

Cloe Screen Hepatocyte Stability

cyprotex*in vitro* ADME

Background Information



'Human tissues, including freshly prepared hepatocyte, cryopreserved hepatocytes, and freshly isolated liver slices, provide cellular integrity with respect to enzyme architecture and contain the full complement of drug metabolizing enzymes.'

FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling (September 2006)

- The liver is the most important site of drug metabolism in the body. Approximately 60% of marketed compounds are cleared by hepatic CYP-mediated metabolism¹.
- 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 studies

Cyprotex's hepatocyte stability assay can be extended to profile the main metabolites that are formed. Options include a low resolution analysis to identify whether metabolites are formed, or a cross species comparison to identify potential differences in metabolism which could in turn help to interpret pharmacology and toxicity data. We can also perform ion-transition analysis in order to understand the derivation of metabolites.

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)

Test Compound Concentration

3 μ M (different concentrations available)

DMSO Concentration

0.25 %

Incubation Time

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

Compound Requirements

50 μ L of 10 mM solution

Analysis method

LC-MS/MS quantification

Assay Controls

Known substrates which undergo either phase I or phase II metabolism

Heat-inactivated hepatocyte control incubation for each compound

Vehicle control incubation

Data Delivery

Intrinsic clearance

Standard error of intrinsic clearance

Half life

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 differences and metabolite profiling studies.

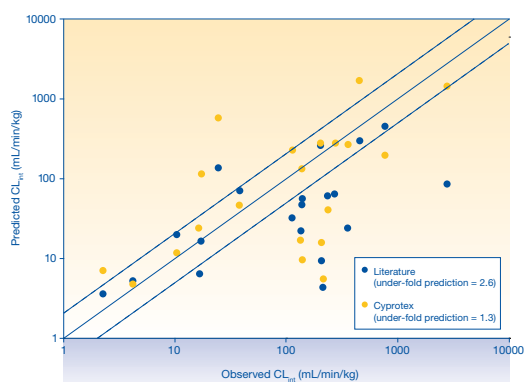


Hepatocyte Stability Assay

20 compounds were assessed in Cyprotex's human hepatocyte stability assay on three separate occasions. Data were scaled to *in vivo* intrinsic clearance and compared with observed values.

Figure 1

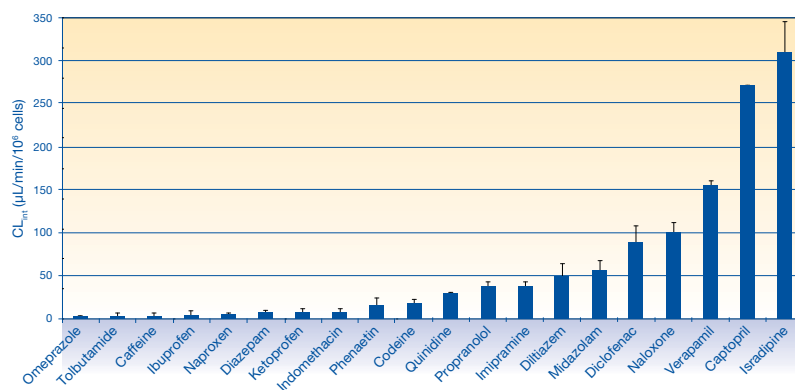
Cyprotex's human hepatocyte stability data and literature values^{2,3,4,5} were scaled to *in vivo* intrinsic clearance (predicted CL_{int}) and compared to observed values of intrinsic clearance in 20 compounds.



Human hepatocyte CL_{int} ($\mu\text{L}/\text{min}/10^6$ cells) from the Cyprotex assay and from literature^{2,3,4,5} were scaled to *in vivo* CL_{int} ($\text{mL}/\text{min}/\text{kg}$) using a hepatocellularity of 99×10^9 cells/g liver and a human liver weight of 21.4 g liver/kg. CL_{int} predictions were assessed for the predicted error (difference between the predicted and observed *in vivo* value). The bias of CL_{int} prediction was assessed from the geometric mean of the ratio of predicted and observed value and the fold under-prediction calculated. The data from the Cyprotex assay showed greater predictive capability when compared with data from the literature. Using literature values, the fold under-prediction was 2.6. Using Cyprotex values, the fold under-prediction was 1.3.

Figure 2

Graph illustrating intrinsic clearance data for 20 compounds generated in Cyprotex's hepatocyte stability assay. The data show the mean \pm standard deviation of 3 separate incubations.



The graph shows consistency of data between separate runs of the assay. Pooled hepatocytes typically from 5 different donors are used for the human hepatocyte stability assay to reduce the problems associated with inter-individual variability.

References

- McGinnity DF *et al.*, (2004) *Drug Metab Dispos* **32**:1247–1253.
- Soars MG *et al.*, (2002) *J Pharmacol Exp Ther* **301**(1): 382-90.
- Shibata Y *et al.*, (2002) *Drug Metab Dispos* **30** (8): 892-896.
- Lau YY *et al.*, (2002) *Drug Metab Dispos* **30** (12): 1446-1454.
- McGinnity DF and Riley RJ (2004) *Drug Metab Rev* **36** (S1): 211.