

Hepatic Uptake Assay

Background Information



'Experiences of sub-optimal drug exposure due to drug transporter interplay have supported incorporation of studies aimed at understanding the interactions between compounds and drug transporters much earlier in drug discovery.'

¹Riley RJ, Foley SA, Barton P, Soars MG & Williamson B (2016) *Expert Opin Drug Metab Toxicol* **12(2)**; 201-216

- Intrinsic clearance can be influenced by several processes including hepatic uptake, efflux, biliary excretion and drug metabolism².
- The predominant transporters involved in human hepatic uptake include OATPs, NTCP, OCTs and OATs³. These transporters determine intracellular concentrations which can influence clearance as well as potential DDI and hepatotoxicity.
- Inter-individual variability in hepatic uptake is also likely for substrates of hepatic uptake transporters which exhibit polymorphisms.
- Through its parent company, Evotec, Cyprotex are able to offer a hepatic uptake assay which utilises the media loss⁴ approach, and determines the hepatic uptake intrinsic clearance.

Protocol

Cells

Cryopreserved rat hepatocytes

Test Article Concentration

1 μ M (different concentrations available)

Method

Media loss

Incubation Time

0, 0.17, 0.5, 1, 1.5, 2, 5, 10, 20, 30, 60 min

Replicates

n=2

Test Article Requirements

50 μ L of 10 mM solution

Analysis Method

LC-MS/MS quantification

Assay Controls

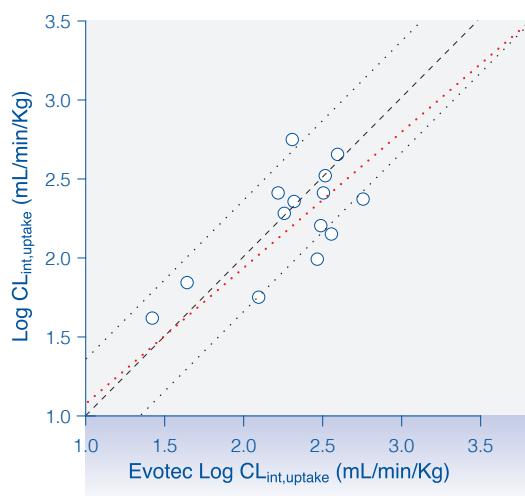
Atorvastatin (positive control for uptake)
Dextromethorphan (negative control for uptake and positive control for CYP activity)

Data Delivery

Uptake intrinsic clearance ($CL_{int,uptake}$)
(μ L/min/ $\times 10^6$ cells)

Figure 1

Relationship between rat uptake intrinsic clearance (using Evotec's hepatic uptake assay) with values reported in the literature^{2,5,6,7,8,9} for a set of 14 compounds.

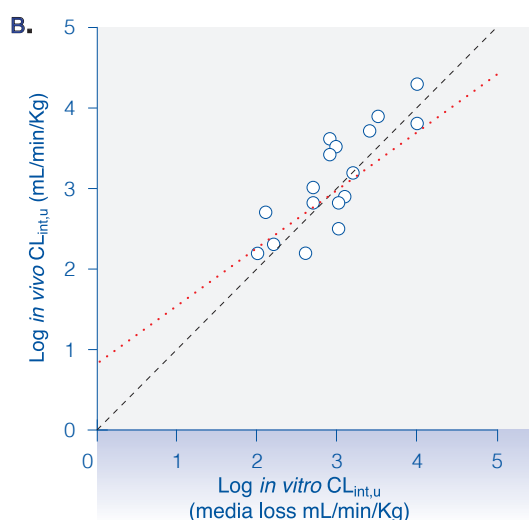
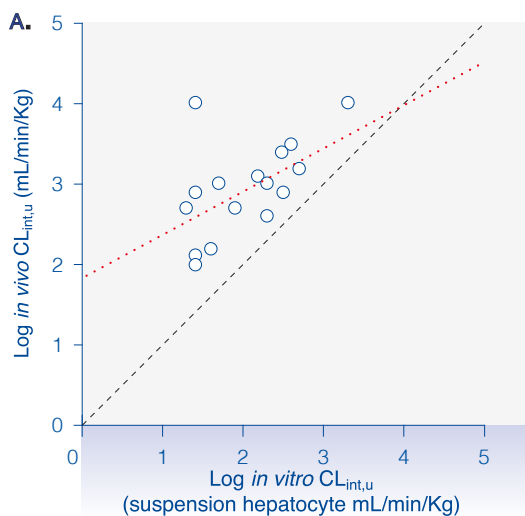


In addition to robust human *in vitro* data, confidence in understanding and predicting preclinical species *in vivo* clearance is essential before extrapolation to human *in vivo* clearance for NCEs¹⁰.

To gain insight and understanding into how transporter mechanisms that may contribute to clearance *in vivo*, early data are often generated in preclinical species such as the rat. Further, the human transporters OATP1B1 and OATP1B3 are orthologous to the rodent specific transporter Oatp1b2¹¹.

Figure 2

Correlation of rat *in vitro* and *in vivo* intrinsic clearance for a set of 17 test compounds determined using A). a standard suspension rat hepatocyte stability assay and B). the media loss assay.



The data generated by Evotec are in broad agreement with those reported in the literature from a range of labs as illustrated in Figure 1. Further, in contrast to the standard suspension hepatocyte stability model (Figure 2A), the scaled *in vitro* rat uptake intrinsic clearance data from the Evotec model demonstrates a strong correlation with *in vivo* rat intrinsic clearance (Figure 2B) demonstrating the advantages of the media loss approach.

References

- ¹ Riley RJ *et al.*, (2016) *Expert Opin Drug Metab Toxicol* **12**(2); 201-216
- ² Maeda K and Sugiyama Y (2013) *In Transporters in Drug Development – Discovery, Optimization, Clinical Study and Regulation*. Ed. Sugiyama Y and Steffansen B. 121-154
- ³ Annaert P *et al.*, (2007) *In Drug Transporters: Molecular Characterization and Role in Drug Disposition*. Ed. You G and Morris ME. 359-410
- ⁴ Soars MG *et al.*, (2007) *Drug Metab Dispos* **35**(6); 859-865
- ⁵ Ishiguro N *et al.*, (2006) *Drug Metab Dispos* **34**(7); 1109-1115
- ⁶ Paine SW *et al.*, (2008) *Drug Metab Dispos* **36**(7); 1365-1374
- ⁷ Gardiner P & Paine SW (2011) *Drug Metab Dispos* **39**(10); 1930-1938
- ⁸ Ménochet K *et al.*, (2012) *Drug Metab Dispos* **40**(9); 1744-1756
- ⁹ Ménochet K *et al.*, (2012) *J Pharmacol Exp Ther* **341**(1); 2-15
- ¹⁰ Grime K and Riley RJ (2006) *Curr Drug Metab* **7**; 251-264
- ¹¹ Hagenbuch B and Meier PJ (2003) *Biochim Biophys Acta* **1609**(1); 1-18