In vitro ADME

Hepatic Uptake Assay

Background Information

- Intrinsic clearance can be influenced by several processes including hepatic uptake, efflux, biliary excretion and drug metabolism.
- The predominant transporters involved in human hepatic uptake include OATPs, NTCP, OCTs and OATs. 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 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\textsubscript{int,uptake}) (μL/min/x10\textsuperscript{6} cells)

'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.'

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. 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.

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
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