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In vitro **ADME**

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.'

[1]Riley 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 metabolism[2].
- The predominant transporters involved in human hepatic uptake include OATPs, NTCP, OCTs and OATs[3]. 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 loss[4] approach, and determines the hepatic uptake intrinsic clearance.

Protocol

Cells
Cryopreserved rat hepatocytes

Test Article Concentration
1 µM (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 µL of 10 mM solution

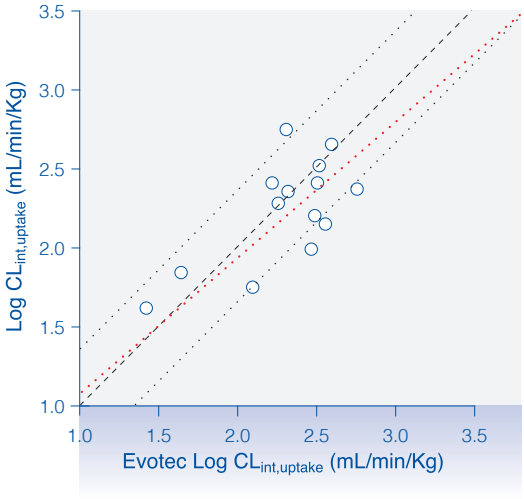
Analysis Method
LC-MS/MS quantification

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

Data Delivery
Uptake intrinsic clearance ($CL_{int,uptake}$) (µL/min/x10^6 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.

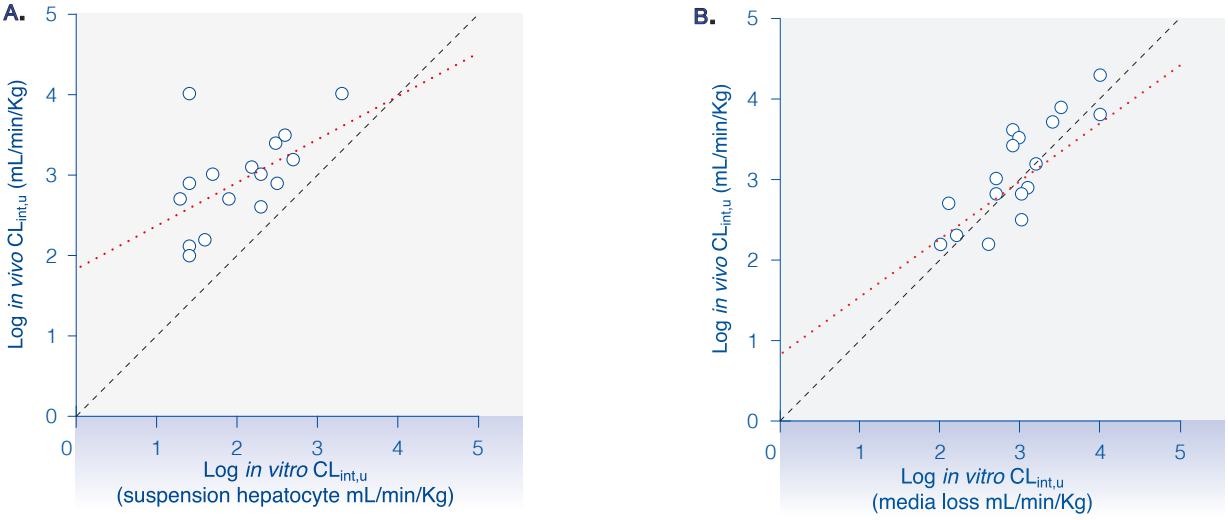


In addition to robust human *in vitro* data, confidence in understanding and predicting preclinical species *in vivo* clearance is essential before extrapolation to human *in vivo* clearance for NCEs[10].

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 Oatp1b2[11].

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
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[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