Human iPS-derived hepatocytes and cardiomyocytes for drug toxicity testing

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INTRODUCTION

Pharmaceutical candidate compounds require an extended period of time and high developmental costs before reaching the market. However, many candidate compounds are eliminated in the development process. Candidate compound attrition is frequently based on hepatotoxicity and cardiotoxicity (torsades de pointes). Although human cell lines are widely used for drug toxicity testing, they are subject to high lot-to-lot variability. Moreover, it is difficult to perform long-term tests with the same donor when using human primary cells due to their limited supply.

MATERIALS & METHODS

ReproHepato Type ITM Kit (1 kit for 96-well plate) (ReproCELL Cat. No. RCESD008)

- Cells, 1 vial (8.25 million cells/vial)
- Thawing Medium, 1 bottle
- Maintenance Medium, 1 bottle
- Assay Medium, 1 bottle
- Supplements

Hepatotoxicity Assay

- Acetaminophen (Sigma A7068)
- Aminodone hydrochloride (Sigma A8423)
- Cyclophosphamide monohydrate (Sigma C20768)
- Flutamide (Sigma F9397)
- CellTiter-Glo™ Cell Viability Assay (Promega #G7571)
- Cytotoxicity Detection KitPLUS (LDH) (Roche #4744936)
- ARVOX3 (PerkinElmer Japan)

ReproCardio2TM Kit (1 kit for 96-well plate) (ReproCELL Cat. No. RCESC008)

- Cells, 3 vials (3.3×10⁷ single cells/vial)
- Maintenance culture medium (80mL) x 2
- Coating solution (30mL) x 1
- Low attachment plate for aggregated cells
- Attachment plate for thin-layer / single cells

Immunocytochemistry

Primary antibodies
- Anti-α-SM/SMA (1:1000)
- Anti-MLC-2V/2A (1:500)
- Anti-Troponin (AbD 1:500)
- Anti-Vimentin (Millipore 1:100)

Secondary antibody
- Alexa Fluor™488 (Life Technologies)

Electrophysiological Assay
- Equipment: aMED, AXION, MCS
- Coating: Fibronectin

CONCLUSIONS

- Human iPS-derived hepatocytes (ReproHepato) and cardiomyocytes (ReproCardio2) express normal markers matching their particular cell types.
- ReproHepato cells show similar response to primary hepatocytes in standard in vitro toxicity assays.
- ReproHepato cells facilitate HCS analysis of hepatotoxicity.
- ReproCardio2 cells enable MESA assays for in vitro prediction of cardiotoxicity.

Drug Toxicity Testing using ReproHepato ATP and LDH Assay

<table>
<thead>
<tr>
<th>Test Agent</th>
<th>1 nM</th>
<th>3 nM</th>
<th>10 nM</th>
<th>30 nM</th>
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<tbody>
<tr>
<td>Flutamide</td>
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<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>100</td>
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<tr>
<td>cheapest</td>
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MEA assay using ReproCardio2

<table>
<thead>
<tr>
<th>Test Agent</th>
<th>Field Potential Duration (FPD)</th>
<th>Beat Rate Changes</th>
<th>µM</th>
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<tr>
<td>Azithromycin</td>
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<td>120</td>
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<td>Moxifloxacin</td>
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<td>100</td>
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<tr>
<td>Clarithromycin</td>
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<tr>
<td>Telithromycin</td>
<td>101</td>
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</table>

FIGURE 1. ReproHepato expresses standard hepatocyte markers for drug metabolizing enzymes and drug transporters. The mRNA levels of various hepatocyte specific markers measured by qPCR relative to primary hepatocytes (set to 100).

FIGURE 2. ReproHepato cells show toxic responses to standard cardiotoxins. After exposure of ReproHepato to hepatotoxins for 48 hours, ATP content and LDH release were measured, showing concentration-dependent reduction of cell viability.

FIGURE 3. ReproHepato cells facilitate HCS analysis of toxicity. ATP and LDH Assay

FIGURE 4. ReproCardio 2 cells express standard cardiomyocyte markers. α-MHC and ß-MHC are the representative cardiac markers during differentiation from iPS cells and expressed in heart. MLC-2A and MLC-2V are myosin specific markers for the atrium of the mammalian heart and for the ventricle of the mammalian heart, respectively. Cardiac troponin T (cTnT) is a specific marker in human heart and a thin filament protein which takes part in muscle contraction. Cx43 is a connexin gap junction protein.

FIGURE 5. ReproCardio 2 cells are sensitive to electrophysiological effects of drugs by MEA. Thin-layer ReproCardio 2 cells were exposed to various concentrations of drugs from 300 pM to 30 µM. The cells were analyzed by MEA assay using an MCS system, with 2 minutes of drug exposure and 2 minutes of recording. A) Field Potential Duration (FPD) and beat rate changes were measured for potential cardiotoxins. B) Three different lots of ReproCardio 2 were exposed to various concentrations of E4031. The three different lots exhibit similar Field Potential Duration, indicating low lot-to-lot variability.