Effect of Transfecting HepG2 with Human CYP Enzymes on Chemical Toxicity

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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/hPOR) 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, HepaRG™ cells (HPRGC10) and Upcyte ® human hepatocytes were obtained from ATCC, and ThemoFisher 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–Glo assay kits(V8421, V8321, V8791, V9001), CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) kit (G3580) and CellTiter-Glo® ATP assay kit (G7571) were purchased from Promega (Madison, WI). Naphthoflavone (N5757), clopidogrel (C0614), sulfaphenazole (S0758), ketoconazole (K1003), aflatoxin B1 (A6636), chlopramazine (C8138), rifampicin (R3501), cyclophosphamide (C0768), acetaminophen (A7085), 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 wells plate 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-H (CYP2C9) 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 uM sulfaphenazole (CYP2C9) or 1 µM ketoconazole (CYP3A4).

Results

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

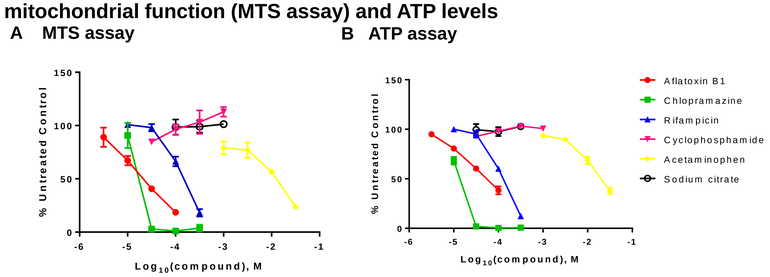

Figure 1. Assessment of cell viability of HepG2 cells after treatment with various compounds. HepG2 cells were plated in 96 well plates as 5000 cells per well. Cells were treated with multiple compounds of concentration range from 3 µM to 30 mM. for 72 hrs. Cell viability was assessed with Promega CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) (A) and CellTiter-Glo® ATP assay kit (B). Each point represents mean ± SD, n=3.

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

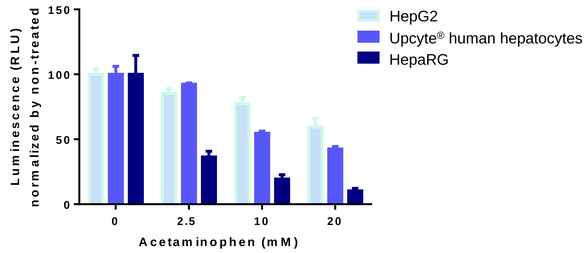

Figure 2: HepG2 cells are more resistant to acetaminophen toxicity compared with Upcyte® human hepatocytes or HepaRG cells. This result implies the relatively low CYP activity in HepG2 cells which reported by other groups. Concentration-dependent reduction in cell viability is shown in three cell lines treated with acetaminophen. 5000 cells per well were plated in 96 well plate. Cells were treated with 2.5 mM, 10 mM, 20 mM acetaminophen for 72 h. Cell viability was assessed with Promega CellTiter-Glo® ATP assay kit (B). Each bar represents mean ± SD, n=3.

Table 1. HepG2 cell lines available at Hera BioLabs.

1	HepG2	7	HepG2-CYP2C8
2	HepG2 CYP reductase KO	8	HepG2-CYP2C9
3	HepG2-CYP1A1	9	HepG2-CYP2C19
4	HepG2-CYP1A2	10	HepG2-CYP2D6
5	HepG2-CYP2A6	11	HepG2-CYP2E1
6	HepG2-CYP2B6	12	HepG2-CYP3A4

Note: HepG2 CYP reductase (hPOR) KO cell line has premature stop codon in exon 3 (total 15 exons)
HepG2-CYP cells stably express human CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4, respectively

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

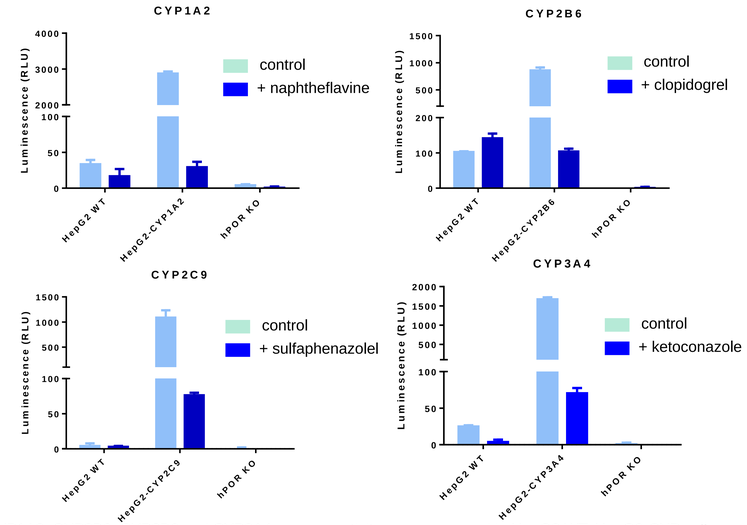

Figure 3. CYP1A2, CYP2B6, CYP2B6 and CYP3A4 enzyme activity were measured in HepG2 WT, HepG2-CYP cells and CYP reductase (hPOR) KO HepG2 cells. Cells were plated in 96 wells plate as 15000 cell/well and cultured for 72 h. Then, cells were incubated with their corresponding substrates (materials and methods). CYP activity was measured with P450 –Glo assay kits (Promega). Specific CYP activity in transfected cells was confirmed with CYP-selective inhibitors, namely 5 µM naphthoflavone (CYP1A2) 1 µM clopidogrel (CYP2B6) 2 uM sulfaphenazole (CYP2C9) or 1 µM ketoconazole (CYP3A4). Each bar represents mean ± SD, n=3.

Figure 4: An assessment of CYP3A4-mediated Aflatoxin B1 cytotoxicity in three cell lines.

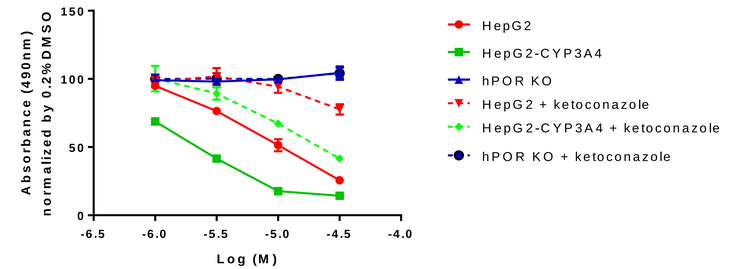

Figure 4. Detection of Aflatoxin B1 cytotoxicity. 5000 cells per well were plated in 96 well plates and adapted to cell culture conditions overnight. Cells were treated with various concentrations of aflatoxin B1 with or without 1µM ketoconazole (CYP3A4 inhibitor). In cells treated with aflatoxin B1, concentration-dependent cytotoxicity (based on loss of cell viability) was enhanced by the expression of CYP3A4 and suppressed by CYP reductase knockout. In HepG2 WT cells and HepG2-CYP3A4 cells, co- incubation with10 µM ketoconazole protected against the toxic effect of aflatoxin B1. Cell viability signal was assessed with Promega CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS). Each point represents mean ± SD, n=3.

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 B1cytotoxicity 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.