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Evaluation of neutral red uptake assay using Balb/c 3T3 cells as a screening test to predict skin phototoxicity potential

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In order to evaluate the neutral red uptake assay as an alternative method for phototoxicity test, we compared the potential of phototoxicity in vitro in cultured human fibroblasts and 3T3 fibroblast cells derived from Balb/c mice. Both fibroblasts were exposed to various known phototoxic chemicals (promethazine, neutral red, chlorpromazine, chlortetracycline, amiodarone, bithionol, 8-methoxypsoralen) and non-phototoxic chemical (ammonium laureth sulfate) and irradiated with 5 J/cm² of UVA. Cell viability (IC₅₀) was measured by neutral red uptake (NRU) assay. The ratio of IC₅₀ value of chemicals in the presence and absence of UVA was determined by the cut-off value. The phototoxic (PT) potential of test chemicals in NRU assay was assessed by determining the photoirritation factor (PIF) with a cut-off value of 5. In both 3T3 and human fibroblasts, all known phototoxic chemicals (promethazine, neutral red, chlorpromazine, chlortetracycline, amiodarone, bithionol, 8-methoxypsoralen) were positive (over 5 of PIF value), whereas non-phototoxic chemical ALS was negative. However, bithionol was negative in human fibroblasts. These results suggest that NRU PT assay using fibroblasts could be applicable for prediction of the skin phototoxicity potential and 3T3 PT may be more reliable.