Low Intrinsic Clearance determination using Primary Human Hepatocytes in monoculture compared to co-culture with non-parenchymal stromal cells.

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Abstract

- Definition of intrinsic clearance (CL_{int}) values of less than 1µL/min/million human hepatocytes is challenging since this implies a drug half-life of 12 hours or less in an hepatocyte CL_{int} assay
- Drug metabolizing enzyme (DME) activities decline to low levels after 4 hours in primary human hepatocyte suspension incubations[1] but pooled multi-donor platable hepatocytes in monoculture on collagen-coated plates have shown slower decline of activity for important DMEs and stability is maintained for 8-12 hours of incubation[2]
- HµREL Corporations novel co-culture of hepatocytes with stromal cells has shown sustained activity of DMEs up to 2-3 weeks in culture[3]
- We compared the two formats of cultured primary human hepatocytes (5-donor pool, XenoTech) & the potential to provide improved clearance prediction for drugs with high metabolic stability
- Enzyme activity changes (EAC) during the incubation were monitored by determining rate of metabolite formation for DME selective probe substrates at various times through the incubations
- Results demonstrate that hepatocytes plated in both monoculture (10 hour incubation) and in co-culture (72 hour incubation) can robustly produce CL_{int} values less than 1µL/min/million cells
- Both systems predict human clearance well but the combination of high hepatocyte density & DME activity stability up to 72 hours, as well as assay-ready plate delivery, makes the HµREL co-culture system a preferable method for determination of low CL_{int}

Objectives

- To compare low CL_{int} determination & prediction of human *in vivo* clearance using pooled platable hepatocytes in monoculture & co-culture of hepatocyte/non-parenchymal stromal cells.

Methods

- CL_{int} was determined for a set of substrates (Table 1) with primary human hepatocytes in monoculture for 10 hours as well as in co-culture for 72 hours. EAC was monitored with a CYP-selective substrate cocktail (Midazolam, Diclofenac, Bufuralol, S-Mephenytoin, Phenacetin) assessing formation of DME specific metabolites for 1 h at different time points after plating.

Monoculture
Pooled human hepatocytes (lot 1310168) were purchased from XenoTech, thawed and seeded into collagen-I-coated 24 well plates for 4 hours, according to XenoTechs plating protocol. Incubations were then started.

Co-culture
Pooled human hepatocytes (lot 1310168) were co-cultured with non-parenchymal stromal cells for six days at HµREL, US before subsequent shipping of assay ready 24 well plates to AstraZeneca labs in Sweden. Plates were acclimatized for 24 hours before start of incubation at day 7 after seeding.

- Fresh culture media containing typically 1µM substrate was added to cells & aliquots of media were withdrawn from each well during incubations in order to determine CL_{int}.
- Substrates & metabolites were quantified using LCMSMS. CL_{int} was calculated from 'parent drug-loss' plots & scaled into *in vivo* CL_{int}.
- Mathematical scaling was performed using the following parameters & method: 120 million hepatocytes per g liver, 24 g liver per kg bodyweight. Scaled *in vivo* CI_{int} values were corrected with a regression line off-set approach to predict *in vivo* hepatic CL_{int}[4]. The scaled *in vivo* CL_{int} values were compared to back-scaled (well-stirred model) *in vivo* CL_{int} from human plasma clearance.

Results and Discussion

- EAC (decline) for major CYPs gives a window of approximately 10 hours for log-linear compound disappearance (metabolism) from monocultures. In contrast, EAC (decline) in the co-culture system is considerably more stable over 72 hours (data not shown).
- CL_{int} values in the range of 0.2 to 34 µL/min/million hepatocytes were determined. A suspension assay format gave calculable CL_{int} values for only 11 of the 21 compounds whereas 17/21 values were determinable from the monoculture data and 19/21 from the co-culture assay (Figure 1, Table 1). Typical compound depletion profile used for CL_{int} determinations are shown in Figure 2.
- Monoculture and co-culture predicted *in vivo* CL_{int} to within 2 to 3-fold for the majority of tested compounds: Monoculture average fold difference (AFD) of 1.8; Co-culture AFD of 2.6 (Table 1).

Figure 1.

Success rate in determination of CL_{int} in different hepatocytes CL_{int} assays.

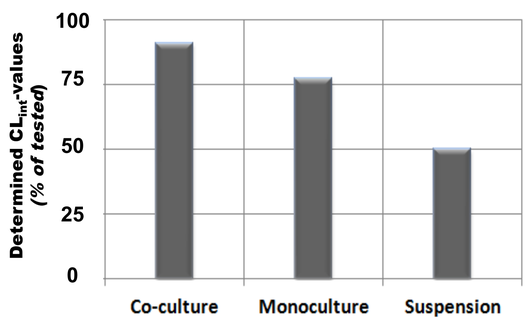
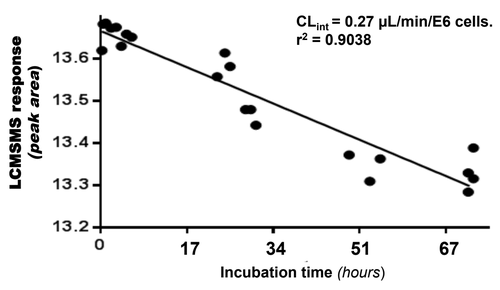


Figure 2.

Disopyramide disappearance from media in H*µ*RELs co-culture.



Conclusions

- Plated human hepatocytes in 10 hour monoculture incubations & in stromal cell co-culture for 72 hours predict *in vivo* clearance to within 2 to 3-fold for compounds metabolised by key DMEs
- Both systems show high success rate for determination of CL_{int} in the 0.2 to 34 µL/min/million hepatocyte range (CL_{int} below 1 is readily measurable)
- H*µ*RELs assay-ready plates improve success rate for CL_{int} determination

Table 1. Compound information, observed CL_{int}-values and *in vivo*-prediction data. (Between 2 and 6 replicate incubations with *in vivo* predicted compounds).

Test compound properties			Observed *in vitro* CL_{int}			Observed *in vivo* CL_{int}	Predicted *in vivo* CL_{int}			
Compound	Ion class	DME	Co-culture	Monoculture	Suspension		Co-culture		Monoculture	
				µL/min/million cells		mL/min/kg	mL/min/kg	Fold [a]	mL/min/kg	Fold [a]
AZ001	Neutral		n.v.	0.4	n.v.	2.1 [b]	n.v.	n.v.	1.9	-1.1
Theophylline	Neutral	1A2	n.v.	n.v.	n.v.	1.4 [5]				
Disopyramide	Base	3A4	0.4	0.2	1.5	0.9 [6]	1.5	1.6	0.9	-1.1
S-warfarin	Neutral	2C9>3A4	0.7	n.v.	n.v.	0.1 [5]	0.4	6.5	n.v.	n.v.
AZ002	Acid		0.8	n.v.	n.v.					
AZ003	Base		0.8	n.v.	n.v.					
AZ004	Neutral		0.8	0.4	n.v.					
Metoprolol	Base	2D6	0.9	1.4	n.v.	37.1 [6]	8.8	-4.2	12.3	-3.0
Diazepam	Neutral	2C19>3A4	1.3	0.8	n.v.	0.5 [5]	1.2	2.3	0.8	1.5
AZ005	Base		1.4	1.6	n.v.					
Imipramine	Base	2C9>2D6>3A4>1A2	1.7	8.6	4.5	41.5 [5]	15.3	-2.7	50.9	1.2
AZ006	Neutral		1.8	1.0	n.v.					
AZ007	Neutral		1.9	n.v.	n.v.					
AZ008	Neutral		2.0	1.3	4.2					
AZ009	Acid	3A4	3.3	1.2	3.6	4.7 [b]	2.3	-2.1	1.1	4.4
Ketoprofen	Acid	UGT2B2	4.3	3.9	6.6	2.4 [5]	8.1	3.3	7.5	3.1
Verapamil	Base	3A4	4.9	11.1	18.5	39.7 [5]	17.2	-2.3	31.9	-1.2
Sildenafil	Base	3A4 > 2C9/2C19	6.2	7.0	9.9	8.6 [5]	7.2	-1.2	7.9	1.1
Ketanserin	Base	non-CYP AKR/CR	14.1	8.3	33.1	10.1 [6]	15.3	1.5	14.7	1.5
AZ010	Base		27.3	28.4	5.9					
Carvedilol	Base	2D6>2C9	34.2	26.3	37.2	15.4 [5]	19	1.2	15.6	1.0

[a] Fold difference predicted versus observed *in vivo* CL_{int} [b] In-house clinical data

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