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 characterized *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 (C_{max}/EC_{50}, relative induction score, R_3) and mechanistic models (next effect model and PBPK model), are being proposed to predict clinical CYP3A4 induction using *in vitro* data from primary human hepatocytes. In light of the large 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 inducers. 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 6β hydroxylase activity) and mRNA expression were measured as endpoints using LC-MS/MS and RT-PCR, respectively. EC_{50} and E_{max} were estimated from concentration-dependent induction response curves. A Relative Induction Score (RIS) model (as well as other 2 basic models, C_{max}/EC_{50} and R_3) was employed using EC_{50} and E_{max} data for the model drugs, and resulting RIS data were evaluated for the ability to predict potential clinical CYP3A4 inducers/non-inducers. It was found that all 3 models correlated very well (R^2>0.9) with clinical clearance 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 obtained from Sigma-Aldrich. Compound stock solutions were prepared by dissolving compound in DMSO and serially diluting the solutions in DMSO. Final working solutions were prepared by diluting the stock solutions 1000X 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 I-coated plate at a density of 80,000 cells per well. Corning Matrigel® matrix was added to cell monolayer at a concentration of 0.25 mg/mL 24 hours after seeding. After being dosed 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 6β-hydroxytestosterone was measured by LC-MS/MS. After enzyme assay, mRNA was isolated from cells using a Qiagen RNeasy® 96-kit. CYP3A4 mRNA expression level was determined using Applied Biosystems two-step protocol using a 7300 Real-time PCR system.

Data analysis. EC_{50} and E_{max} were determined from concentration-dependent induction response curves by fitting the curves to a sigmoidal 3 parameter function of SigmaPlot™ (Systat Software Inc.). RIS and R_3 were calculated using unbound C_{max} from the literature[1,3] and equations described below: $RIS = (E_{max} \times C_{max,ub})/(EC_{50} + C_{max,ub})$, $R_3 = 1/(1 + d \times E_{max} \times C_{max,ub}/(EC_{50} + C_{max,ub}))$ with d = 1. A calibration curve was generated by plotting the induction paramter (e.g., RIS) against observed midazolam AUC change using the Hill 3 parameter function of SigmaPlot. Prediction accuracy using HepatoCells was evaluated by assesing 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 (R^2>0.9).
- 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 (R^2>0.9) 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 E_{max} and EC_{50}

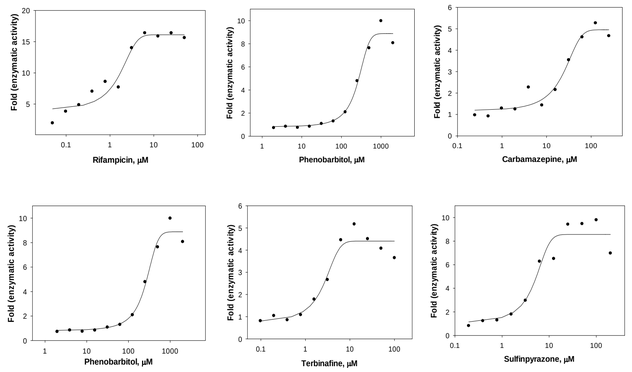

Figure 1. Concentration-dependent CYP3A4 induction response curves were plotted using fold induction data of enzymatic activity from a group of know inducers (mRNA data showed similar concentration dependent response curve). Examples are shown here for 6 model compounds using one lot of Corning HepatoCells. All curves are fitted using SigmaPlot sigmoidal 3 parameter function, with R^2 all greater than 0.9.

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

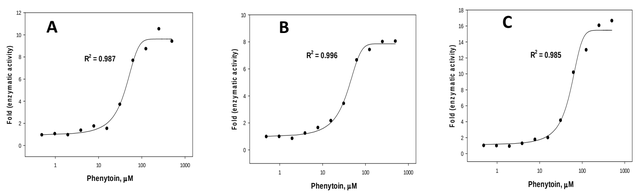

Figure 2. Three different lots of Corning HepatoCells showed similar concentration-dependent induction response to model compounds, suggesting consistent performance of HepatoCells. **(A)** Lot PR2; **(B)** Lot PR3A; **(C)** Lot PR3B.

Table 1: E_{max} and EC_{50} Determination Using Concentration-dependent Induction Response Curves

Test Compound	Concentration Range, µM	Lot PR2			Lot PR3A			Lot PR3B		
		E_{max} (Fold)	EC_{50} (µM)	R^2	E_{max} (Fold)	EC_{50} (µM)	R^2	E_{max} (Fold)	EC_{50} (µM)	R^2
Rifampicin	0.049-50	16.1	1.2	0.95	10.1	0.6	0.96	25.0	1.4	0.95
Phenytoin	0.49-500	9.6	39.5	0.99	7.9	34.3	1.00	15.5	51.9	0.98
Carbamazepine	0.24-250	5.0	18.0	0.96	3.9	14.2	0.93	3.5	23.4	0.90
Phenobarbitol	1.95-2000	8.9	247.7	0.98	6.2	211.0	0.99	14.8	330.7	0.98
Terbinafine	0.1-100	4.4	2.2	0.95	3.3	1.3	0.92	6.9	4.2	0.98
Sulfinpyrazone	0.2-200	8.6	4.6	0.93	8.0	6.2	0.95	15.3	9.2	0.96
Probenecid	0.1-100	31.3	40.7	0.94	40.5	52.8	0.93	36.5	43.8	0.96
Pioglitazone	0.1-100	36.5	5.4	0.97	39.5	5.5	0.98	62.4	9.8	0.96
Dexamethasone	0.24-250	34.1	43.5	0.97	27.7	28.0	0.98	42.1	42.8	0.95
Nifedipine	0.1-100	9.9	4.1	0.96	10.3	3.9	0.98	11.0	4.9	0.98
Omeprazole	0.1-100	19.4	3.8	0.97	14.3	3.8	0.97	21.4	5.1	0.98

Table 1. EC_{50} and E_{max} were determined as described in Materials and Methods. All 3 lots of Corning HepatoCells showed good fitting with R^2>0.9 for all compounds tested.

Figure 3: Calibration Curves Using 3 Different *In Vitro* Induction Parameters

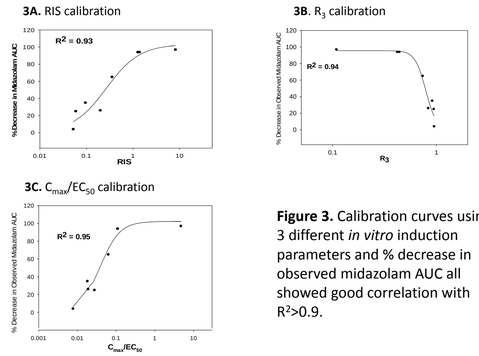

Figure 3. Calibration curves using 3 different *in vitro* induction parameters and % decrease in observed midazolam AUC all showed good correlation with R^2>0.9.

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

Compounds	Observed AUC change	RIS from Corning HepatoCells			RIS from Primary Human Hepatocyte			Average RIS		%CV	
		Lot PR2	Lot PR3A	Lot PR3B	Lot 295	Lot 312	Lot 318	HepatoCells	PHH	HepatoCells	PHH
Dexamethasone	19	0.0042	0.0053	0.0053	0.001	0.001	0.0004	**0.0050**	**0.0008**	**12.7%**	**43.3%**
Terbinafine*	25	0.047	0.059	0.040	0.027	0.024	0.007	**0.0485**	**0.0193**	**20.1%**	**55.8%**
Nifedipine*	4	0.048	0.052	0.045	0.019	0.007	0.011	**0.0484**	**0.0123**	**7.4%**	**49.5%**
Pleconaril*	35	0.087	0.095	0.101	0.025	0.029	0.011	**0.0944**	**0.0217**	**7.5%**	**43.6%**
Omeprazole	-25	0.189	0.139	0.154	0.008	0.019	0.019	**0.161**	**0.0153**	**16.0%**	**41.4%**
Pioglitazone*	26	0.190	0.199	0.178	0.012	0.011	0.025	**0.189**	**0.0160**	**5.5%**	**48.8%**
Troglitazone*	65	0.513	0.355	0.650	0.12	0.2	0.03	**0.506**	**0.1167**	**29.2%**	**72.9%**
Phenobarbitol	61	0.902	0.729	1.158	0.88	2.4	1.6	**0.930**	**1.63**	**23.2%**	**46.7%**
Carbamazepine*	94	1.25	1.074	0.723	1.1	1.1	9.3	**1.02**	**3.83**	**26.5%**	**123.5%**
Phenytoin*	94	1.50	1.381	1.908	1	1.3	1.8	**1.60**	**1.37**	**17.3%**	**29.6%**
Rifampicin*	97	10.93	8.175	16.201	7	11	6.4	**11.77**	**8.13**	**34.7%**	**30.7%**

Table 2. 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 %CV is 18.2% for HepatoCells, and 53.2% for primary human hepatocytes).

Figure 4: Correlation Analysis of Observed Midazolam Victim Drug AUC Change (%) vs. Predicted AUC Change (%) from RIS

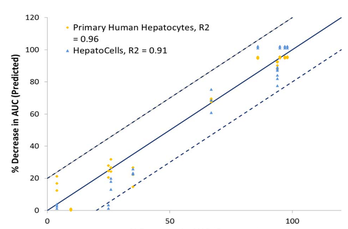

Figure 4. Calibration curve was fitted to a Hill 3 parameter model (SigmaPlot), and the corresponding equation was used to calculate predicted AUC change using *in vitro* induction parameter RIS. The predicted AUC change was then plotted against observed AUC change to determine the accuracy of prediction.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

References

1. USFDA Draft Guidance for Industry: Drug Interaction Studies (2012).
2. EMA Guidance on Investigation of Drug Interaction (2013).
3. Fahmi OD, et al. Drug Metab Dispos 40:2204-2211 (2012).
4. Fahmi OD, et al. Drug Metab Dispos 38:1605-1611 (2010).

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