BCRP Substrate Identification for Screening and Regulatory Studies

Background Information

- BCRP (breast cancer resistance protein; ABCG2) is an important efflux transporter. It is expressed in the gastrointestinal tract, liver, kidney, brain endothelium, mammary tissue, testis, and placenta¹.

- The ITC¹, the EMA guideline² and the draft FDA guidance³ recommend investigating BCRP due the clinical importance of BCRP in the absorption and disposition of drugs. Furthermore clinically relevant genetic polymorphisms of ABCG2 have been shown to have an impact on the pharmacokinetics and toxicity of marketed drugs.

- Caco-2 cells express BCRP. The EMA² and draft FDA³ regulatory guidelines recommend polarised Caco-2 cell monolayers as one of the preferred methods for evaluating the role of BCRP in the efflux of new chemical entities.

- The assay investigates bidirectional transport across the cell monolayer in the presence and absence of the selective BCRP reference inhibitor, fumitremorgin C, to determine if active efflux is occurring, and whether this efflux is mediated by BCRP.

- Where Caco-2 cell assays indicate a compound has inherently low passive permeability, then BCRP membrane vesicles can be used as an alternative in vitro test system to identify BCRP substrates (assay available on request).


Protocol

- **Test Article Concentrations**
  - Screening study: 10μM plus/minus inhibitor (different concentrations available)
  - Regulatory study: 1, 10, 50 and 100μM (different concentrations available) plus inhibition at two substrate concentrations (1 and 10μM).

- **Assay Conditions**
  - Apical to basolateral and basolateral to apical in presence and absence of 10μM fumitremorgin C

- **Number of Replicates**
  - 2 (screening) or 3 (regulatory)

- **Incubation Time**
  - 120 min (screening) or 90 min (regulatory)

- **Analysis Method**
  - LC-MS/MS quantification

- **Integrity Marker**
  - Lucifer Yellow

- **Data Delivery**
  - P_{eff}
  - Efflux ratio in presence and absence of fumitremorgin C
  - Recovery (%)
‘BCRP is highly expressed in normal human tissues including the small intestine, liver, brain endothelium, and placenta. Therefore, BCRP has been increasingly recognized for its important role in the absorption, elimination, and tissue distribution of drugs and xenobiotics.’

The expression and functional activity of BCRP in our Caco-2 cells were determined. Relative mRNA expression levels (relative to housekeeping gene) of the main transporters were analysed by qRT-PCR. BCRP mRNA was expressed in the cells and had comparable relative expression levels with MDR1 (0.041 ± 0.011 for BCRP and 0.047 ± 0.014 for MDR1). Functional activity of BCRP was determined by investigating the inhibition of the BCRP substrate, estrone 3-sulfate, by a number of BCRP and P-gp inhibitors.

Figure 1

Graph showing effect of the selective BCRP inhibitor, fumitremorgin C (FTC) and the selective P-gp inhibitor, verapamil, on the efflux of the BCRP substrate, estrone 3-sulfate.

Estrone 3-sulfate efflux was not inhibited by the P-gp inhibitor, verapamil, but was inhibited by the selective BCRP inhibitor, fumitremorgin C (FTC), showing selectivity of estrone 3-sulfate as a BCRP substrate. Data show the mean ± standard deviation.

References