Use of the HμREL® human hepatocyte co-culture model to determine intrinsic clearance and elucidate metabolic pathways of stable compounds

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Abstract

Hepatocyte suspensions are commonly used to predict clearance of new chemical entities. However, incubation times are relatively short, in order to maintain cell viability and enzymatic activity, which may limit the accuracy of prediction of clearance for stable compounds. The aim of this work was to determine the clearance of a range of compounds in three systems; HμRELhumanPool™ co-culture (72 h), 2D monoculture (24 h) and suspension (2 h), utilising the same batch of cryopreserved human hepatocytes (5-donor pool). Data generated from these assays were then scaled to predict *in vivo* human clearance to assess the suitability of each system in accurately determining clearance of stable compounds. Both plated systems predicted *in vivo* clearance well, following regression correction, however the HμREL® human hepatocyte co-culture model demonstrated superior assay sensitivity, enabled by a longer incubation time in comparison to the 2D monoculture. Following regression correction, human *in vivo* clearance was predicted within 2-fold for 12 out of 13 compounds assessed in the HμREL® co-culture model.

Furthermore, the utility of the HμREL® co-culture model as an *in vitro* model for the generation of metabolites for stable compounds was demonstrated. Eight compounds were incubated in the three *in vitro* systems and metabolic profiling was performed using high resolution accurate mass spectrometry. The benefit of the longer incubation time of the HμREL® co-culture system on the sensitivity of detection of metabolites, which were not observed in the other *in vitro* systems, was demonstrated. For example, diazepam, disopyramide, tolbutamide and warfarin were all shown to form metabolites in the HμREL® co-culture system that were either present in lower amounts or entirely absent in 2D monoculture or suspensions of human hepatocytes.

These results demonstrate the application of the HμREL® co-culture model, both in determining the clearance of stable compounds and in establishing the metabolic profile of a compound, realised by the longer incubation period.

Introduction

- There is an increased prevalence of low clearance compounds in drug discovery driven by a need to minimise dose, improve exposure and prolong half-life[2,3].
- Determining an accurate *in vitro* measurement for low clearance compounds using standard methods is challenging due to issues with low viability or enzymatic activity at longer incubation times.
- HμREL® provide a co-culture of primary hepatocytes (5 donor pool) and non-parenchymal stromal cells, which have been designed to maintain their cellular function for use in long term culture.
- Thus, the capability of the HμRELhumanPool™ co-culture model, over a 72 h incubation period, to provide an accurate assessment of CL_{int} for stable compounds and identify metabolic pathways was established in comparison to plated monoculture and suspension formats utilising the same batch of cryopreserved human hepatocytes.

Methods

- Pooled cryopreserved human hepatocytes were purchased from HμREL® corporation (lot HU1021) for use in suspension and in a 96-well plated format.
- For the suspension method, test compounds (1 µM, 0.25 % DMSO) were incubated with a suspension of hepatocytes (0.5 x 10^6 cells/mL). At six time points (0, 10, 20, 40, 60, 120 min), aliquots were removed and quenched by addition to acetonitrile.
- For the plated method, cells were thawed and plated onto 96-well collagen-coated plates. Seeding media was replaced after 6 h with a serum-free incubation media. 24 h after seeding, the cells were incubated with test compounds (1 µM, 0.1 % DMSO) at 37 °C, 5 % CO_2. Each time point was performed as a separate incubation. At six time points (0, 1, 2, 4, 6 and 24 h) aliquots were removed and quenched by addition to acetonitrile.
- HμREL*humanPool*™ 96-well hepatic co-culture plates were purchased from HμREL® corporation (lot HU1021). Following 6 days co-culture and shipment of cells, media was replaced and cells allowed to acclimatise for ~20 h. Test compounds (1 µM, 0.1 % DMSO) were incubated as described for plated monoculture, however incubation times were 0, 2, 6, 24, 48 and 72 h.
- Following centrifugation of the samples, internal standard was added to sample supernatants prior to analysis using Cyprotex generic LC-MS/MS methods.
- Metabolite profiling was carried out on eight of the compounds (bupropion, diazepam, disopyramide, imipramine, quinidine, tolbutamide, verapamil and warfarin) in all three systems with analysis performed on a Waters Xevo® G2-S QTof. All sample time points were processed against the 0 min sample using Metabolynx XS (Waters Ltd) using mass defect filtering with the dealkylation tool to establish which and how many metabolites are formed. Data was reviewed to confirm the validity of the detected metabolites and to ensure correct assignment of the metabolite observed. Metabolites were reported where they were observed in one of the systems at greater than 1 % of total drug related material.

Results

	HμREL plated co-culture (72 h)	Monoculture plated hepatocytes (24 h)	Suspension hepatocytes (2 h)
	CL_{int} (µl/min/10^6cells)		
Theophylline	-0.0987	0.119	0.215
Disopyramide	0.217	0.0526	-5.43
Warfarin	0.537	0.0522	-2.78
Diazepam	0.544	0.373	-5.07
Metoprolol	0.727	0.182	1.60
Tolbutamide	1.04	0.799	-6.42
Prednisolone	0.353	1.19	7.59
Quinidine	0.361	0.846	6.15
Imipramine	1.24	1.42	13.9
Ketoprofen	4.36	4.89	-4.15
Bupropion	6.42	11.4	78.1
Verapamil	14.5	15.9	113
Diclofenac	30.6	28.1	170
Carvedilol	54.2	33.5	113
Number of compounds falling into typical classification bands:			
<Lower limit or statistically insignificant	**1**	**5**	**8**
Low clearance (CL_{int} < 3.5)	**8**	**4**	**0**
Medium clearance (CL_{int} 3.5-19)	**3**	**3**	**2**
High clearance (CL_{int} > 19)	**2**	**2**	**4**

Table 1. *In vitro* CL_{int} values generated using HμREL® system (n=3), monoculture plated hepatocytes (n=3) and suspension heps data (n=1) generated in HU1021. Lower limit of quantification (LOQ; based on extrapolation of half-life to 3 x incubation time) determined to be 0.143, 0.411 and 3.85 µL/min/10^6 cells respectively.

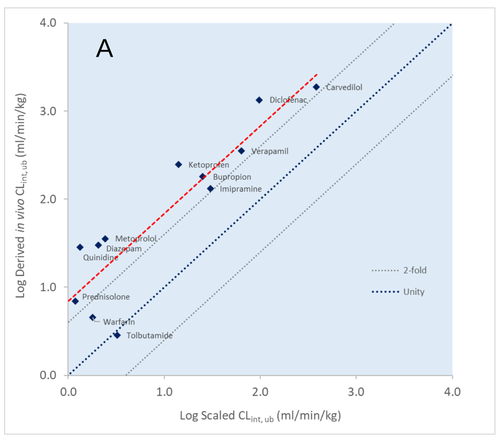

Figure 1. A) Chart displaying *in vitro-in vivo* scaling of HμREL® data for donor HU1021 before, and B) following, regression analysis. *In vitro* data was scaled to *in vivo* CL_{int} (scaled CL_{int}) using human physiological scaling factors[4]. The average fold error (AFE) prior to regression analysis was 6.88 (Log derived/scaled). Regression line correction applied, as described previously by Sohlenius-Sternbeck et al., 2012[4].

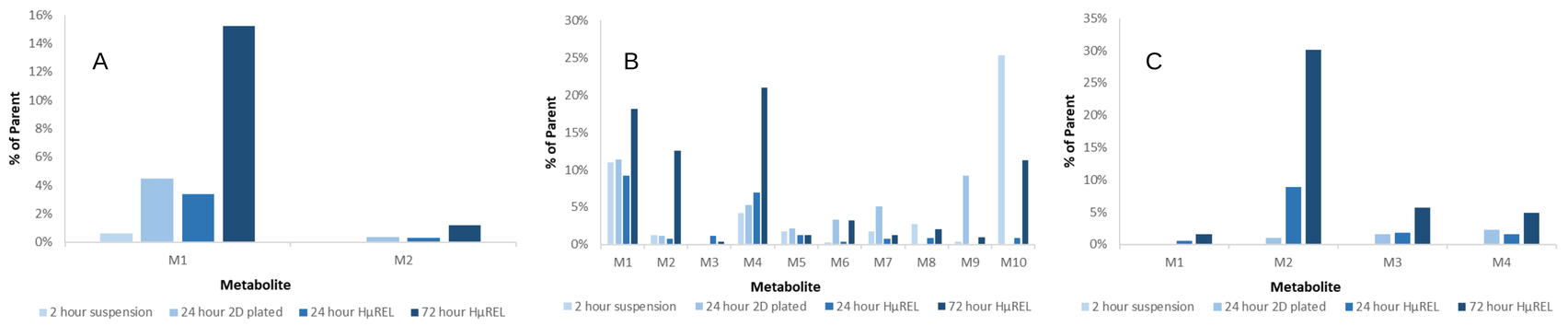

Figure 2. Identified metabolites from incubations in hepatocyte suspensions (2 h), plated monoculture (24 h) and HμREL® co-culture (24 and 72 h), expressed as % of parent, for A) Diazepam, B) Imipramine and C) Warfarin. Designated metabolites with transformation were for diazepam (A), M1 (demethylation); for imipramine (B), M1 (demethylation), M4 (oxidation), M10 (oxidation + glucuronidation); for warfarin (C), M2 (reduction). For illustrative purposes, the transformation of all metabolites is not displayed here.

- As a result of the improved long-term viability of the HμREL® co-culture model, an increased number of statistically significant CL_{int} values above LOQ could be determined with 13, 9 and 6 CL_{int} values out of a 14 compound set generated when comparing HμREL® co-culture, monoculture and suspension hepatocytes, respectively (Table 1).
- Generally, lower *in vitro* CL_{int} values were generated for compounds classified as high clearance in the HμREL® and plated monoculture models, in comparison to the suspension model. Loss of linearity with time could contribute to this.
- Following regression correction, human *in vivo* clearance was predicted within 2-fold in the HμREL® co-culture model for 12 out of 13 compounds (Figure 1).
- Broadly, an increased amount (as % of parent) of main metabolites for compounds such as bupropion, diazepam, disopyramide, tolbutamide and warfarin was observed with the HμREL® co-culture model in comparison to monoculture and suspension hepatocytes.
- The HμREL® co-culture model was also capable of generating a broad number of metabolites, comparable to monoculture and suspension hepatocytes, for compounds such as imipramine, quinidine and verapamil. Diazepam, imipramine and warfarin are shown as examples in Figure 2.

Conclusions

- Of the fourteen validation compounds assessed, thirteen had CL_{int} values that were reproducibly and accurately determined above the LOQ in the HμREL® model compared with six compounds in suspension hepatocyte incubations (2 h) and nine compounds in monoculture hepatocytes (24 h). The HμREL® model's CL_{int} data were comparable to values reported in literature[1,5].
- Prior to regression correction, both the plated monoculture hepatocytes and the HμREL® co-culture systems under-predicted the *in vivo* clearance, consistent with literature[1,4]. Following regression correction, the HμREL® human hepatocyte co-culture model, predicted *in vivo* clearance for twelve of the compounds within 2-fold of the observed values, with tolbutamide being overpredicted. Overall, this demonstrates the superior assay sensitivity of the HμREL® human hepatocyte co-culture model, enabled by a longer incubation time in comparison to plated monoculture or suspension hepatocyte models.
- Furthermore, the extended incubation time of the HμREL® human hepatocyte co-culture model proved beneficial in metabolite profiling studies with a number of low clearance compounds, providing a wider range of metabolites at detectable levels for metabolite identification and structural elucidation compared with hepatocyte suspension assays. The metabolites identified provided evidence of both CYP and non-CYP enzymatic activity in the HμREL® co-culture system.

References

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