Effect of Transfeting HepG2 with Human CYP Enzymes on Chemical Toxicity

Wei Zhang1, Tssten Yeshi3
1Hera Biolabs, Inc. Lexington, KY, USA

Abstract

HepG2, a human liver cancer-derived cell line, is one of the most commonly used cell systems in toxicity screening with the advantages of unlimited life-span and supply, and stable phenotype. However, low and inconsistent expression and activity of drug metabolizing enzymes, especially the Phase I cytochrome P450 (CYP) enzymes, could lead to missed or underestimated cytotoxicity of test compounds. To provide an in vitro cell system to identify specific CYP-mediated cytotoxicity, here we evaluated the cytotoxicity of selected chemicals in HepG2 cells stably transfected with individual human CYP enzymes and NADPH cytochrome P450 oxidoreductase (CYP reductase/POR) knockout (KO) HepG2 cells. HepG2 cell lines that express CYP1A2, CYP2B6, CYP2C9 or CYP3A4 showed significantly higher specific CYP activity compared with HepG2 wild type (WT) cells. The activity of all the four CYP enzymes was abolished in CYP reductase KO cells, as expected. CYP3A4-mediated hepatotoxicity of aflatoxin B1 was well demonstrated in this system, showing HepG2-CYP3A4 cells were much more sensitive to aflatoxin B1 compared with HepG2 WT cells whereas CYP reductase KO HepG2 cells were highly resistant. This study illustrates the utility of human CYP-expressing HepG2 cell lines to study the role of specific human CYP enzymes in chemical-induced cytotoxicity.

Materials and Methods

Cells and reagents: HepG2 cells, HepG2ΔhPOR cells (HPRGΔhPOR) and Upcyte® human hepatocytes were obtained from ATCC, and ThermoFisher Scientific Inc, respectively. HepG2 cells stably transfected with individual human CYP enzymes were provided by Takeda Pharmaceutical Company Limited (Osaka, Japan). They were established by selecting transformants which express CYP stably (Toxicol. In Vitro 2001 Jun;15(3):245-56.). P450-350 assay kits (V8421, V8321, V8791, V9001). CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) kit (G5880) and CellTiter-Glo® ATP assay kit (G7571) were purchased from Promega (Madison, WI). Naphthoflavone (N757), clopidogrel (C3614), sulfaphenazole (S7578), ketoconazole (K1003), aflatoxin B1 (A6366), chloramphenicol (C3138), rifampin (R9051), cytochrome P450 (CYP6), acetaminophen (AT085), and sodium citrate (PHR1416), were from Sigma Aldrich (St. Louis, MO).

Cytotoxicity assay: Cells were plated in 96 well plates overnight. Cells were treated with multiple compounds of indicated concentrations for 72 hrs. Cell viability was assessed with Promega CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) and/or CellTiter-Glo® ATP assay kit.

CYP activity measurements: Cells were plated in 96 well plates as 15000 cell/well and cultured for 72 h. Then, cells were incubated with 6 µM Luciferin-1A2 (CYP1A2), 3 µM Luciferin-2B6 (CYP2B6), 100 µM Luciferin-3A4 (CYP3A4) and 3 µM Luciferin-IPA (CYP3A4) for 1 hour, 2 hours, 4 hours and 1 hour respectively. CYP activity was measured with P450-Glo assay kits. Specific CYP activity in transfected cells was confirmed with CYP-selective inhibitors, namely 5 µM naphthoflavone (CYP1A2), 1 µM clopidogrel (CYP2B6) 2 µM sulfaphenazole (CYP2C9) or 1 µM ketoconazole (CYP3A4). A complete list of chemicals treated in HepG2 WT and HepG2-CYP transfected and CYP reductase KO cells is provided in Supplementary Materials.

Results

Figure 1: Evaluation of chemical cytotoxicity in HepG2 cells based on mitochondrial function (MTS assay) and ATP levels

A MTS assay

B ATP assay

Figure 2: Comparative toxicity of acetaminophen in HepG2, Upcyte® human hepatocytes and HepaRG based on ATP levels

Figure 3: CYP activity in HepG2 WT cells, HepG2-CYP cells and HepG2-CYP reductase KO cells

Table 1. HepG2 cell lines available at Hera BioLabs.

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>HepG2 Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>HepG2-CYP1A1</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>HepG2-CYP2B6</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>HepG2-CYP3A4</td>
</tr>
</tbody>
</table>

Note: HepG2 CYP1A2 reductase (hPOR) KO cell line has premature stop codon in exon 3 (total 15 exons)

Table 2. CYP activity in HepG2 cell lines

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>HepG2 Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>HepG2-CYP1A2</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>HepG2-CYP2B6</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>HepG2-CYP3A4</td>
</tr>
</tbody>
</table>

Note: HepG2 CYP1A2 reductase (hPOR) KO cell line has premature stop codon in exon 3 (total 15 exons)

Conclusions

1. We provide HepG2 cells stably transfected with a number of individual human CYP enzymes. The extent of the increased expression varies from 10 to over 200 fold depending on the individual enzyme being expressed.
2. We have created a CYP reductase KO HepG2 cell line.
3. A cell line overexpressing CYP3A4 is more sensitive to Aflatoxin B1 cytotoxicity than the corresponding WT cell line.
4. Together these cell lines can be used for reaction phenotyping—determining, directly which CYP is primarily responsible for the metabolism of a new drug.
5. By incubating a specific cell line with a well characterized model substrate of that specific CYP and see if the metabolism of the model substrate is inhibited by a new compound, these cells can be used to screen if any CYPs are inhibited by the new compound.