In Vitro Assessment of Drug-Induced Liver Injury (DILI) using a High Content Cellular Imaging System

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Society of Toxicology Annual Meeting, March 10-14, 2013

INTRODUCTION

Drug-induced liver injury (DILI) is a leading cause of drug failing during clinical trials and being withdrawn from the market. Idiosyncratic drug hepatotoxicity has not been well predictive because of its low concordance with either standard in vitro cytotoxicity screening assay results or regulatory animal study findings. Implementing an in vitro cell-based predictive assay early in the drug discovery process would help improve early compound attrition and develop safer drug candidates. The Thermo Scientific ToxInsight® IVT platform and DILI Assay Cartridge offer the tools to determine the hepatotoxicity risk of a compound by measuring multiple biomarkers of hepatotoxicity in individual cells. The high content imaging approach increases the sensitivity and specificity for predicting hepatotoxicity by simultaneously detecting five multiplexed cellular targets and properties associated with cell loss, DNA content, cellular redox stress, and mitochondrial stress. Combining this testing system with a proper cellular model will further improve the predictability of human hepatotoxicity cost effectively.

OBJECTIVES

To test the potential hepatotoxicity of nine reference test articles in three hepatocyte model systems using the ToxInsight DILI Assay Cartridge. The specificity and sensitivity of the assay will be evaluated.

METHODOLOGY

- Thermo Scientific ToxInsight®
- Cell Model of Choice:
  - Human hepatoma Hep G2 cell line
  - Induced Pluripotent stem cell (iPSC)-derived human hepatocytes
  - Fresh human primary hepatocytes
- Culture cells in a 96-well plate at 37°C overnight.
- Treat the cells with test article, vehicle control, and negative control.
- B concentration responses in triplicate
- 10X Cross if possible with 1.2 dilutions up to 10 concentrations and 0.5% neat DMSO concentrated.
- Incubate with test reagents for 22-24 hours.
- Scan the cells with four fluorescent probes:
  - Hoechst 33342
  - Cell tracker
  - Inuclea DNA integrity
  - Mitochondrial membrane (MMD)
  - Reduced glutathione levels
  - ROS Dye
  - Phosphatidylserine Exposure
  - Mitochondria Dye
  - Mitochondrial membrane potential

Cell Imaging on the ToxInsight®
- Data Analysis to quantify the multi-channel image outputs

- Hepatotoxicity prediction using the Thermo Scientific DILI Assay Excel Template

COMPOUNDS TESTED

<table>
<thead>
<tr>
<th>Compound Drug’s Trade Name</th>
<th>Cmax (µg/mL)</th>
<th>Pharmacological Action</th>
<th>Known to be Hepatotoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylbutazone</td>
<td>12.5X</td>
<td>Cyclooxygenase inhibitor</td>
<td>Yes</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>12.5X</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>12.5X</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Aflatoxin B1</td>
<td>2500X</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Aspirin</td>
<td>12.5X</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Fluoxetine</td>
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<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Melatonin, Circadin</td>
<td>0.00557</td>
<td>Circadian rhythm hormone, antioxidant, anticonvulsant, free radical scavenger</td>
<td>No</td>
</tr>
</tbody>
</table>

COMMENTS

- All three human hepatocyte models were stained efficiently with the four dyes, and allowed for quantification of all five cellular targets with the specific imaging protocol established for each hepatocyte model on the ToxInsight® system.
- The Hep G2 cells demonstrated a 100% specificity and 80% sensitivity with the nine reference compounds that were tested in the in vitro DILI assay.
- The sensitivity for the toxicity curves is shifted for some test articles, based on which source of hepatocytes they were tested.
- 50% of the order of toxicity for the test articles, differs on the source of hepatocytes.
- The rank order of toxicity for the test articles, differs on the source of hepatocytes.

RESULTS

- All three human hepatocyte models can be used in the in vitro DILI high content imaging assay using the ToxInsight® system.
- From the nine compound reference set, the three human hepatocyte models demonstrated high specificity and sensitivity, except for the lack of Aflatoxin B1 toxicity in the Hep G2 cells.
- The lack of toxicity of Aflatoxin B1 at the concentrations tested in the Hep G2 cells in the in vitro DILI assay is most likely due to the lack of metabolic activity in this human hepatomas cell line. Both the iPSC-derived hepatocytes and primary hepatocytes are known to maintain more hepatocyte functions and metabolic activities. The benefit of iPSC-derived hepatocytes over primary hepatocytes include their availability and data reproducibility.
- The use of an 80% concentration for each test article in triplicate helps to capture the potential hepatotoxic effects of a test article.
- The sensitivity for individual toxicity cellular parameters appears to vary depending on the source of hepatocytes. This may suggest that one source of hepatocytes provide a better hepatotoxicity model for a series of test articles.

REFERENCES & ACKNOWLEDGEMENTS


I would like to acknowledge MPI Research management team for supporting the work involved in establishing the present data set. I would like to acknowledge Carter Cliff from CDI for arranging the collaborative efforts between MPI Research and CDI research teams.