The effect of chronic dosing regimens on genotoxicity assessment

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1. Introduction

- There is an effort globally to develop quick, accurate non-animal tests to predict the safety of novel compounds being produced.
- The US National Academy of Sciences' report, 'Toxicity Testing in the 21st Century [TT21C]: A Vision and a Strategy' envisions that most of this safety testing will eventually be carried out in vitro using human cells.
- Current tests which identify cellular toxicity, specifically genotoxicity, include the bacterial (Ames) mutation assay and the in vitro micronuclear assay.
- These use a single, acute chemical dose, which requires administration with solvent, and exposure is over a short time period (3-6 hours).
- This short exposure time is not realistic to everyday human exposure, which is often repeated and over a period of time.
- Previous studies have investigated the effect of split (chronic) doses of DNA alkylating agents methylmethane sulphonate (MMS) and N-methyl-N-nitrosourea (MNNU) on TK6 human lymphoblast cells, over five and ten days.
- This study aims to condense these previous time frames into periods closer in scale to current recommended safety tests, i.e., less than 24 hours.
- This project will also extend to further chemicals, which have differing chemical and DNA damaging properties, and will assess the utility of passive dosing (PD) methods to enable chronic dosing of cells.

2. Methods

- Day 1: Seed TK6 cells
- Day 2: Count cell concentration
- Day 3: Take second cell concentration count and calculate relative population doubling (RPD)

3. Results

- Generally, an increase in MMS dose decreased cell viability (RPD) across all three dosing regimens.
- A single 9 hr dose caused a significant increase in chromosome damage at MMS doses 0.7 and 1.0µg/ml.
- Three 1/3rd total dose 3 hr treatments with MMS caused a significant increase in chromosome damage compared to solvent control at 1.0µg/ml only.
- There was no significant increase in chromosome damage when the dose was split into 9 treatments within the same time period.

4. Discussion

- Three 3 hr 1/3rd total doses decrease the genotoxicity of MMS, measured using the in vitro micronuclear assay, whereas single 9 hr total doses eliminates toxicity, compared to a 9 hr acute treatment.
- This decrease in genotoxicity could be a result of lower chemical exposure, but could also indicate the efficiency and time frame of DNA repair mechanisms, which may not be able to cope with a higher exposure.
- A decrease in toxicity following split dosing may also indicate the safety of repeat exposures in everyday situations.

5. Future work and passive dosing

- Future work will involve continuation of these chronic and acute studies in MCL-5 and Hep G2 cells, which can metabolise carcinogens such as benz[a]pyrene.
- Benz[a]pyrene is a hydrophobic organic compound (HOC), which can be difficult to dissolve within the test cell culture medium.
- A novel method for dosing with HOCs is passive dosing (PD), which involves the concentration dependent release of chemical from a polydimethylsiloxane (PDMS) membrane into the test medium.
- This method can provide a constant exposure of chemical and eliminate many disadvantages of using a traditional 'solvent spiking' method, as the previous chronic studies have used.
- PD can be used in vitro testing to represent actual biological concentrations and can compensate for the losses detailed in Figure 5.
- Future work within this project will compare the effect of a PD system to chronic and acute dosing in order to better define toxicity testing.

6. References and acknowledgements


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