A physiologically-relevant in vitro screening assay for cytotoxic reactive metabolite formation would aid the elimination of drug candidates with hepatotoxic liability from further development.

**Introduction**

- Reactive metabolism is a major mechanism of drug toxicity, especially in drug induced liver injuries (DILI).
- Individual differences in drug metabolizing enzyme activities may lead to individual differences in toxic responses.
- Reactive metabolism is one of the hypothetical mechanisms for idiosyncratic DILI.
- A physiologically-relevant in vitro screening assay for cytotoxic reactive metabolite formation would aid the elimination of drug candidates with hepatotoxic liability from further development.

**Metabolism of xenobiotics with the potential to cause toxicity by forming cytotoxic reactive metabolites**

GSH represents the first line of cellular defense against the toxicity of reactive metabolites. Reduction of toxicity via increasing GSH contents would indicate that a toxicant would exhibit its toxicity via the formation of cytotoxic reactive metabolites.

**MetMax™ Human Hepatocyte GSH Rescue Assay for the Identification of Drugs with the Potential to Form Cytotoxic Reactive Metabolites**

- HEK-293 cells were supplied to us by Carol Loretz and Albert P. Li*.
- A MetMax™ GSH Rescue Assay for the identification of drugs with the potential to form cytotoxic reactive metabolites was performed on a pool of 10 (5 male and 5 female) human donors using proprietary procedures (patent pending).
- The use of a multiple donor pool eliminates results unique to an individual, allowing results to be representative of the human population.

**GSH attenuation of cytotoxicity of protoxicants in the metabolically incompetent target cell line (HEK263) in the presence of MetMax™ human hepatocytes is an indication of the formation of cytotoxic reactive metabolites.**

**MetMax™ Human Hepatocytes (Patent Pending):**

**MetMax™ Hepatocytes and intact hepatocytes contain complete drug metabolizing enzyme pathways due to the presence of all cellular organelles.**

- Intact Hepatocytes
- MetMax™ Hepatocytes
- Microsomes
- S9

**Complete Drug Metabolizing Enzyme Activities in MetMax™ Hepatocytes**

**Scientific Rationale**

**Hypothesis**

**Results**

**Summary and Conclusions**

- Two protoxicants, acetaminophen and cyclophosphamide, that are known to form cytotoxic, reactive metabolites upon hepatic metabolism were evaluated for their cytotoxicity in HEK-263 cells in the presence and absence of GSH with and without exogenous metabolism by MetMax™ Human Hepatocytes.
- The cytotoxicity of both protoxicants was increased by MetMax Human Hepatocytes, confirming the formation of cytotoxic metabolites.
- GSH (20 mM) effectively eliminated the cytotoxicity of the protoxicants in the presence of metabolic activation, thereby confirming MMHH-mediated formation of cytotoxic reactive metabolites.
- GSH (20 mM) did not diminish the cytotoxicity of the protoxicants in the absence of metabolic activation, suggesting that the toxicity was likely due to the inherent cytotoxicity of the parent compounds.

**MetMax™ Human Hepatocytes GSH-Rescue Assay can be used to identify xenobiotics with the potential to cause toxicity by forming cytotoxic reactive metabolites.**