Low Clearance HμREL® Co-culture Assay

Background Information

'Optimization of clearance is one of the more significant challenges for a drug discovery project. Identification of the rate in preclinical species and optimization in human are major goals in most projects.'

[1]Grime *et al*., (2013) *Mol Pharm* **10**: 1191-1206.

- Low clearance compounds are increasingly prevalent in drug discovery[2], with the emphasis on reducing the metabolic clearance of new chemical entities (NCEs) in order to minimise dose, improve exposure and prolong the half-life.
- Determining an accurate *in vitro* measurement for clearance prediction of such compounds in human hepatocyte suspensions may not be possible due to the short incubation times required to maintain viability/activity. Although longer term studies using simple plated mono-cultures of cryopreserved human hepatocytes can be used for a more accurate determination of low CL_{int}, drug metabolising enzyme activities start to decline by 12 hr[3], thus leading to inaccuracies in the intrinsic clearance values.
- Hepatocytes contain the full complement of hepatic drug metabolising enzymes (both phase I and phase II), making them the 'gold standard' for use in metabolic studies. 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[4].
- The low clearance method developed utilises HμREL*humanPool*™ co-culture models over a 72 hr incubation period, allowing for more accurate assessment of CL_{int} for low clearance NCEs.

Protocol

Cells
HμREL*humanPool*™ (5 donor)

Species
Human

Test Article Concentration
1 μM (different concentrations available)

Incubation Times
0, 2, 6, 24, 48 and 72 hr

Test Article Requirements
50 μL of a 10 mM DMSO solution

Analysis Method
LC-MS/MS quantification

Assay Controls
Prednisolone
Ketoprofen

Data Delivery
Intrinsic clearance
Standard error of intrinsic clearance
Half-life

Related Services

Hepatocyte stability

Table 1

Comparison of human mean *in vitro* intrinsic clearance data generated by Cyprotex, alongside publications by Hultman *et al.*, 2016[5] and Bonn *et al.*, 2016[6] where the HµREL® co-culture model was used.

Drug	Ion Class	Major Drug Metabolising Enzyme	Cyprotex HµREL® CL_{int} 72 hr (µL/min/10^6 cells)	Hultman HµREL® CL_{int} 70 hr (µL/min/10^6 cells)	Bonn HµREL® CL_{int} 72 hr (µL/min/10^6 cells)
Theophylline	Base	CYP1A2	Below limit of quantification	Not reported	Below limit of quantification
Disopyramide	Base	CYP3A4	0.22	0.3	0.4
Warfarin	Neutral	CYP2C9, CYP3A4	0.54	0.6	0.71
Diazepam	Neutral	CYP2C19, CYP3A4	0.54	1.20	1.35
Metoprolol	Base	CYP2D6, CYP3A4	0.73	1.00	0.78
Tolbutamide	Acid	CYP2C9	1.04	Not reported	Not reported
Prednisolone	Neutral	CYP3A4	0.35	Not reported	Not reported
Ketoprofen	Acid	UGT	4.36	5.90	4.3
Verapamil	Base	CYP3A4, CYP1A2, CYP2C9	14.5	16.8*	Not reported
Imipramine	Base	CYP2C9, CYP2D6, CYP3A4, CYP1A2	1.24	19.6*	1.7
Diclofenac	Acid	CYP2C9, UGT2B7	30.6	Not reported	Not reported
Carvedilol	Base	CYP2D6, CYP2C9	54.2	Not reported	34.2
Quinidine	Base	CYP3A4	0.36	0.60	Not reported
Bupropion	Base	CYP2B6, CYP1A2, CYP2A6, CYP3A4, CYP2E1	6.42	Not reported	Not reported

* measured over 3 hr rather than 70 hr

Figure 1

Comparison of CL_{int} values generated using Cyprotex's HµREL® low clearance assay (media sampling method) in 3 separate assays, based on n=1 per assay.

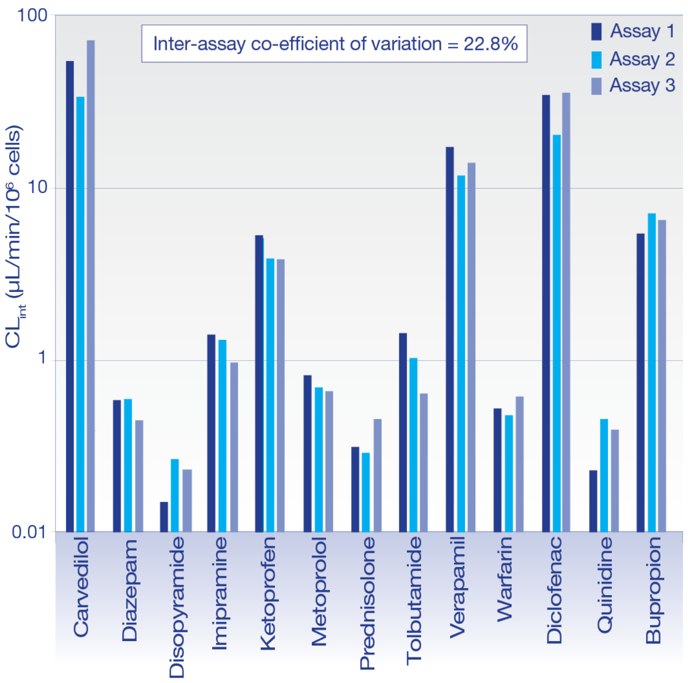
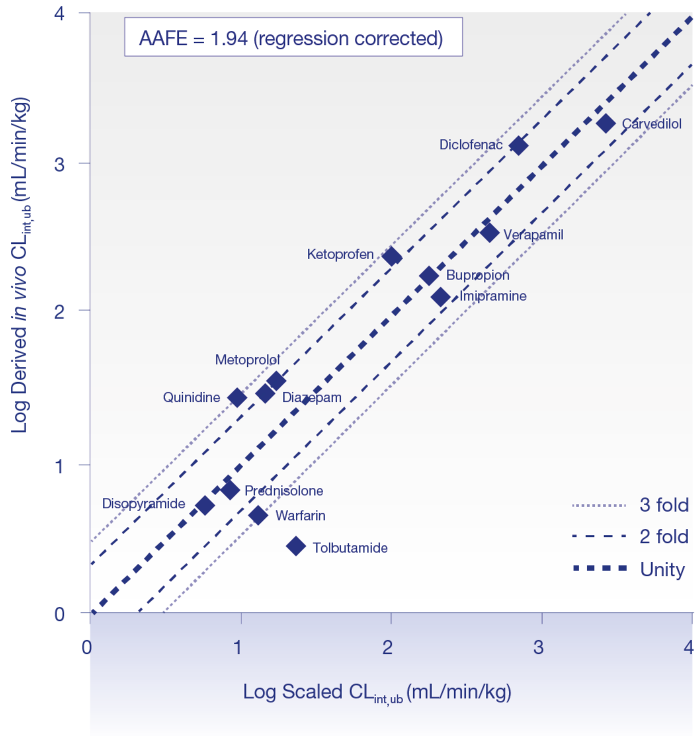


Figure 2

In vitro-in vivo scaling of HµREL® data for donor HU1021 following application of regression line correction, as described previously (Sohlenius-Sternbeck et al., 2012[7]).



References

1 Grime KH *et al.*, (2013) Application of *in silico*, *in vitro* and preclinical pharmacokinetic data for the effective and efficient prediction of human pharmacokinetics. *Mol Pharm* **10(4)**; 1191-1206

2 Di L *et al.*, (2012) A novel relay method for determining low-clearance values. *Drug Metab Dispos* **40(9)**; 1860-1865

3 Hutzler JM *et al.*, (2015) Low-turnover drug molecules: A current challenge for drug metabolism scientists. *Drug Metab Dispos* **43**; 1917-1928

4 Novik E *et al.* (2010) A microfluidic hepatic coculture platform for cell-based drug metabolism studies. *Biochem Pharmacol* **79(7);** 1036-1044

5 Hultman I *et al.* (2016) Use of HµREL human coculture system for prediction of intrinsic clearance and metabolite formation for slowly metabolized compounds. *Mol Pharmaceutics* **13(8);** 2796-2807

6 Bonn B *et al.* (2016) Determination of human hepatocyte intrinsic clearance for slowly metabolised compounds: Comparison of a primary hepatocyte/stromal cell co-culture with plated primary hepatocytes and HepaRG. *Drug Metab Dispos* **44(4)**; 527-533

7 Sohlenius-Sternbeck AK *et al.* (2012) Practical use of the regression offset approach for the prediction of *in vivo* intrinsic clearance from hepatocytes. *Xenobiotica* **42(9)**; 841-853

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