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In vitro **ADME & PK**

Preclinical Species Hepatic Oatp Uptake Transporter Inhibition

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).'

[4]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 an inhibitor of an hepatic active uptake transporter in those species. Whilst not usually performed for a compound on the back of IC_{50} data indicating a positive interaction potential for inhibition of human OATP1B1, were a sponsor to consider conducting an *in vivo* interaction study in a preclinical species (e.g. Cynomolgus monkey)[3] then it would be beneficial to understand the *in vitro* inhibitory potential of the molecule against that preclinical species Oatp in order to put it into context with exposure and aid interpretation of the data.
- Cyprotex's preclinical species hepatic Oatp transporter inhibition assay determines if your compound is an inhibitor of key preclinical species transporters.

Related Services

Human SLC Transporter Inhibition

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

Probe Substrate
[^{3}H]-Estradiol 17β-glucuronide

Test Article Concentrations
Single concentration (for batches of 6 compounds) OR
6 concentrations plus 0 µM (final test compound concentrations dependent on customer requirements)

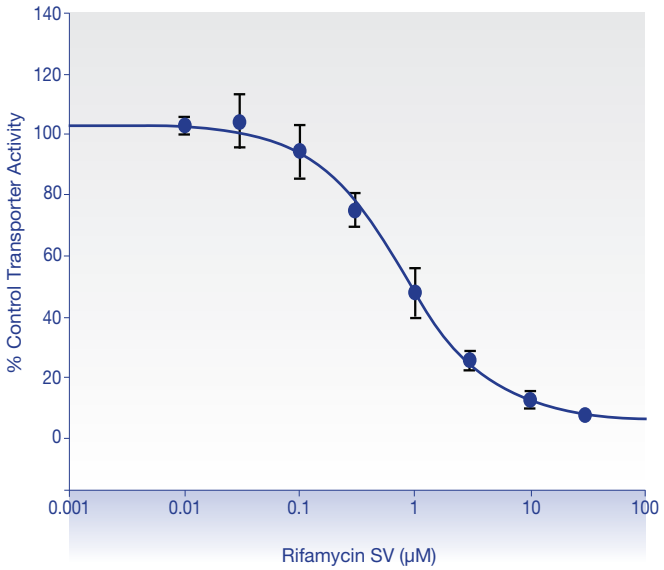
Time Points
Dependent on transporter

Analysis Method
Radiochemical detection using scintillation counting

Data Delivery
Percentage inhibition (single concentration) OR IC_{50}

Figure 1

Mean rat Oatp1b2-mediated estradiol 17ß-D-glucuronide (1 µM) transport in the presence of a range of concentrations of rifamycin SV expressed as a percentage of vehicle control.



The results represent the mean ± standard deviation of 3-9 replicate wells (triplicate wells per incubation condition performed on three separate occasions).

Table 1

Inhibition of rat Oatp1b2, dog Oatp1b4 and cynomolgus monkey Oatp1b1-mediated transport of the prototypical substrate estradiol 17ß-glucuronide.

Transporter	Substrate	Inhibitor	IC_{50} ± standard error (µM)
Rat Oatp1b2	Estradiol 17β-glucuronide	Rifamycin SV	0.992 ± 0.188
Dog Oatp1b4	Estradiol 17β-glucuronide	Rifamycin SV	0.314 ± 0.0623
Cyno Oatp1b1	Estradiol 17β-glucuronide	Rifamycin SV	0.120 ± 0.0246

The incubation conditions for each of the species have been fully characterised for our chosen Oatp substrate, estradiol 17ß-glucuronide, based on time linearity and uptake kinetics (V_{max} and K_m).

The chosen substrate concentration is at least seven-times lower than the determined K_m, and as such IC_{50} equates to K_i (assuming competitive inhibition).

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 Ufuk A *et al.*, (2018) In Vitro-In Vivo Extrapolation of OATP1B-Mediated Drug-Drug Interactions in Cynomolgus Monkey. *J Pharmacol Exp Ther* **365(3)**: 688-699.
4 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.