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In vitro **ADME**

BCRP Substrate Identification for Screening and Regulatory Studies

Background Information

'ABCG2 is a high-capacity efflux transporter with wide substrate specificity recognizing large, hydrophobic molecules of either negative or positive charge, organic anions, and sulfate conjugates.'

[4] Chen Z *et al*., (2010) *Int J Cancer* **126(4)**; 841-851

- 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 placenta[1].
- The ITC[1], the EMA guideline[2] and the draft FDA guidance[3] 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 EMA[2] and draft FDA[3] 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_{app}

Efflux ratio in presence and absence of fumitremorgin C

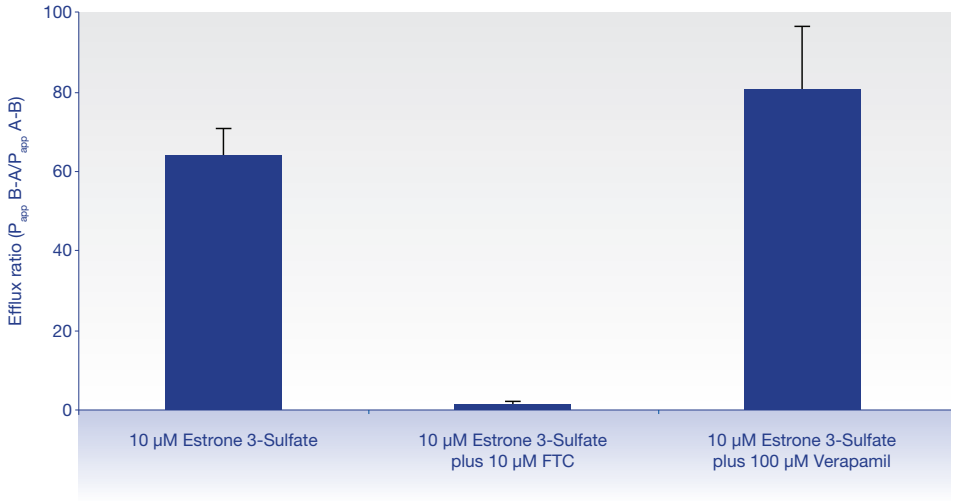
Recovery (%)

‘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.’[5]

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

[1] The International Transporter Consortium (2010) *Nat Rev Drug Disc* **9**; 215–236
[2] The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012)
[3] Draft FDA Guidance for Industry – Drug Interaction Studies - In Vitro Metabolism and Transporter-mediated Drug-Drug Interaction Studies, October 2017
[4] Chen Z *et al*., (2010) Suppression of ABCG2 inhibits cancer cell proliferation. *Int J Cancer* **126(4)**; 841-851
[5] Zhanglin N *et al*., (2010) Structure and function of the human Breast Cancer Resistance Protein (BCRP/ABCG2). *Curr Drug Metab* **11(7)**; 603-617