Micronucleus Induction by 5-Methoxypsoralen in the Photo Reconstructed Skin Micronucleus Assay: Development of a Human-Relevant NAM for Identifying Photo-genotoxic Substances

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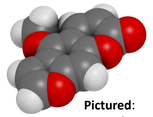
BACKGROUND

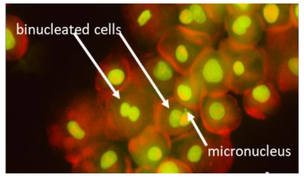


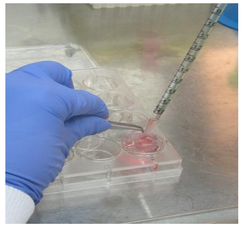
Pictured: 5-Methoxypsoralen (5-MOP) molecule

To address a lack of suitable non-animal tools to screen novel ingredients in personal care products for mutagenic/clastogenic activity after solar light exposure, we initiated a three-phase program to develop a new approach methodology (NAM) which integrates established UVA/UVB/visible light (UV/vis) photo-activation techniques to the reconstructed skin micronucleus (RSMN) assay. The first phase of the program established a repeat exposure photo-irradiation schedule in reconstructed human epidermal (RhE) tissues, and the second phase was conducted to identify a photo-genotoxic substance for use as a positive control in subsequent third phase studies focused upon test method evaluations. An ideal positive control should be non-mutagenic in the absence of photo-activation, but is recognized to have human-relevant photo-induced mutagenic or carcinogenic etiology or, at minimum, is known to induce micronuclei in replicating mammalian cells after photo-irradiation.

MATERIALS AND METHODS

Several putative positive control candidates were proposed, including 8-methoxypsoralen (8-MOP, which has been extensively reported to induce squamous cell carcinoma after long-term PUVA therapy), 5-MOP, and Sparfloxacin. Initial dose range finding assays in the presence and absence of UV/vis light were conducted on each of the candidate substances, followed by at least two definitive photo RSMN trials. In brief, the apical surfaces of the RhE tissues were dosed daily for a total of three days with 10 µL of the test substance diluted in the solvent, acetone, and immediately irradiated with either 1.5 or 2 J/cm² UV/vis light. After the 72 hour repeat exposure regimen, the tissues were trypsinized to maximize collection of the proliferative basal cells, and the cells were prepared for micronucleus scoring following standard fluorescence microscopy scoring methods for the RSMN assay. In parallel with the test substance, a negative control, 10 µL of acetone, was tested, as well as a UV/vis only control (whereby untreated RhE tissues were irradiated with either 1.5 or 2 J/cm² UV/vis light). The standard RSMN positive and negative controls, mitomycin C and acetone, were also tested in parallel in the absence of UV/vis light to monitor for standard test system responses. At least two tissues in each treatment group were assessed for binucleation frequency and induction of micronuclei in the definitive trials.



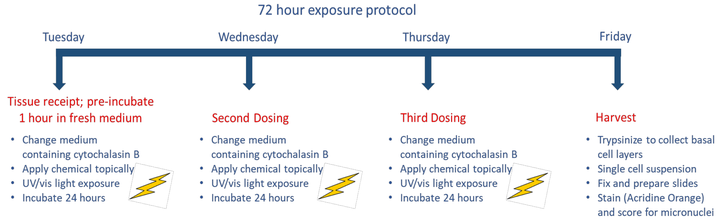


Pictured: Trypsinization of tissues

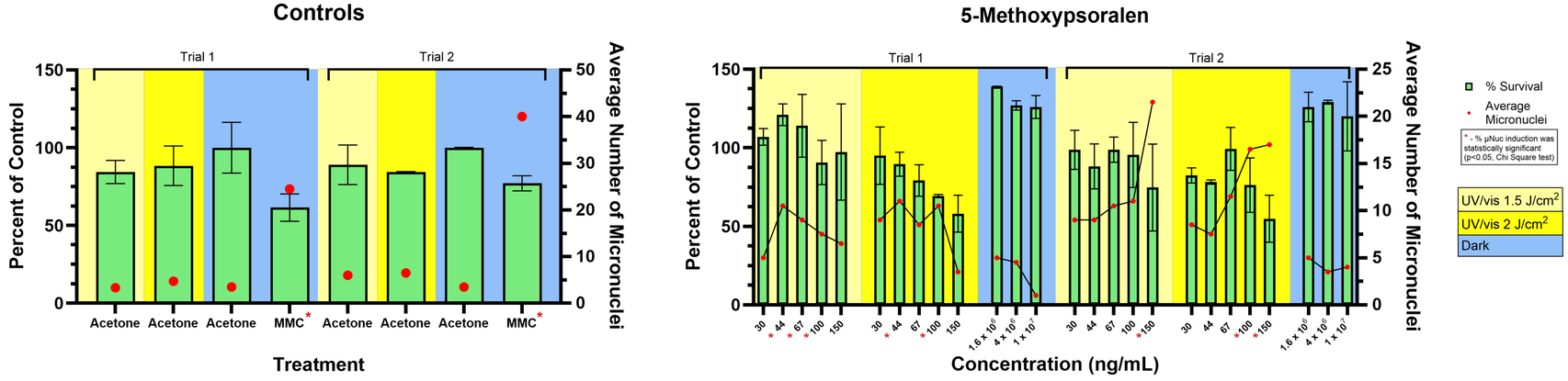
PHASE 2 STUDY DESIGN

In the initial studies of the three putative positive controls, only UVA/vis light was used. Since this only resulted in minimal increases in micronuclei induction by 8-MOP and 5-MOP, subsequent investigations included UVB in the UV/vis light irradiation of the tissues (the ratio of UVA:UVB was 20:1).

- A Dermalight SOL 3 solar simulator, equipped with a UVA/UVB H1 filter (320 to 400 nm) delivering 1 J/cm² per 10 minutes was used.
- The following photo irradiation treatment groups were selected and evaluated in the definitive trials:
  - 2 J/cm² – 20 minutes exposure per day
  - 1.5 J/cm² – 15 minutes exposure per day
  - 0 J/cm² – 20 minutes exposure per day, as a dark exposure control
- Each tissue was harvested and assessed for binucleation frequency and induction of micronuclei



PHASE 2 RESULTS



Both 8-MOP and 5-MOP caused high levels of photo-induced cytotoxicity, requiring lower doses with up to 5 orders of magnitude dilutions in the UV/vis exposure groups compared to those tested in the absence of UV/vis exposure. Whilst both 8-MOP and 5-MOP showed a dose-related induction of micronuclei in the presence of UV/vis exposure, 5-MOP exhibited somewhat less photo-cytotoxicity at photo-genotoxic doses and thus was chosen for subsequent testing. Relative binucleation rates in RhE tissues treated with 5-MOP at the highest dose of 150 ng/mL in the presence of 1.5 J/cm² UV/vis irradiation were considerably higher than those in the presence of 2 J/cm² UV/vis irradiation; the latter were at or slightly above the threshold for acceptable cytotoxicity. In the two definitive trials, the baseline micronucleus induction rates in the solvent control in the absence of UV/vis exposure were both 0.35%. Exposure to 1.5 and 2 J/cm² UV/vis increased micronucleus induction rates to 0.33% and 0.60%, respectively, in the first trial, and to 0.47% and 0.65%, respectively, in the second trial. After treatment with 5-MOP, the maximum statistically significant induction of micronuclei in the two definitive trials was 1.15% and 2.3% in the presence of 1.5 J/cm² UV/vis, and 1.10% and 1.75% in the presence of 2 J/cm² UV/vis.

CONCLUSIONS

The results of the two definitive trials demonstrate that under the testing conditions identified, 5-MOP acts as a suitable positive control to evaluate photo-genotoxicity since it gave reproducible positive results. Additionally, the UV/vis exposure of 1.5 J/cm² provided better performance relative to the 2 J/cm² exposures. The micronucleus induction was lower in the 1.5 J/cm² UV/vis irradiated solvent controls, and cytotoxicity was lower at the highest doses of 5–MOP after exposure to 1.5 J/cm² compared to those exposed to 2 J/cm² according to the binucleation rates, thus allowing for the potential to test higher test substance doses to increase test method sensitivity.

PHASE 3 DIRECTION

Once results from Phase 2 are available, 5-MOP data can be further evaluated to develop and establish specific criteria for testing the positive control, as well as for establishing criteria for a valid test. By applying the learnings from Phase 2, the optimized protocol will focus on the exposure range finding experiments in both the presence and absence of UV irradiation to ensure the appropriate selection of doses and UV/vis exposures for the unknown test substances.

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