cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

Preclinical Species Hepatic Oatp Uptake Transporter Substrate Identification

Background Information

'It has become increasingly clear that there are significant differences between rodents, dog, monkey, and human in the substrate specificity, tissue distribution, and relative abundance of transporters. These differences complicate cross-species extrapolations, which is important when attempting to predict human pharmacokinetics (PK) of drug candidates and assess risk for drug–drug interactions (DDIs).'

[3]Chu X *et al*., (2013) *Expert Opin Drug Metab Toxicol* **9(3)**: 237-252.

- The SLC (solute carrier) family transport a wide range of different solutes across biological membranes using diverse energy coupling mechanisms[1].
- One of the most important human SLC transporters expressed in human liver is OATP1B1 which is responsible for the hepatic uptake and rate-determining elimination of a wide range of endogenous compounds and drugs that are substrates[2].
- Species differences in drug transporters with regard to their tissue distribution, expression levels and substrate specificity can be problematic for preclinical cross-species extrapolation of drug disposition (clearance) and DDI potential to human.
- The use of *in vitro* cell test systems that each overexpress the major hepatic Oatp transporter of preclinical species (Oatp1b2, Oatp1b4 or Oatp1b1 for rat, dog or Cynomolgus monkey, respectively) may be useful towards understanding whether a molecule is a substrate of a hepatic active transporter in those species. Such knowledge may assist in the interpretation of any observed liver accumulation *in vivo*, or help towards qualitatively understanding an underprediction of *in vivo* hepatic clearance from *in vitro* microsomal clearance data for that preclinical species.
- Cyprotex's preclinical species hepatic Oatp transporter substrate identification assay determines if your compound is a substrate of key preclinical species transporters.

Related Services

Human SLC Transporter Substrate Identification

Protocol

Test System
Mammalian HEK293 cells transiently overexpressing a single preclinical species transporter (rat Oatp1b2, dog Oatp1b4 or Cynomolgus monkey Oatp1b1)

Control vector-transfected HEK293 cells

Test Article Concentrations
Options: -

- Single concentration (typically 1 µM), single timepoint for 7 compounds
- Two concentrations (typically 1 and 10 µM), single timepoint for 3 compounds
- Two concentrations (typically 1 and 10 µM), two timepoints for a single compound

Time Points
Typically, 2 min or 2 and 20 min (depending on customer requirements)

Analysis Method
MicroBeta® scintillation counter (radiolabelled substrates)

LC-MS/MS analysis (non-radiolabelled substrates)

Data Delivery
Cellular uptake and fold accumulation

Figure 1

Uptake of ^{3}H-estradiol 17ß-glucuronide (1 µM) in rat Oatp1b2-transfected HEK293 cells and control HEK293 cells over 1.5 min in the presence and absence of the inhibitor rifamycin SV (30 µM).

To confirm transporter involvement in the uptake of estradiol 17ß-glucuronide in the rat Oatp1b2-transfected cells, the inhibitor rifamycin SV was included in the incubations. This reduced the uptake ratio by well over 50% with the transporter uptake giving similar levels (~ 2 fold uptake ratio) as observed in the control cells.

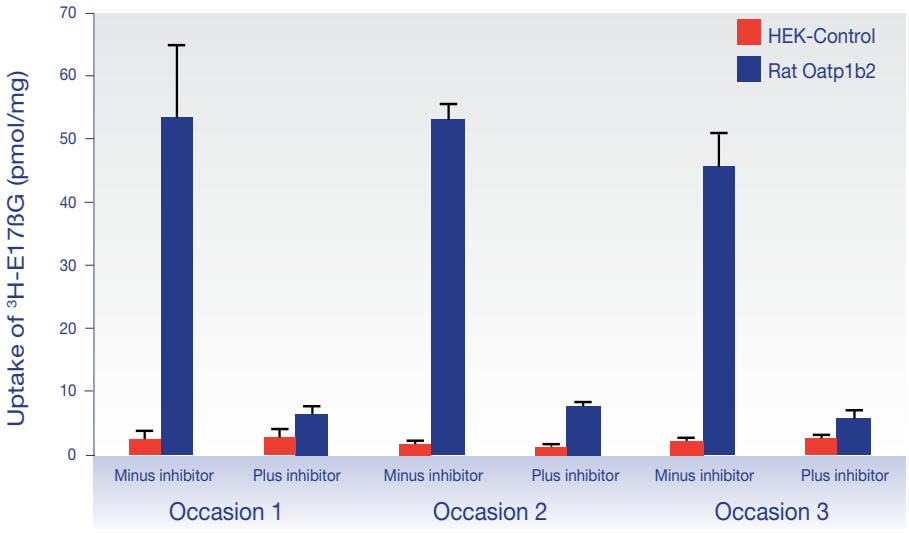


Figure 2

Uptake of ^{3}H-estradiol 17ß-glucuronide (1 µM) in dog Oatp1b4-transfected HEK293 cells and control HEK293 cells over 3 min in the presence and absence of the inhibitor rifamycin SV (10 µM).

To confirm transporter involvement in the uptake of estradiol 17ß-glucuronide in the dog Oatp1b4-transfected cells, the inhibitor rifamycin SV was included in the incubations. This reduced the uptake ratio by well over 50% with the transporter uptake giving similar levels (< 2 fold uptake ratio) as observed in the control cells.

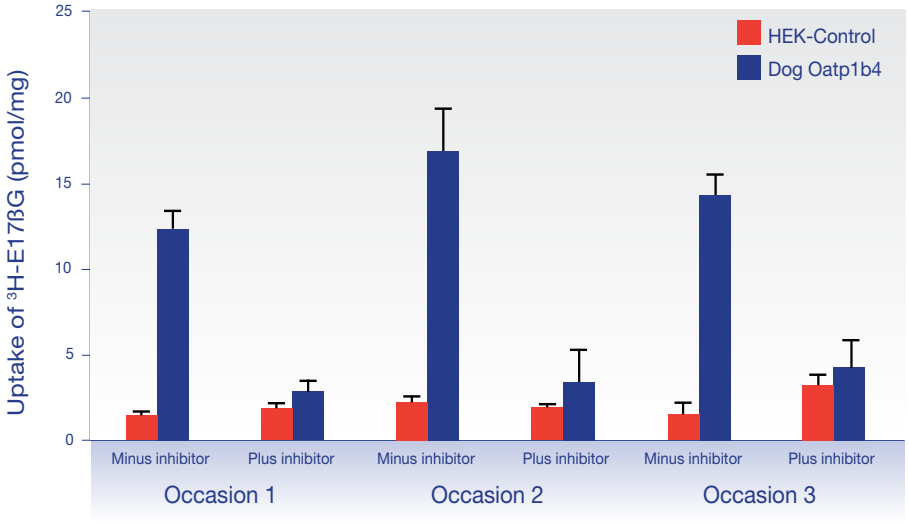
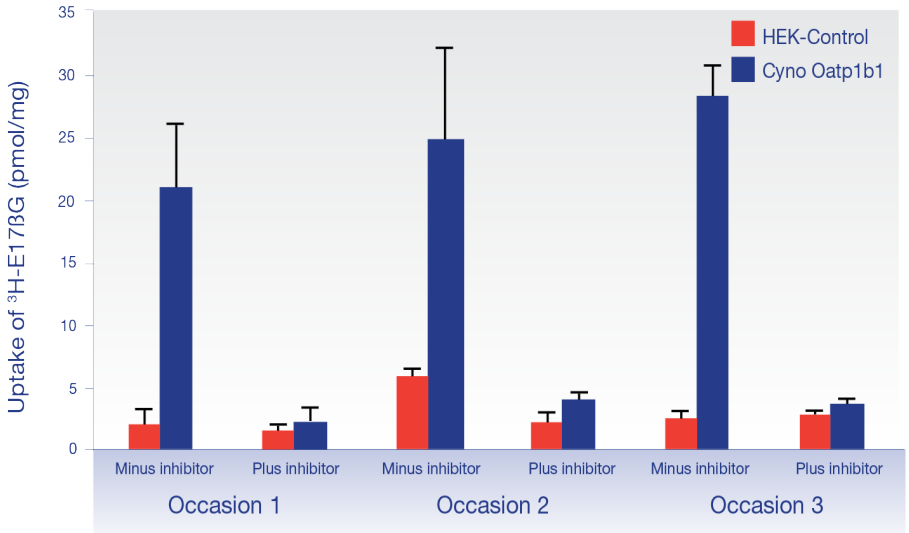


Figure 3

Uptake of ^{3}H-estradiol 17ß-glucuronide (1 µM) in Cynomolgus monkey Oatp1b1-transfected HEK293 cells and control HEK293 cells over 2 min in the presence and absence of the inhibitor rifamycin SV (3 µM).

To confirm transporter involvement in the uptake of estradiol 17ß-glucuronide in the Cynomolgus monkey Oatp1b1-transfected cells, the inhibitor rifamycin SV was included in the incubations. This reduced the uptake ratio by well over 50% with the transporter uptake giving similar levels (< 2 fold uptake ratio) as observed in the control cells.



References

[1] Schlessinger A *et al.*, (2013) Molecular modeling and ligand docking for solute carrier (SLC) transporters. *Curr Top Med Chem* **13(7)**; 843-856.

[2] Shitara Y *et al.*, (2013) Clinical significance of organic anion transporting polypeptides (OAPTs) in drug disposition: their roles in hepatic clearance and intestinal absorption. *Biopharm Drug Dispos* **34**: 45-78.

[3] Chu X *et al.*, (2013) Species differences in drug transporters and implications for translating preclinical findings to humans. *Expert Opin Drug Metab Toxicol* **9(3)**: 237-252.