HμRELhumanPool™: Combining the phenotypic breadth of pooled primary hepatocytes with the stable, long-enduring cellular competency of HμREL® co-culture technology

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INTRODUCTION

Accurately predicting the pharmacokinetic and toxicological properties of drugs as early as possible in the development process can materially reduce the number of clinical failures, and hence the total cost of developing a drug. As such, there is an accelerating drive to identify systems that offer greater utility in predicting human physiological responses to xenobiotics. Current systems provide *in vivo* data obtained using whole animals or *in vitro* using microsomes, isolated hepatocytes and cell lines such as HepG2. Due to the distinct advantages and disadvantage associated with data generated by each system, researchers will typically employ most if not all of these systems to investigate the disposition of a compound under development.

To reduce the use of whole animals and the costs associated with them researchers are constantly working on the development of stable and effective hepatocyte cell-based *in vitro* systems that can provide more relevant data earlier in the development process. Isolated hepatocytes or their enzymes comprise the base of most *in-vitro* DMPK and Toxicity assays. Because most hepatospecific functions are typically lost in the first days of culture, they are of limited use in cases where compounds clear slowly, or where metabolites are generated over extended periods of time. To address this limitation, HμREL has developed and extensively characterized, long-term, stable hepatocyte co-culture models in four species (Human, Dog, Monkey, and Rat). In the course of characterizing the single donor HμREL*human*™ model it became evident that clearance predictions and metabolite formation rates are significantly influenced by donor-dependent levels of enzyme activity and that there is large donor-to-donor variability in activity. To account for variability in single donor cryopreserved hepatoyctes, in this study we have characterized a 5-donor pooled lot of primary human hepatocytes in the HμREL co-cuture platform.

MATERIALS AND METHODS

Hepatocyte Co-cultures

A cryopreserved 5-donor pooled HμREL hepatic co-culture model was tested for long term function as well as its ability to predict clearance rates of low turnover compounds. Cryopreserved hepatocytes and non-parenchymal stromal cells were cultured with HμREL Platinum*Heps*™ media. Cells were seeded into collagen coated 96-well plates at the density of 30,000 hepatocytes. After culturing for 7 days to stabilize the cultures, cells were treated with drug compounds.

Metabolic Activity

Enzyme	Substrate	Concentration (μM)
CYP3A4	Midazolam	5
CYP2D6	Dextromethorphan	20
CYP2C9	Tolbutamide	20

CYP substrates and incubation concentrations are given in the table above. All incubations were carried out in triplicate on customer days 1, 7 and 14. Substrates were incubated for 60 minutes. Reactions took place in a humidified incubator at 37° C, in 5% CO_2. Collected supernatants were stored at –20° C until further LC-MS analysis (Applied Biosystems API 4000).

Clearance Prediction Evaluation

Several low-moderate clearance compounds (1 μM final concentration) were incubated using HμREL 96 well plates containing 30,000 hepatocytes/well to determine intrinsic clearance. HμREL provided their proprietary dosing and maintenance media. Cells were dosed with 125 μl media with 1 media. Time-points were taken at 15 min, 12 hours, 24 hours, 48 hours, 72 hours. 100 μl were removed at each time point and quenched with 100 μl 100% Acetonitrile containing 0.5 μM terfenadine. Samples were filtered and analyzed by LC/MS/MS. Liquid chromatography was done by Agilent 1200. Injections were made by LEAP HTS Pal autosampler. MS/MS used was Applied Biosystems API 4000.

Estimation of Hepatic Clearance

k=slope of time vs LN%Remaining

$$t_{\frac{1}{2}} = \frac{\ln(2)}{k} \qquad V = \frac{\text{inc.volume (uL)}}{\text{total\#cells} \bullet 10^6}$$

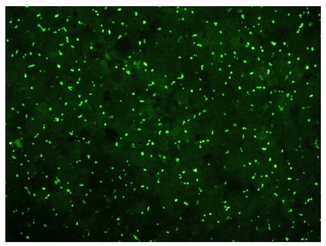
$$CL_{int}\,(\text{ml/min/}10^6\text{ cells}) = \frac{\ln(2) \times V}{t_{\frac{1}{2}}}$$

Qh (mL/min/kg)	20
Liver Weight/Body Weight (g/kg)	21.4
# of cells per incubation *10^6	0.03
total incubation volume (mL)	0.125
million cells/g liver	120

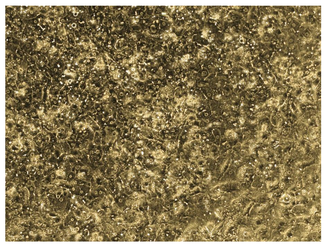
$$CL_{int,u}\,(\text{ml/min/kg}) = CLint / fu_{inc} \times \frac{\#\text{ cells}}{\text{g liver}} \times \frac{\text{liver wt}}{\text{body wt}}$$

$$CL_H = \left[\frac{Q_{H,B} \bullet fu \bullet CLu_{int,H}}{Q_{H,B} + fu \bullet CLu_{int,H}/(C_B/C_P)}\right]$$

FIGURE 1: Morphology and canalicular formation of HμRELhumanPool™ co-culture model (day 14)



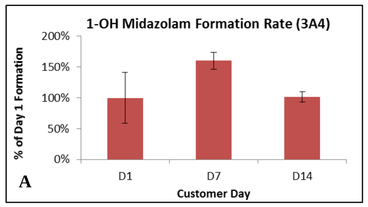
Canalicular Formation

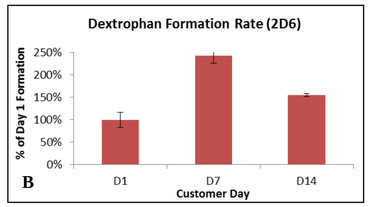


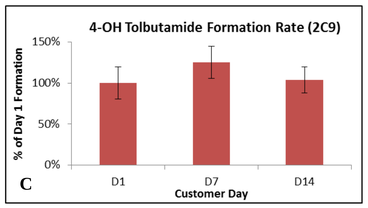
Phase Image

Morphology and canalicular formation in HμRELhumanPool™ co-culture models at 14 days of culture. 10x magnification.

FIGURE 2: 14 customer days of enzymatic function

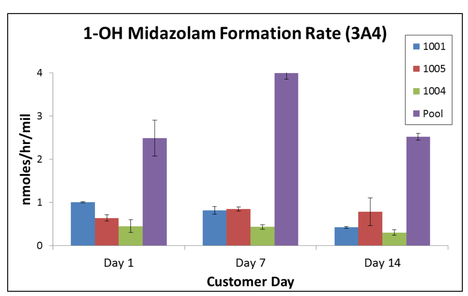






Metabolic Activity characterized by CYP specific metabolite formation. **A)** CYP 3A4 activity measured by the rate of midazoam conversion to 1-OH midazolam. **B)** CYP 2D6 activity measured by the rate of dextromethorphan conversion to dextrophan. **C)** CYP 2C9 activity measured by the rate of tolbutamide conversion to 4-OH tolbutamide. The concentrations of the metabolites were monitored over one hour in culture and normalized to day 1 formation rates.

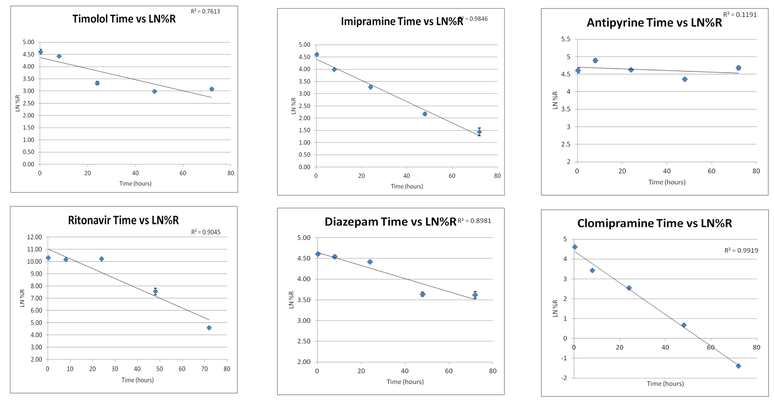
FIGURE 3: Single vs. Pooled Donor Function



Pooled hepatocytes have 3-4 fold higher activity when compared to single donor lots

FIGURE 4: Time vs LN%R clearance graphs

Concentration vs Time Curves- Pooled Hepatocytes



Time-points were taken at 15 min, 12 hours, 24 hours, 48 hours, 72 hours. 100 μl was removed at each time point and quenched with 100 μl 100% Acetonitrile containing 0.5 μM terfenadine.

FIGURE 5: Clearance prediction data: Single vs. pooled donor

Compound	CLh (mL/min/kg)	CLp Literature (mL/min/kg)	Hurel CLh (mL/min/kg) X 2	Fold Difference (Literature/ Hurel)
timolol	1.00	7.8	2.00	-3.90
timolol pooled	3.00	7.8	6.00	-1.30
imipramine	3.80	13.5	7.60	-1.78
imipramine pooled	2.50	13.5	5.00	-2.70
antipyrine	1.70	0.6	3.40	-5.70
antipyrine pooled	0.74	0.6	1.48	-2.43
clomipramine	5.30	10.9	10.60	-1.03
clomipramine pooled	2.60	10.9	5.20	-2.10
diazepam	0.10	0.38	0.20	-1.90
diazepam exp 2	0.10	0.38	0.20	-1.90
diazepam pooled	0.11	0.38	0.22	-1.73
ritonavir	1.80	1.2	3.60	3.33
ritonavir exp 2	0.23	1.2	0.46	-2.61
ritonavir pooled	1.00	1.2	2.00	1.67

Multiplying hepatic clearance values (estimated using HμREL pooled donor hepatocyte co-culture system) by a factor of 2 results in ALL substrate clearance predictions within 3-fold of literature values.

CONCLUSIONS

- Pooled cryopreserved hepatocytes plated in HμREL's co-culture model can be maintained with the stable, long-enduring cellular competency for up to 21 days from the start of the cultures.
- Based on the preliminary data presented here, pooled hepatocytes have 3-4 fold higher activity when compared to single donor lots.
- The data demonstrates that using a scaling factor of 2 resulted in ALL substrate clearance predictions within 3-fold of *in vivo* literature values.

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