The Assessment of Phototoxicity Using the 3T3 Neutral Red Uptake (NRU) Phototoxicity Assay and a Modified Photo-Direct Peptide Reactivity Assay (DPRA)

Gamsion, A., Herpel, T., Lamm, M., Sadowski, N., Sheehan, D., Hilberer, A.
Institute for In Vitro Sciences, Inc., Gaithersburg, MD USA

Abstract 3147

Introduction

Alternative methods, including the validated 3T3 Neutral Red Uptake (NRU) Phototoxicity assay (OECD 422) may be used as a pre-clinical test to address phototoxicity. Currently, there are no validated alternative test methods to identify phototoxicants; however, there are several validated alternative test methods to address skin sensitization, including the Direct Peptide Reactivity Assay (DPRA) (OECD 420). To address phototoxicity, we performed the standard DPRA with a modification to include a UV-exposure (3 J/cm²) as described by Hayato, et al. (2016). The Dermapath SOL 3 solar simulator equipped with H Y 11 filter (320-400 nm) was used for all photo-irradiation. The amount of cysteine (Cys) peptide depletion was determined immediately after UVA exposure (432 nm, time 0) and at 2 hour intervals up to a 26 hour period (See Figure 3). We utilized the 3T3 Phototoxicity assay (Figure 1a) in combination with a modified photo-DPRA assay (Figure 1b) to determine if these assays are capable of (1) identify compounds with phototoxicity potential and 2) discriminate between photomutants and photolabile agents.

Materials & Methods

To establish proof of concept, we selected a small subset of seven compounds: aldehydes and photolabile agents (chlorpromazine (chlor), 6-methylcoumarin (6-MC), and amiodarone), photoallergen (Hexachlorophene), and peptides which were selected by a seven peptide modification. The reactivity of each compound was determined via each assay. For each test compound exposed in the presence and absence of UVA-visible light. The % depletion for each compound exposed in the presence of UVA (dotted lines) and absence of UVA (solid lines) over a time course is presented in Figure 2. Two time points were used to calculate the Area Under the Curve (AUC) for phototoxicity, and concentration-depletion curves were calculated for each test compound exposed in the presence and absence of UVA-visible light.

To ensure that the UVA exposure didn’t affect the Cys peptide, the peptide was exposed to 12 J/cm² of UVA exposure and showed 0% depletion (time 0 or 24 hours), indicating peptide stability after UVA exposure.

The clinical classification of each compound (photomutants and/or photolabile) was assigned using references cited from Ahmad et al. (2016), Maibach & Honari (2014), and Onoue et al. (2017). The phototoxicity potential was evaluated in the 3T3 Phototoxicity Assay by the Mean Photo Effect (MPE) values. A compound was predicted to have phototoxicity if the MPE was >0,150 and no phototoxicity if the MPE was <0,100 (OECD TG 432). The peptide depletion cut-offs described in OECD TG 440, were used as guidance (i.e., peptide depletion >13.8% was considered to be positive for sensitization potential). A summary of the results for each compound is presented in Table 1.

Conclusions & Future Directions

All compounds identified as phototoxic in the 3T3 NRU assay showed differences (>21.0%) in reactivity in the presence of UVA as compared to the absence of UVA.

Hexachlorophene, which did not trigger a phototoxic response in the 3T3 NRU assay (i.e., MPE <0,100), would be more likely to be classified as a photomutant if used in the DPRA assay.

The peptide depletion in the presence of UVA was very high (>90%) for Anthracene, a photoirritant, throughout the course of testing (up to 24 hours) of the Photo-DPRA assay.

Although we were not able to discriminate between photomutants and photolabile agents, our tiered approach was able to identify compounds which posed a phototoxic hazard (i.e., photomutant and photolabile). The Ahamed model; evaluation of a larger subset of compounds; and use of lysine peptide DPRA were noted increases in peptide depletion for some compounds in the absence of UVA, more notably as the reactivity time nears 24 hours.

Future Directions: Investigation of the use of other validated skin sensitization methods with a photo-irradiation modification, as well as an RÆ model; evaluation of a larger subset of compounds; and use of lysine peptide combinations of the Direct Peptide Reactivity Assay (DPRA) (b) and the UVA-Depletion assay (a) to determine the potential of a compound to induce phototoxicity.

Figure 1. Step by step procedures for the 3T3 Phototoxicity Assay and the Photo-DPRA assay

Results

Figure 2. Selected dose response curves from the 3T3 Phototoxicity Assay. The concentrations chosen were based on the highest testing limits of the OECD TG 422 (i.e., 1000 µg/mL) or based on the cytotoxic profile of a dose range-finding assay. The relative viability was calculated one test concentration in the presence (yellow boxes) and absence (blue boxes) of UVA. Each yellow or blue box represents relative viability of an individual well at each concentration (6 total replicates per UVA or dark exposure). Area shaded in grey was used to calculate the MPE values.

Figure 3. Photo-DPRA time course reactive depletion of test compounds in the presence (+UV) and absence (-UV) of UVA-visible light. After 1 hour of dark exposure, the depletion of cysteine (Cys) peptide was determined (time 0), and at 2 hour intervals thereafter (up to 24 hours). Dotted line represent Cys depletion for each test compound exposed to dark light exposure (-UVA) and solid lines represent Cys depletion for each test compound exposed to UVA light exposure (+UV).

Table 1. Summary results for 3T3 Phototoxicity Assay (MPE values) and photo-DPRA (% depletion of Cys peptide for each compound in the presence (+UV) and absence (-UV) of UVA exposure (time 0) and in dark exposure (time 0). Classification of each compound using results from alternative test methods (i.e., in vivo and clinical classification using referenced literature (Ahamed, et al., 2016); Maibach & Honari (2014) and/or Onoue et al. (2017)). Positive responses for photomutagen and photolabile agents in red, negative responses in green; and mixed responses (n.g. photomutant and non-photolabile or non-photomutant and photolabile) are green/white.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Average Depletion</th>
<th>Clinical</th>
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<tbody>
<tr>
<td>6-methylcoumarin</td>
<td>21.0%</td>
<td>Photomutant, Photoallergen</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>63.1%</td>
<td>Photomutant, Photoallergen</td>
</tr>
<tr>
<td>Anthracene</td>
<td>84.7%</td>
<td>Photomutant, Non-Phototolable</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>68.5%</td>
<td>Photomutant, Non-Phototolable</td>
</tr>
<tr>
<td>Hexachlorophene</td>
<td>42.5%</td>
<td>Non-Photomutant, Non-Photoallergen</td>
</tr>
<tr>
<td>Cinnamic Aldehyde</td>
<td>48.5%</td>
<td>Non-Photomutant, Non-Photolabile</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>3.2%</td>
<td>Non-Photomutant, Non-Photolabile</td>
</tr>
</tbody>
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References

Ahamed, M., & Honari, H. (2014). Dermatological phototoxicity: A new model; evaluation of a larger subset of compounds; and use of lysine peptide DPRA.
