

In vitro ADME & PK

P-glycoprotein (P-gp) Substrate Identification Assay for Screening and Regulatory Reporting Purposes

Background Information



'P-gp has an important role in limiting entry of various drugs into the central nervous system. In addition, it also plays a part in the intestinal absorption and in the biliary and urinary excretion of drugs.'

¹The International Transporter Consortium (2010) Membrane transporters in drug development. *Nat Rev Drug Discov* **9(3)**: 215-236

- P-gp (P-glycoprotein; MDR1, ABCB1) is an important efflux transporter. It is expressed in the gastrointestinal tract, liver, kidney and brain endothelium¹.
- The ITC¹, the EMA guideline² and the draft FDA guidance³ recommend investigating P-gp due to the clinical importance of P-gp in the absorption and disposition of drugs.
- Madin Darby canine kidney (MDCK) cells transfected with the human MDR1 gene overexpress P-gp. The EMA² and draft FDA³ regulatory guidelines recommend polarised MDCK-MDR1 cell monolayers as one of the preferred methods for evaluating the role of P-gp in the efflux of new chemical entities.
- The assay investigates bidirectional transport across the cell monolayer in the presence and absence of a P-gp reference inhibitor, elacridar (screening) or cyclosporin A (regulatory), to determine if active efflux is occurring, and whether this efflux is mediated by P-gp.
- Where MDCK-MDR1 cell assays indicate a compound has inherently low passive permeability, then P-gp membrane vesicles can be used as an alternate *in vitro* test system to identify P-gp substrates (assay available upon request).

Protocol

Test Article Concentration

Screening study - 10 μ M plus/minus reference inhibitor (different test compound concentrations available)

Regulatory study - 1, 10, 50 and 100 μ M (different concentrations available) plus inhibition at two substrate concentrations (1 and 10 μ M)

Assay Conditions

Apical to basolateral and basolateral to apical in presence and absence of elacridar (2 μ M; screening) or cyclosporin A (10 μ M; regulatory)

Number of Replicates

2 (screening) or 3 (regulatory)

Analysis Method

LC-MS/MS quantification

Integrity Marker

Lucifer Yellow

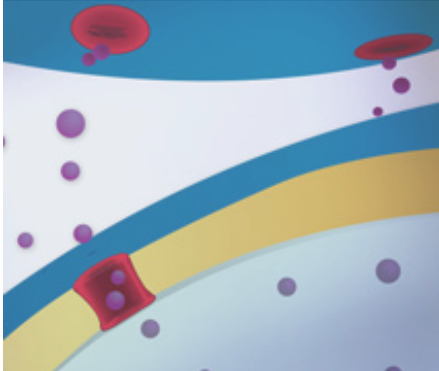
Data Delivery

P_{app}

Efflux ratio in presence and absence of reference inhibitor

% Recovery

Bidirectional transport in the presence and absence of a reference P-gp inhibitor can be used to determine if active efflux is occurring and whether the efflux is mediated by P-gp.

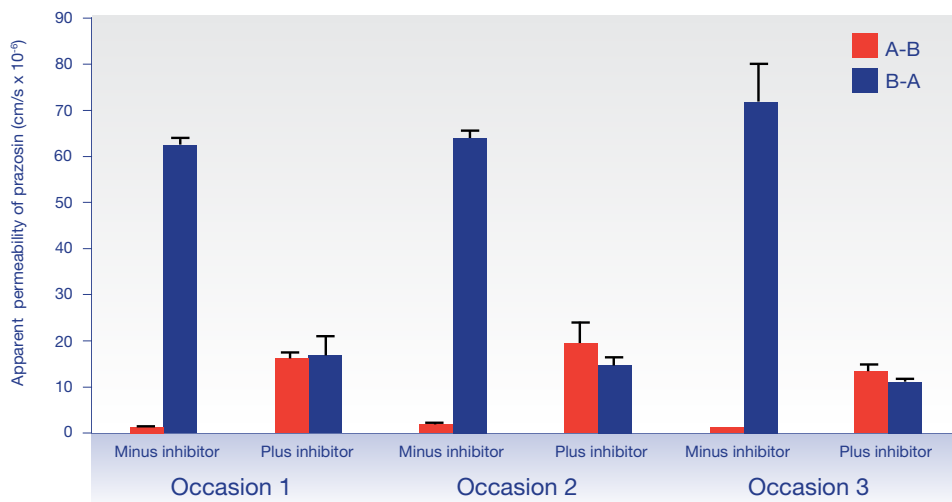


P-gp Substrate Identification Assay

Cyprotex's P-gp substrate identification assay can be used either for screening for potential substrates of P-gp or for regulatory confirmation of a P-gp substrate.

Figure 1

Graph showing effect of the P-gp inhibitor, cyclosporin A (10 μ M) on the efflux of the P-gp substrate, prazosin. Data show the mean \pm standard deviation



Functional activity of P gp in MDCK MDR1 polarised cell monolayers was demonstrated by investigating the inhibition of efflux of the P gp substrate prazosin by the reference inhibitor cyclosporin A.

References

- ¹The International Transporter Consortium (2010) Membrane transporters in drug development. *Nat Rev Drug Disc* 9: 215-236
- ²The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012)
- ³Draft FDA Guidance for Industry – Drug Interaction Studies – In Vitro Metabolism and Transporter-mediated Drug-Drug Interaction Studies, October 2017