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In vitro **ADME & PK**

Hepatocyte Stability

Background Information

'Human hepatocytes have become the "gold standard" for evaluating hepatic metabolism and toxicity of drugs and other xenobiotics *in vitro*.'

[1]LeCluyse EL and Alexandre E (2010) *Methods Mol Biol* **640**; 57-82

- The liver is the most important site of drug metabolism in the body. Approximately 60% of marketed drugs are cleared by hepatic CYP-mediated metabolism[1].
- Hepatocytes contain the full complement of hepatic drug metabolising enzymes (both phase I and phase II) maintained within the intact cell.
- Hepatocytes can be used to determine the *in vitro* intrinsic clearance of a compound.
- The use of species-specific cryopreserved hepatocytes can be used to enable an understanding of interspecies differences.
- Hepatocytes can be used to profile for metabolites formed by both phase I and phase II enzymes.

Follow on metabolite profiling and structural elucidation

Cyprotex's hepatocyte stability assay can be extended to profile the metabolites that are formed. Cyprotex's biotransformation services are supported by high resolution, accurate mass spectrometry. These services can provide information on an individual species' metabolite profile, or a cross-species comparison to identify potential differences in metabolism which could in turn help to interpret pharmacology and toxicity data. Structural elucidation can also be performed on the potential metabolites' MS/MS fragmentation data. All biotransformation studies are performed by a dedicated team of experts.

Please refer to our Metabolite Profiling and Identification section for further details.

Protocol

Cells
Cryopreserved hepatocytes

Species
Human, rat, mouse, dog, primate, minipig, rabbit, guinea pig (other species available on request)

Test Compound Concentration
1 µM (different concentrations available)

DMSO Concentration
0.25%

Incubation Time
0, 5, 10, 20, 40 and 60 min

Compound Requirements
50 µM of 10mM solution

Analysis Method
LC-MS/MS quantification

Assay Control
Known substrates which undergo either phase I or phase II metabolism
Vehicle control incubation

Data Delivery
Qualified and calculated intrinsic clearance
Standard error of qualified and calculated intrinsic clearance
Half life

To find out more contact **enquiries@cyprotex.com**

Hepatocytes have the full complement of hepatic drug metabolising enzymes within an intact cell and so are a popular in vitro model for determining intrinsic clearance, interspecies difference and metabolite profiling studies.

The liver is the main organ of drug metabolism in the body. Hepatocytes contain both phase I and phase II drug metabolising enzymes, which are present in the intact cell, and provide a valuable *in vitro* model for predicting *in vivo* hepatic clearance.

Hepatocytes are incubated with the test compound at 37°C. Samples are removed at the appropriate time points into acetonitrile to terminate the reaction. Following centrifugation, internal standard is added and the supernatant is analysed by LC-MS/MS. The disappearance of test compound is monitored over a 60 minute time period.

The ln peak area ratio (compound peak area/internal standard peak area) is plotted against time and the gradient of the line determined.

Figure 1

In vitro in vivo clearance correlation in Cyprotex's human hepatocyte stability assay. *In vitro* CL_{int} data, for 29 literature compounds including acid, base and neutral compounds, was scaled (mL/min/kg Scaled $CL_{int,ub}$) and compared to derived values of *in vivo* intrinsic clearance (Derived *in vivo* $CL_{int,ub}$) back-calculated from observed *in vivo* clearance using the well-stirred model. Dashed line shows line of regression. Dotted lines show 2-fold of unity line (solid)..

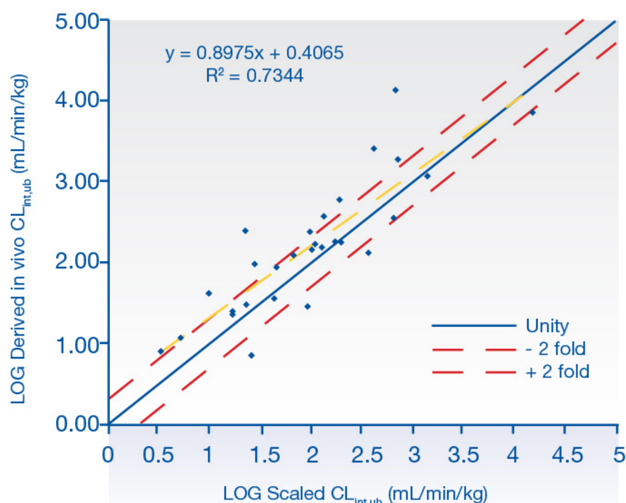
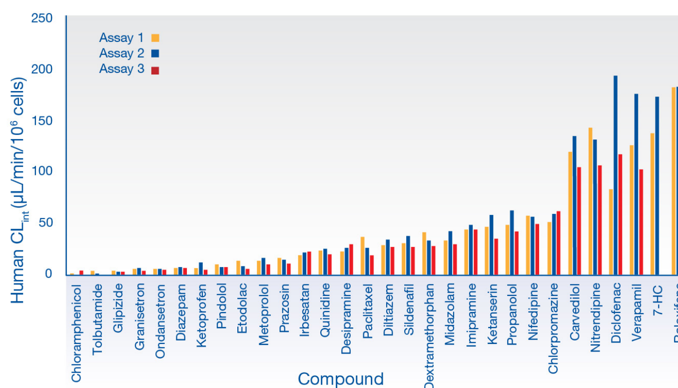


Figure 2

Comparison of CL_{int} values generated in 3 separate assays, based on n=1 per assay. Incubations performed using human hepatocytes, 0.5 x 10^6 cells/mL, Williams E media, 1 µM substrate concentration.



A range of literature compounds were assessed in the Cyprotex hepatocyte stability assay (1µM; 0.5 million cells/mL; 60 min incubation; n=3 assays) and intrinsic clearance (CL_{int} µL/min/10^6 cells) determined. Predicted *in vivo* CL_{int} (mL/min/kg) values were determined using a hepatocellularity of 120 x 10^6 cells/g liver[2] and a human liver weight of 25.7 g/liver/kg[3], and taking into account fuinc (fraction unbound *in vitro* incubation). Observed *in vivo* $CL_{int,ub}$ were back-calculated from observed hepatic clearance using the well-stirred model, human liver blood flow of 20.7 ml/min/kg[4] and fu_b (fraction unbound in blood)[4].

References

1 LeCluyse EL and Alexandre E (2010) Isolation and culture of primary hepatocytes from resected human liver tissue. *Methods Mol Biol* **640**; 57-82

2 Barter ZE *et al.*, (2007) Scaling factors for the extrapolation of *in vivo* metabolic drug clearance from *in vitro* data: reaching a consensus on values of human microsomal protein and hepatocellularity per gram of liver. *Curr Drug Metab* **8(1)**; 33-45

3 Davies B. and Morris T. (1993) Physiological parameters in laboratory animals and humans. *Pharma Res* **10(7)**; 1093-1095

4 Riley RJ *et al.*, (2005) A unified model for predicting human hepatic, metabolic clearance from *in vitro* intrinsic clearance data in hepatocytes and microsomes. *Drug Metab Dispos* **33(9)**; 1304-1311

5 Houston JB. (1994) Utility of *in vitro* drug metabolism data in predicting *in vivo* metabolic clearance. *Biochem Pharmacol* **47(9)**; 1469-1479

6 Sohlenius-Sternbeck AK (2006) Determination of the hepatocellularity number for human, dog, rabbit, rat and mouse livers from protein concentration measurements. *Toxicol. In Vitro* **20**; 1582-1586