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In vitro **ADME & PK**

P-glycoprotein Inhibition

Background Information

'*In vitro* inhibition studies are recommended to investigate whether the investigational drug inhibits any of the transporters known to be involved in clinically relevant *in vivo* drug interactions.'

[4]The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012)

- P-gp is one of the most well-recognised efflux transporters expressed in many tissues including the intestine, brain and kidney[1].
- Inhibition of P-gp has shown to be responsible for several clinical drug-drug interaction. For example, clarithromycin can inhibit the transport of the P-gp substrate digoxin resulting in a clinically significant elevation of plasma exposure and a decrease in renal clearance[2].
- The International Transporter Consortium[1], the draft FDA guidance[3] and the EMA guideline[4] recommend investigating P-gp due to P-gp's clinical importance in the absorption and disposition of drugs.
- Cyprotex use MDCK-MDR1 cells to identify P-gp inhibitors using a range of test inhibitor concentrations in the presence of the clinically relevant probe substrate digoxin. This method conforms with the recommended methods within the International Transporter Consortium white paper[1], the draft FDA drug interactions guidance[3] and the EMA drug interactions guideline[4].

Protocol

Substrate
5 µM [^{3}H]-Digoxin
(clinically relevant substrate)

Test Article Concentrations
Seven point IC_{50}

Direction
Unidirectional (basolateral to apical)

Inhibitor Preincubation Time
30 min

Incubation Time
90 min

Growth Period
4 days

Analysis Method
Liquid scintillation counting

Integrity Marker
Lucifer Yellow

Data Delivery
IC_{50} (derived from corrected B-A P_{app})

Interference at the level of ATP binding cassette (ABC) and other transporters is increasingly being identified as the mechanism behind clinically important drug-drug interactions[5].

Table 1

Inhibition of P-gp-mediated digoxin transport by literature inhibitors.

Inhibitor	Mean IC_{50} ± Standard Deviation (n=3)
Cyclosporin A (positive control)	0.931 ± 0.0574
Ketoconazole	8.83 ± 4.09
Verapamil	54.7 ± 10.3
Elacridar	0.284 ± 0.0452

The MDCK-MDR1 cell test system using the P-gp substrate digoxin is able to correctly identify known literature P-gp inhibitors with a range of different potencies.

The incubation conditions have been fully characterised for our chosen P-gp substrate, digoxin, based on time linearity and chosen substrate concentration being at least ten-times lower than the reported K_m, and as such IC_{50} equates to K_i (assuming competitive inhibition).

References

1. The International Transporter Consortium (2010) *Nat Rev Drug Disc* **9**; 215-236.
2. Wakasugi H *et al*. (1998) *Clin Pharmacol Ther* **64**; 123-128.
3. Draft FDA Guidance for Industry - In vitro metabolism- and transporter-mediated drug-drug interaction studies (2017).
4. The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012).
5. Marchetti S *et al.* (2007) *Oncologist* **12**; 927-941.