A Need for an Exogenous Activating System for the Evaluation of Xenobiotic Toxicity

- Drug metabolism is a key determinant of toxicity. Drug metabolism may increase toxicity via the formation of metabolites with higher toxic potential than the parent drugs (metabolic activation), or lower toxicity due to the transformation of toxic drugs to less toxic metabolites (detoxification).
- In vitro hepatotoxicity evaluation using primary hepatocytes allows the detection of metabolism-dependent toxicity due to the inherent drug metabolizing enzyme activities.
- Cytotoxicity assays using non-hepatic target cells (e.g., cardiomyocytes for cardiotoxicity) which do not have drug metabolizing enzyme activities may not be relevant to toxicity observed in vivo.
- While the liver post-mitochondrial supernatant (liver S9) has been used successfully as a metabolic activating system for in vitro genotoxicity assays, it is not applicable for in vitro cytotoxicity assays due to the inherent cytotoxicity of the S9-cofactor mixture.
- We report here on a novel exogenous metabolic system, MetMax™ human hepatocytes (MMHH), as a potentially useful method for the incorporation of hepatic metabolism in cell based in vitro toxicity assays.

A Need for an Assay to Identify Drugs/Drug Candidates that Can Be Metabolized to Cytotoxic Reactive Metabolites

Drugs that are metabolically activated may have the following complications in drug development:

- Safety findings in preclinical animal species may not accurately reflect human effects due to species differences in drug metabolism.
- Idiosyncratic drug toxicity that, because of its low incidence, cannot be readily detected in Phase I, II and III clinical trials due to the limited number of patients.
- A large majority of drugs with idiosyncratic hepatotoxicity are known to form cytotoxic reactive metabolites.

There is a need to identify drug candidates with the potential to form cytotoxic reactive metabolites so that they can be excluded from further development to avoid the advancement of a drug with idiosyncratic toxicity.

MetMax™ Human Hepatocytes (Patent Pending): Permeabilized Hepatocytes Supplemented with Metabolic Enzyme Collectors

- MetMax™ Cryopreserved Human Hepatocytes (MMHH) Metabolize Protoxicants (P) to Toxic Metabolites (TM) Which Diffuse Out of the Hepatocytes into the Culture Medium and Cause Cytotoxicity in the HEK293 cells

Similar Drug Metabolizing Enzyme Activities between Conventional and MetMax™ Human Hepatocytes

- MetMax™ Hepatocyte (Permeabilized)

Experimental Procedures

- MetMax™ Human Hepatocytes (MMHH): MMHH were prepared from a pool of 10 (five male and five female) human donors using proprietary procedures (patent pending). The use of a single donor pool eliminates results unique to an individual, allowing results to be representative of the human population.
- Co-factor-Specified MMHH: MMHH was supplemented with the following cofactors to direct the pathway of metabolism:

Metabolic Activation Assay for Protoxicants: HEK293 cells, a commonly used cell line for in vitro cytotoxicity assays, were supplied to us by the US EPA. The cells were maintained in T-75 flasks as log growing cells in DMEM containing 10% fetal bovine serum. On the day of experimentation, the cells were trypsinized and reseeded in the maintenance medium. Cell concentration was quantified via hemocytometer counting. Cell concentration was adjusted to 500,000 cells/ml. The cell suspension was plated into 384-well white cell culture plates with transparent bottom at a cell density of 5000 cells/well (10 uL of 50,000 cells/ml) and incubated overnight in a cell culture incubator with a humidified atmosphere of 5% carbon dioxide before the commencement of drug treatment for the evaluation of cytotoxicity.

There were two treatment conditions: 1. HEK293 cells without metabolic activation; and 2. HEK293 cells with metabolic activation. To group 1, 10 uL of the hepatocyte medium without MetMax™ human hepatocytes was added. To group 2, 10 uL of Co-factor-Specified MetMax™ human hepatocytes was added. Treatment was initiated by an addition of 10 uL of test articles at 3X of the final desired concentration. After the addition of test articles, the plates were returned to the cell culture incubator where they were incubated for 24 hours followed by quantification of cell viability.

Quantification of Cell Viability: Cell viability was determined at the end of the treatment duration using cellular ATP as endpoint quantified by luminescence using the ATPlite (PerkinElmer) kit as per manufacturer’s instructions, and the PerkinElmer Victor3 Multimode Plate Reader. Results are presented as Relative Viability calculated using the following equation:

Relative Viability (%)= [Luminescence (treatment)/Luminescence (solvent control)] x 100

Results

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (uM) of Drug Cytotoxicity in the Presence of Pathway Specific MetMax Human Hepatocytes</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MMHH/oxidation</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>293</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>105.92</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>25.65</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>7.04</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>19.41</td>
</tr>
</tbody>
</table>

Summary

Five toxic drugs were evaluated for their cytotoxicity in HEK-293 cells in the presence and absence of Pathway-Specific MetMax™ Human Hepatocytes.

- Oxidation-mediated Metabolic Activation: The cytotoxicity of troglitazone, nefazodone, acetaminophen, and cyclophosphamide was enhanced by MMHH-oxidation, confirming that MMHH-oxidation can be used in the identification of protoxicants with toxicity directed by cytotoxic metabolites.
- Oxidation-Mediated Detoxification: Terfenadine cytotoxicity was reduced by MMHH-oxidation, confirming the parent drug is more cytotoxic than its oxidative metabolites. The observation is consistent with the known drug-drug interaction associated with terfenadine. Inhibition of CYP3A4 by ketoconazole or erythromycin are known to lead to cardiotoxicity due to decreased oxidation of the parent compound.
- Conjugation-Mediated Detoxification: Of all the conjugation pathways, GSH-conjugation led to the most substantial decreases in cytotoxicity for all five drugs evaluated, including terfenadine. The results confirm previously reported findings that cytotoxic reactive metabolites are key contributors to the toxicity of acetaminophen, cyclophosphamide, and nefazodone.

Conclusions

- MetMax™ Human Hepatocytes represent an effective exogenous metabolic activation system for the provision of human-specific drug metabolizing enzyme activities for the evaluation of chemical toxicity in cell based in vitro cytotoxicity assays.
- Pathway-Specific MMHH can be used to identify key detoxification pathways.
- MMHH represent a useful tool in drug development to minimize the development of drugs with unacceptable human toxicity, including the elusive idiosyncratic drug toxicity.
- In drug development, the assay can be used to identify drug candidates with their toxicity modified by drug metabolizing enzymes. Such drug candidates should be avoided as there may be species-differences in toxicity due to species-differences in drug metabolism. Furthermore, there may be individual differences in drug toxicity due to individual differences in drug metabolism in the human population.
- Identification of key detoxification pathways using Pathway-Specific MMHH may aide the elucidation of the mechanism of toxicity as well as the identification of at-risk human populations with their detoxification capacities compromised by environmental and genetic factors.