like cosmetic ingredients or products, it is necessary to define their safe handling and use. Predictive tests to identify and characterize these substances historically has been based on animal tests, but since 2009 the use of animals for testing cosmetics and relative ingredients has been prohibited by European law in March 2013. Therefore, in recent years, the need of non-animal alternative methods (e.g. in vitro approaches) to identify skin sensitization hazard potential of these chemicals has become urgent.

One of the non-animal alternative assays accepted at the OECD (OECD 2016c) is the human cell line activation test (h-CLAT), which measures in vitro dendritic cell activation by the assessment of the expression of CD54 and CD86 in human THP-1 monocytic cell line. This method evaluates the variation in the expression of these two specific membrane antigens induced by sensitizing substances by means of flow cytometric analysis. Antigens are detected via specific monoclonal antibodies (mAbs) labelled with fluorochromes such as Fluoresceine-isothiocyanate (FITC). Unfortunately, the emitted fluorescence is overlapped by a natural level of cell fluorescence, called autofluorescence. This event induce an overestimation of the generated fluorescence signals and generate the need of troubleshooting in data analysis and interpretation.

The aim of this study was to find an alternative parameter (such as cell shape and morphology) more sensitive than fluorescence to evaluate skin sensitization potency of chemicals.

For this purpose THP-1 cells were cultured in RPMI-1640 medium with 0.05 mM 2-mercaptoethanol supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin. After thawing, cell morphology was examined with an Olympus BX51 microscope and replication rate was monitored by calculating the doubling time.

For the experiments, cells were seeded at density of 0.2x106 cells/ml in 75 cm2 culture flask and cultured for 48 hours at 37°C with 5% CO2. Then cells were transferred in a 24 well plate (1x106 cells/well) and treated with different allergens and non-allergens substances. For control analysis, a set of cells was not exposed to any chemical and has been maintained in culture medium. After a 24 hours period of incubation, cells were washed twice in phosphate-buffered saline and flow cytometric analysis was performed with Partec Cy-Flow-Space cytometer. We observed a significant change in the forward scatter (FSC) of cells treated with skin sensitizing substances. No changes were observed in control cells and in cells treated with non-sensitizers.

Our data suggest that well-known sensitizing chemicals, like 2,4-dinitrochlorobenzene (DNCB) and nickel sulfate hexahydrate ($NiSO_4$), are able to induce morphological changes in THP-1 cells, as demonstrated by significant variations in the FSC. For these considerations, the measure of FSC can be used as a sensitive, fast and low-cost method for the analysis of sensitizing substances allowing to discriminate between sensitizers and non-sensitizers.