Corning® HepatoCells: An In Vitro Screening Tool for Predicting Clinical CYP3A4 Induction

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Abstract

Induction-mediated drug-drug interactions need to be carefully characterised in vitro for drug candidates in order to determine and/or predict their induction potential in patients. Currently, both the FDA and EMA recommend using primary human hepatocytes as an in vitro test system for this purpose. Several models ranging from simple to complex, such as basic models (Cmax/EC50, relative induction score, R3, and mechanistic model) and PK/PD models, are being proposed to predict clinical CYP3A4 induction in vitro data from primary human hepatocytes. In light of the large ligand-lot-to-lot variation inherent in primary human hepatocytes, Corning HepatoCells (derived from primary human hepatocytes) was evaluated for its applicability as an in vitro screening tool for predicting clinical CYP3A4 inducibility. Three lots of cells were treated with a group of known clinical strong, moderate/weak inducers, and non-inducers at more than 8 concentrations each. Both enzymatic activity (testosterone β4 hydroxylase activity) and mRNA expression were measured as endpoints using LC-MS/MS and RT-PCR, respectively. EC50 and Emax were estimated from concentration-dependent induction response curves. A Relative Induction Score (RIS) model (as well as other 2 basic models, EC50, and R3) was employed using EC50 and Emax for the data for model drugs, and resulting RIS data were evaluated for the ability to predict potential clinical CYP3A4 inducer/non-inducer. It was found that all 3 models correlated well (R2=0.93) with clear clinical data of CYP3A4 substrates. The results also showed that all 3 lots of Corning® HepatoCells behaved similarly to primary human hepatocytes in terms of prediction, with only minor lot-to-lot variations for the 3 lots of cells. In conclusion, Corning HepatoCells can be used as a potential in vitro screening tool for prediction of clinical CYP3A4 induction.

Materials and Methods

Materials: Cryopreserved Corning HepatoCells and Corning hepatocyte maintenance medium (Corning Cat. No. 354882) were obtained from Corning Life Sciences. All test compounds were provided by Sigma-Aldrich. Compound stock solutions were prepared by dissolving compound in DMSO and serially diluting the solutions in EMDS. Final working solutions were prepared by diluting the stock solutions 100X in culture medium.

Thawing, plating, and culturing of the cells: On day 1, HepatoCells were thawed in a 37°C water bath. After removing the cryo-freezing media, the cell pellet was resuspended in culture medium containing 10% FBS, then the cells were seeded in a 96-well Corning BioCoat® Collagen-coated plate at a density of 20,000 cells per well. Corning M9600 matrix was added to cell monolayer at a concentration of 0.5 mg/ml. 24 hours after seeding. After being washed with test compounds at different concentrations for a consecutive 3 days, the cells were washed with fresh culture medium and incubated with 200 µM testosterone for 1 hour to measure enzyme activity. The metabolite β4-hydroxytestosterone was measured by LC-MS/MS. After enzyme assay, mRNA was isolated from cells using a Qiagen RNeasy® 96-wk. CYP3A4 mRNA expression level was determined using Applied Biosystems two-step protocol using a 7500 Real-time PCR system.

Data analysis: EC50 and Emax were determined from concentration-dependent induction response curves by fitting the curves to a sigmoidal 3 parameter model of SigmaPlot® (Systat Software Inc.). RIS and R3 were calculated using unbound EC50 from the literature2 and equations described below: RIS = (Emax/E0 + Cmax/EC50) + 1 – 1/3 * + R3 = (EC50 + Emax) / EC50 + 1 – 1/3 * + A calibration curve was generated by plotting the induction parameter [e.g., RIS] against observed metabolism AUC change using the Hill 3 parameter function of SigmaPlot. Prediction accuracy using HepatoCells was evaluated by assessing the correlation between predicted AUC change with observed AUC change, and was compared with that of primary human hepatocytes.

Conclusions

Corning HepatoCells showed concentration-dependent responses to a group of known clinical CYP3A4 inducers with very good correlation (R2=0.95).

RIS 3 lots of HepatoCells behaved similarly to primary human hepatocytes in terms of estimation of induction parameter (i.e., RIS), with much smaller lot-to-lot variations.

Predicted DDI (AUC change) estimated using RIS data generated with in vitro data from HepatoCells correlated very well (R2=0.95) with clinical clearance data of CYP3A4 substrates.

HepatoCells can be used as a potential in vitro screening tool for prediction of clinical CYP3A4 induction.

Figure 1: Examples of Concentration-dependent Induction Response Curves Used to Determine EC50 and Emax

Figure 2: Consistent Performance between 3 Different Lots of Corning HepatoCells

Figure 3: Examples of Calibration Curves Using 3 Different In Vitro Induction Parameters

Figure 4: Calibration curve was fitted to a Hill 3 parameter model (SigmaPlot), and the corresponding equation was used to calculate predicted AUC change using in vitro induction parameter RIS. The predicted AUC change was then plotted against observed AUC change to determine the accuracy of prediction. Corning HepatoCells have shown similar prediction accuracy as primary human hepatocytes (for both cell models, predicted AUC changes fall within ±20% of prediction).

Table 1: RIS and Emax Determination Using Concentration-dependent Induction Response Curves

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (pM)</th>
<th>Lot P1</th>
<th>Lot P2</th>
<th>Lot P3</th>
<th>Lot P1</th>
<th>Lot P2</th>
<th>Lot P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td>0.1</td>
<td>3.5</td>
<td>1.1</td>
<td>0.95</td>
<td>10.3</td>
<td>3.9</td>
<td>0.98</td>
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<tr>
<td>Nifedipine*</td>
<td>4</td>
<td>0.048</td>
<td>0.052</td>
<td>0.045</td>
<td>0.019</td>
<td>0.007</td>
<td>0.011</td>
</tr>
<tr>
<td>Probenecid</td>
<td>0.0485</td>
<td>0.0193</td>
<td>20.1%</td>
<td>55.8%</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4*</td>
<td>0.506</td>
<td>0.1167</td>
<td>29.2%</td>
<td>72.9%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CYP3A4* prepared cells were cryo-coated in 10% primary hepatocytes in well. HepatoCells correlated similarly to primary hepatocytes in terms of prediction, with only minor lot-to-lot variations for the 3 lots of cells. In conclusion, Corning HepatoCells can be used as a potential in vitro screening tool for prediction of clinical CYP3A4 induction.

Table 2: Comparison of Induction Parameter (RIS) between Corning HepatoCells and Primary Human Hepatocytes

<table>
<thead>
<tr>
<th>Compound</th>
<th>RIS Corning</th>
<th>RIS Primary</th>
<th>% Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td>3.9</td>
<td>3.5</td>
<td>96.7%</td>
</tr>
<tr>
<td>Nifedipine*</td>
<td>1.8</td>
<td>2.1</td>
<td>95.2%</td>
</tr>
<tr>
<td>Probenecid</td>
<td>0.0485</td>
<td>0.0193</td>
<td>20.1%</td>
</tr>
<tr>
<td>CYP3A4*</td>
<td>0.506</td>
<td>0.1167</td>
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</tr>
</tbody>
</table>

Figure 5: Calibration curves using 3 different in vitro induction parameters and % decrease in observed metabolism AUC all showed good correlation with R2=0.95.

Table 3: Three prototype lots of Corning HepatoCells generated similar RIS data as primary human hepatocytes; however, HepatoCells showed much smaller variance than primary human hepatocytes (Average NCE ±10.2% for HepatoCells, and 53.2% for primary human hepatocytes).

Figure 6: Correlation Analysis of Observed Midazolam Victim Drug AUC Change (%) vs. Predicted AUC Change (%) from RIS

Conclusions

• Corning HepatoCells showed concentration-dependent responses to a group of known clinical CYP3A4 inducers with very good correlation (R2=0.95).

• All 3 lots of HepatoCells behaved similarly to primary human hepatocytes in terms of estimation of induction parameter (i.e., RIS), with much smaller lot-to-lot variations.

• Predicted DDI (AUC change) estimated using RIS data generated with in vitro data from HepatoCells correlated very well (R2=0.95) with clinical clearance data of CYP3A4 substrates.

• HepatoCells can be used as a potential in vitro screening tool for prediction of clinical CYP3A4 induction.