Corning® HepatoCells Closely Model the Behavior of Parental Cells for Predicting Hepatotoxicity

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Abstract

Unforeseen hepatotoxicity is one of the primary reasons for aftermarket black box warnings and removal of drugs. As a result the FDA recently recommended the development of novel tools and cell-based assays to more accurately identify drug induced liver injury. Primary human hepatocyte (PHH) culture is presently the gold standard model for studying the ADMET properties of drug candidates. However, due to large lot-to-lot variations and limited lot size of primary hepatocytes, significant time and resources are spent on prequalifying primary hepatocytes. Donor cells should ideally express the complement of Phase I and II drug metabolism enzymes and drug transporters which impact hepatotoxicity. The availability of renewable sources of human hepatocytes that closely model the behavior of parental hepatocytes in primary culture will enable the long-term use of these cell models for comparative pharmacogenomics toxicity studies. In the present hepatotoxicity studies, we compared the performance of Corning HepatoCells, a cell-line derived from primary human hepatocytes, to the parental hepatocyte cells and HepaRG™. Liver cells were seeded into 96 well Corning® Biocoat™ Type I Collagen plates using recommended culture medium containing 10% serum. The next day unbound cells were removed and 25 ug of Corning® Matrigel® was added to each well. Medium was changed every two days. On culture day 5 liver cells were pretreated with a panel of 80 compounds (100uM) that contained toxic and non toxic compounds (Plate 2, Enzo Screen-Well® Hepatotoxicity Library). Cell viability was measured 48 hours after treatment using the Promega CellTiter™ GLO assay and data was normalized to 1% DMSO Controls. Linear regression analysis of hepatotoxicity data revealed that the compound toxicity profile of HepatoCells more closely modeled the behavior of parental donor primary hepatocytes (R^2=0.87) than HepaRG™ (R^2=0.5). Of note, both HepatoCells and the parental cells detected the top 18 toxic compounds in our study. In contrast, 13/18 of these compounds exhibited cytotoxicity when primary hepatocytes from a different donor were used. Taken together, our results suggest that Corning HepatoCells closely model the functional behavior of parental cells and therefore may be used as a primary hepatocyte surrogate to study pharmacogenomic-related cytotoxicity.

Introduction

Corning scientists have developed a novel platform technology to create single-use, high-purity, cryopreserved HepatoCells. We have previously shown that HepatoCells express mRNA for phase I and II drug metabolism enzymes and transporters. The purpose of this study was to determine if HepatoCells modeled the behavior of parental primary human hepatocytes following *in vitro* exposure to known hepatotoxic compounds.

Experiments

Two hepatotoxicity experiments were performed:

Liver Cells	Seeding Density	Culture Medium
Primary Human Hepatocytes[1]	70K/well	CHMM[3]
HepatoCells[2]	70K/well	CCMH[4]
HepaRG™	100K/well	Williams Medium + Tox Supplement[5]

[1]Primary human hepatocytes from two different donors were employed for these experiments:
Experiment 1: Donor 1 is a three year old Caucasian male
Experiment 2: Donor 2 is a nine year old , Caucasian, female
[2]Hepatocells were derived from Donor 2. Experiments 1 and 2 were conducted with HepatoCells produced from two different lots.
[3]CHMM: Corning® Hepatocyte Maintenance Medium (Catalog # 40-550-CV)
[4]CCMH: Corning Culture Medium for HepatoCells
[5]HepaRG™ Tox Medium supplement was purchased from Life Technology and culture medium was prepared according to manufacturer's recommendation.

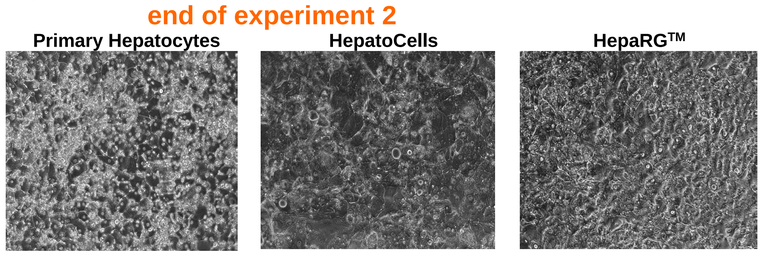
Hepatotoxicity Assay

Liver cells were seeded into 96 well Corning® Biocoat® Type I Collagen plates using recommended culture medium containing 10% serum. The next day unbound cells were removed and 25 ug of Corning® Matrigel® was added to each well to restore membrane polarity. Medium was changed every two days.

Five days later liver cells (e.g. duplicates) were pretreated with a library of 80 compounds (100uM) that contained toxic and non toxic compounds (Plate 2, Enzo Screen-Well® Hepatotoxicity Library).

Cell viability was measured 48 hours after compound treatment using the Promega CellTiter™ GLO assay. Data was normalized to 1% DMSO control. Hepatotoxicity was define as a 50% reduction in cell viability.

Figure 1. Morphology of liver cells (control wells) at the end of experiment 2



After one week culture, HepatoCells maintain a confluent cell monolayer of comprised of cells with prominent nuclei and bile canalicular structures, an indication of the hepatic origin of the cells.

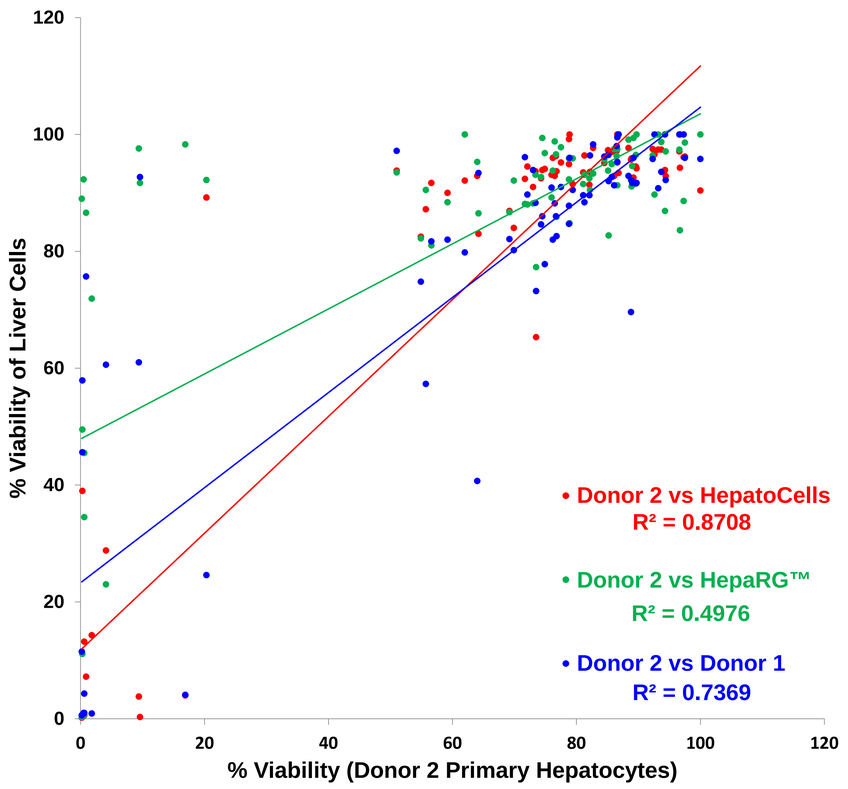
Table 1. Comparison of liver cell models for predicting hepatotoxicity (% viability)

Most Toxic Non Toxic

Compound Name	Hepatotoxicity related to:	Experiment 1 Donor 1	Experiment 1 HepaRG™	Experiment 1 Hepato-Cells	Experiment 2 Donor 2	Experiment 2 HepaRG™	Experiment 2 Hepato-Cells
Ciglitazone	Inhibition of BSEP	14.5	100	0.8	0.2	89	0.2
Thioridazine·HCl	Phospholipidosis	0.6	0.9	0.6	0.2	0.3	0.3
Fluphenazine di·HCl	Increases lipid peroxidation	0.6	0.8	0.6	0.2	0.4	0.3
Promethazine·HCl	Phospholipidosis	57.9	80.5	8.5	0.3	11.1	0.3
Hexylresorcinol	Non-Hepatotoxic control	92.7	92.9	1.8	9.6	91.7	0.3
Tamoxifen	Steatohepatitis	0.8	1.1	0.9	0.4	0.7	0.4
Diethylstilbestrol	Induction of Mallory bodies	0.9	92	0.8	0.5	92.3	0.5
Coralgil	Steatohepatitis	0.9	1.1	0.9	0.5	0.6	0.5
rac Perhexiline Maleate	Steatohepatitis	1	1.1	1	0.5	0.6	0.6
Clotrimazole	Inhibition of BSEP	1	51.9	1	0.6	24.5	0.6
Nordihydroguaiaretic acid	Reactive metabolites	61	92.6	3.8	9.4	97.6	3.8
Lithocholic acid	Inhibition of BSEP	4.1	96.7	7.1	38.9	98.3	4
Ethinyl estradiol	Inhibition of BSEP	75.7	71.6	28.9	0.9	86.6	7.2
Nicardipine·HCl	Induction of Mallory bodies	4.3	63.2	1	0.6	45.5	13.2
(+)Usnic acid	Elevated ALT levels	0.9	57.5	17	1.8	71.9	14.3
Amiodarone·HCl	Induction of Mallory bodies	60.6	80.1	3.4	4.1	23	20.8
Chlorcyclizine·HCl	Phospholipidosis	45.6	84.5	14.4	0.3	49.5	28
Nimesulide	Mitochondrial Toxicity	73.2	93.9	73.6	73.5	77.3	65.3
Colchicine	Inhibition of BSEP	74.8	78.8	79.8	54.9	82.2	82.5
Tacrin·HCl	Bioactivation to Rx metabolites	93.4	93.1	90.3	64.2	86.5	83
Glafenine	Fatal jaundice	80.2	93.3	64.3	69.9	92.1	84
(+)-Griseofulvin	Induction of Mallory bodies	82.1	74.2	74.9	69.2	86.7	86.9
Glibenclamide	Inhibition of BSEP	57.3	82.7	80	55.7	90.5	87.2
Chloroquine Diphosphate	Phospholipidosis	24.6	100	91.1	20.3	92.2	89.2
Desmethoxyyangonin	Elevated ALT levels	82	88.4	81.2	59.2	88.4	90
Propyl gallate	Non-Hepatotoxic control	95.8	100	99.9	100	100	90.4
Methylsticin	Elevated ALT levels	93.9	90	88	73	88.2	91
Phenytoin	Activation to Rx metabolites	89.6	87.4	94.7	82.1	92.5	91.4
Mefenamic acid	Mitochondrial Toxicity	90.5	94.7	81	79.4	95.9	91.5
Diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylate	Induction of Mallory bodies	81.7	76.2	81.7	56.6	81	91.7
Biotin	Non-Hepatotoxic control	91.7	87.6	96.6	89	94.6	91.8
Forskolin	Induction of Mallory bodies	79.8	100	93.3	62	100	92.1
Dihydromethylsticin	Elevated ALT levels	96.1	93.8	93.3	71.7	88.1	92.4
Diltiazem·HCl	Induction of Mallory bodies	84.6	92.3	91.2	74.3	92.7	92.5
Ascorbic acid	Non-Hepatotoxic control	96	93.6	98.3	89.2	99.4	92.6
Seneciophylline	P450 mediated activation	40.7	85.9	95.1	64	95.3	92.9
Sorbitol	Non-Hepatotoxic control	92.2	94.9	94.6	94.4	97.1	92.9
Chlorzoxazone	Activation to Rx metabolites	91.3	89.6	96.2	86.1	97.5	92.9
2,3-Dimethoxy-1,4-naphthoquinone	Activation to Rx metabolites	88.2	97.8	1.1	76.5	98.8	92.9
Cincophen	Fatal jaundice	90.9	98.4	92.6	76	89.2	93.1
Sucrose	Non-Hepatotoxic control	100	100	99.2	86.8	100	93.4
Tolmetin sodium salt dihydrate	Elevated ALT and AST levels	89.6	109.1	107.2	81.1	91.5	93.5
Cortisone	Steatosis	96.4	90.1	94.6	82.2	90.5	93.6
Buspirone·HCl	Inhibition of BSEP	82.6	84.8	88.7	76.8	85.9	93.7
Carbamazepine	P450 mediated activation	88.3	83.4	92	73.4	93.1	93.7
Naproxen	Inhibition of BSEP	97.2	108.1	101.2	51	93.5	93.8
Rifamycin SV sodium salt	Inhibition of BSEP	86	88	93.5	74.5	99.4	93.9
Thiobenzamide	Steatosis	100	100	98.5	94.3	86.9	93.9
Bosentan	Inhibition of BSEP	77.8	87.8	82.3	74.9	96.8	94.1
Adipic acid	Non-Hepatotoxic control	91.7	90.9	92.4	89.7	100	94.2
Methotrexate	Induction of Mallory bodies	100	87.6	98.1	96.7	83.6	94.3
Yangonin	Elevated ALT levels	89.7	92.3	77.3	72.1	88	94.5
Benzoic acid	Non-Hepatotoxic control	91.6	100	100	89.6	96.5	94.5
Prednisone	Steatosis	87.8	90.6	94.2	78.8	92.3	94.9
Tauroursodeoxy cholic acid dihydrate	Inhibition of BSEP	96.2	99.1	100	84.5	95.7	95.1
Gabapentin	Elevated ALT and AST levels	95.3	97.5	94.8	86.6	91.3	95.2
Methylprednisolone	Activation to Rx metabolites	91	82.8	100	77.5	97.8	95.2
Salvinorin A	Formation of hepatotoxic metabolites	69.6	100	100	88.8	92.2	95.7
Acetylsalicylic acid	Elevated levels of aminotransferases	92.1	96.9	94.1	88.9	91.1	95.9
Rifampicin	Interference with bilirubin transport	82	93.1	77.1	76.2	93.8	96
Acetaminophen	Hepatic Necrosis	100	100	100	97.3	88.6	96.1
Tetracycline	Steotosis	96	89.9	100	97.5	98.6	96.2
Nifedipine	Activation to reactive metabolites	86	89.1	86.4	76.7	96.6	96.3
Diclofenac·Na	Mitochondrial Toxicity	88.4	97	84.2	81.3	93.1	96.4
Chloramphenicol	Phospholipidosis	92	95.4	94.6	85.2	82.7	96.5
Gentamicin sulfate	Phospholipidosis	100	100	99	92.6	89.7	96.6
Cholic acid	Induction of Mallory bodies	92.7	92.1	96.2	85.7	94.9	97
Iproniazid Phosphate	Activation to Rx metabolites	100	97.4	100	96.6	97.4	97.1
Sulfasalazine	Steatohepatitis	98	93.5	97.2	86.5	96.3	97.2
Ketoprofen	Steatohepatitis	96.5	92.2	93.3	85.1	93.8	97.3
Aspartame	Non-Hepatotoxic control	90.8	98.2	100	93.2	100	97.4
Glucono δ-lactone	Non-Hepatotoxic control	93.6	91.5	94.5	93.7	98.7	97.4
(+)-Pulegone	Reactive metabolites	95.8	96.4	96.1	92.3	96.4	97.5
Furosemide	Steatosis	98.3	90.4	98.5	82.7	93.3	97.7
Scopolamine·HBr	Elevated ALT levels	92.9	94.1	100	88.4	99.1	97.7
Sulindac	Mitochondrial Toxicity	84.7	94.2	94.4	78.8	95.9	99.2
Collidine	Induction of Mallory bodies	99.5	95.9	100	86.6	97.5	100
(±) Kavain	Elevated ALT levels	96	100	96.1	78.9	84.8	100
1% DMSO	Control	100	100	100	100	100	100

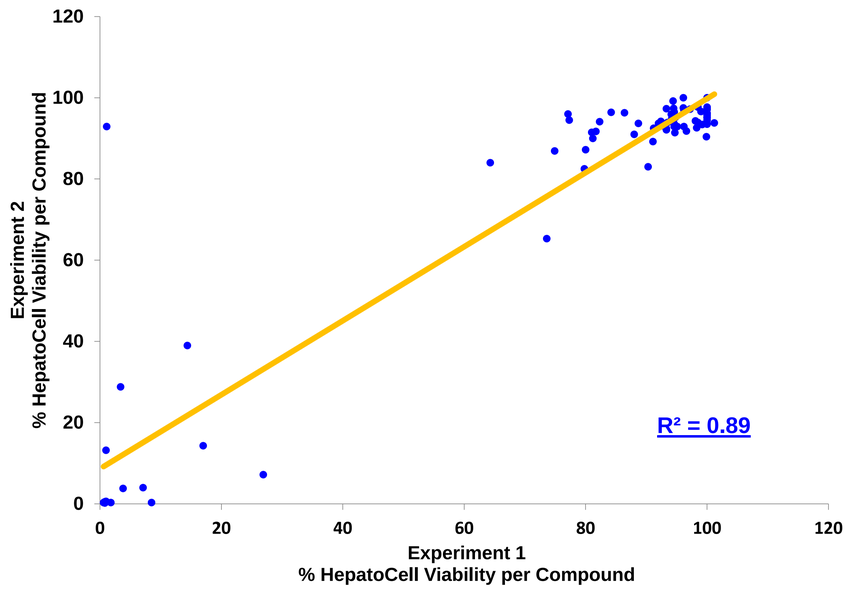
Results from two hepatotoxicity experiments showed that both HepatoCells and the Donor 2 parental cells detected the top 18 most toxic compounds in our study based on cell viability assays. In contrast, 13/18 of these compounds exhibited cytotoxicity when primary hepatocytes from a different donor were used. One compound, hexylresorcinol, was cytotoxic to HepatoCells and Donor 2 but not to Donor 1 and HepaRG™. Of particular note, four compounds (e.g. ciglitazone, clotrimazole, lithocholic acid, and ethinyl estradiol), which are known inhibitors of the bile salt export transporter, were cytotoxic to HepatoCells and parental Donor 2 cells but were not significantly cytotoxic to HepaRG™. Three of these four BSEP inhibitors were also cytotoxic to Donor 1, the exception being ethinyl estradiol. The observation that the viability of HepatoCells mirrors that observed with Donor 2, following compound treatment suggests that the differences observed between cell models reflects the pharmacogenomic differences in Donors.

Figure 2. Relative comparison of liver cell models to predict hepatotoxicity



Linear regression analysis of compound toxicity data from experiments 1 and 2 reveals that HepatoCells, which were derived from Donor 2, closely model the hepatotoxic response of Donor 2.

Figure 3. HepatoCells from two different cell lots reproducibly predict compound toxicity



Experiments 1 and 2 were performed as described using HepatoCells expanded from different lots by different scientists and conducted one year apart. Linear regression analysis of compound toxicity data from these two experiments shows that HepatoCells consistently detect compound toxicity. These observations support the robustness of using HepatoCells for hepatotoxicity screening assays.

Summary

- HepatoCells are a homogeneous cell population that are derived from normal human hepatocytes
- HepatoCells closely model the morphology and behavior of parental primary hepatocytes in hepatotoxicity assays (R^2=0.87)
- The compound hepatotoxicity profile of HepatoCells was reproducible (R^2=0.89)
- We propose that HepatoCells are a suitable surrogate of parental primary hepatocytes for identifying compounds that may exhibit hepatotoxicity