Estrone 3-Sulfate as a Surrogate Breast Cancer Resistance Protein (BCRP) *In Vitro* Probe Substrate for Assessing Drug-Drug Interaction Risk with Rosuvastatin

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Abstract

Inhibition of intestinal Breast Cancer Resistance Protein (BCRP) has been shown to be responsible for several clinical drug-drug interactions (DDIs) including with the common statin co-medication, rosuvastatin [1]. The International Transporter Consortium and regulatory agencies recommend investigating BCRP inhibition liability of candidate drugs in vitro [2, 3, 4]. The FDA draft guidance recommends that a clinical DDI study with a BCRP substrate should be performed if the ratio of theoretical intestinal concentration $[I_2]$ / K_i (IC_{50}) ≥ 10 [4].

Estrone 3-sulfate (E3S) is a common *in vitro* probe substrate that can be used to investigate BCRP inhibition, however its suitability as a surrogate experimental substrate to predict DDIs with rosuvastatin has yet to be established. This study was designed to determine if E3S can be considered as a surrogate *in vitro* BCRP probe using polarised Caco-2 cell monolayers to perform a unidirectional transport assay with drugs that are reported as perpetrating DDIs with rosuvastatin clinically [6]. Six established BCRP inhibitors for which a clinical DDI with rosuvastatin has been characterised were used in this study and top concentrations selected related to the theoretical intestinal concentration $[I_2]$. Caco-2 cells were seeded onto Multiscreen^{TM} plates (Millipore, MA, USA) at a density of $1x10^5$ cells/cm^2 and cultured for 20 days. Inhibitory potency of perpetrator drugs was determined from unidirectional permeability assessment performed in Caco-2 cells. An IC_{50} was determined and $[I_2]$ / IC_{50} ratios were calculated for the therapeutic dose to determine risk of drug-drug interaction as a consequence of intestinal BCRP inhibition.

IC_{50} values determined for perpetrator drugs using rosuvastatin as the BCRP probe were in agreement which those reported in the literature [2]. E3S derived IC_{50} values were within 2.4-fold of rosuvastatin derived IC_{50} values giving good concordance of BCRP DDI potential. E3S is a widely used, and recognised BCRP substrate that can be used towards the *in vitro* identification of BCRP inhibitors. $[I_2]$/ IC_{50} ratios generated using E3S as the BCRP probe were in agreement with those determined using rosuvastatin as the BCRP probe. This suggests that E3S is a suitable surrogate *in vitro* substrate. These findings should be considered when assessing BCRP inhibition liability of investigational drugs, particularly in target patient populations where co-administration with statins is likely due to comorbidities.

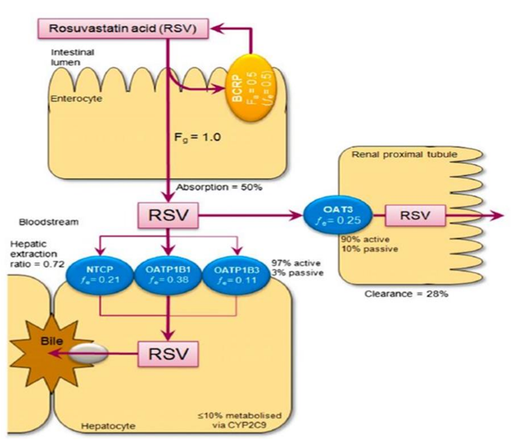


Figure 1: Critical disposition pathways of rosuvastatin (RSV) (taken from Elsby et al; 2016)

Drug	Dose (mg)	$[I_2]$ (µM)	Probe substrate	IC_{50} (µM)	$[I_2]/IC_{50}$ ratio	Potential for intestinal DDI (ratio ≥10)
Eltrombopag	75	678	Rosuvastatin E3S	2.00 3.39	339 200	Yes Yes
Darunavir	600	4382	Rosuvastatin E3S	207 194	21 23	Yes Yes
Lopinavir	400	2545	Rosuvastatin E3S	6.97 3.73	365 682	Yes Yes
Clopidogrel	75	932	Rosuvastatin E3S	87.4 206	11 4.5	Yes Unlikely
Clopidogrel	300	3728	Rosuvastatin E3S	87.4 206	43 18	Yes Yes
Ezetimibe	10	98	Rosuvastatin E3S	2.51 4.95	39 20	Yes Yes
Fenofibrate	67	743	Rosuvastatin E3S	199 >300	3.7 <2.5	Unlikely Unlikely

Table 1: Comparison of BCRP inhibition assessment using rosuvastatin or E3S as *in vitro* probe substrates in Caco-2 polarised cell monolayers

Background

Rosuvastatin is a 3-Hydroxy-3-methylglutaryl-coenzyme-A reductase inhibitor (statin) used in the treatment of dyslipidemia and is a common comedication. The critical disposition pathways of rosuvastatin have recently been determined, for which intestinal breast cancer resistance protein (BCRP) was identified as the rate-determining barrier to rosuvastatin absorption (Figure 1) [1,2].

Inhibition of BCRP has been shown to be responsible for several clinical drug-drug interactions (DDIs) including with rosuvastatin [1]. Inhibition of intestinal BCRP increases absorption of rosuvastatin resulting in an up to 2-fold increase in exposure [2].

As a result, the International Transporter Consortium and regulatory agencies recommend investigating the BCRP inhibition liability of candidate drugs *in vitro* [3,4,5]. The FDA draft DDI guidance recommends that a clinical DDI study with a BCRP substrate should be performed if the ratio of theoretical intestinal concentration $[I_2]$/Ki (IC_{50}) ≥10 [4].

Objective

E3S is a recognised *in vitro* probe substrate that can be used to investigate BCRP inhibition, however its suitability as a surrogate experimental substrate to predict DDIs with rosuvastatin has yet to be proven. This study was designed to determine if E3S can be applied as a surrogate *in vitro* BCRP probe substrate using polarised Caco-2 cell monolayers to perform a unidirectional (basolateral to apical direction) transport assay with drugs that are listed as perpetrating DDIs with rosuvastatin clinically [6].

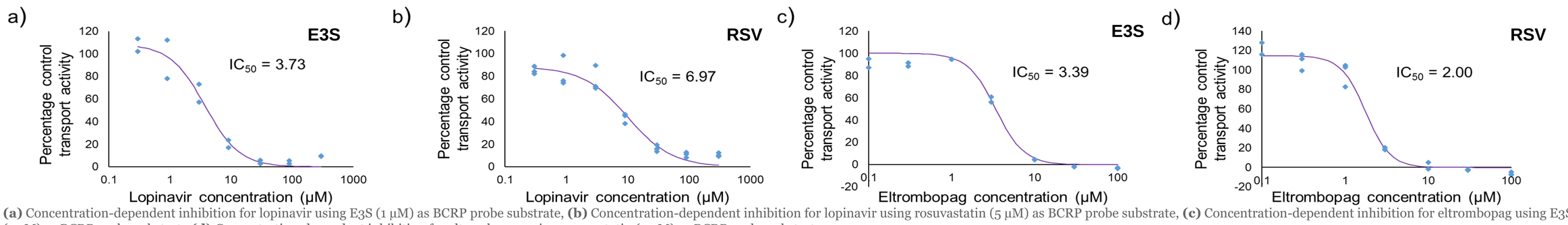
Methods

Six established BCRP inhibitors for which a clinical DDI with rosuvastatin has been characterised were used in this study; namely darunavir, lopinavir, ezetimibe, eltrombopag, fenofibrate and clopidogrel. A top concentration of 300 µM was selected for all drugs tested relating to the theoretical intestinal concentration $[I_2]$, with the exception of eltrombopag, for which a top concentration of 100µM was selected due to solubility constraints.

Caco-2 cells were seeded onto Multiscreen^{TM} plates (Millipore, MA, USA) at a density of $1x10^5$ cells/cm^2 and cultured for 20 days. Inhibitory potency of perpetrator drugs was determined from unidirectional permeability assessment performed in Caco-2 cells as follows:

HBSS (25 mM HEPES, 4.45 mM glucose, pH 7.4) containing probe substrate (1 µM E3S or 5 µM rosuvastatin) was added to the basolateral side of the Caco-2 monolayer in the absence and presence of inhibitor and incubated for 2 hours. Transport of probe substrate was measured in at least duplicate by monitoring its appearance on the apical side of the membrane by LC-MS/MS.

The passive permeability of the probe substrate (observed when BCRP is completely inhibited) was subtracted from the P_{app} determined in the basolateral to apical (B-A) direction in the absence and presence of inhibitor, to give a corrected BCRP-mediated B-A P_{app}. This was subsequently converted to percentage control transport activity and resulting inhibition curves plotted to determine an IC_{50}. $[I_2]$/ IC_{50} ratios were then calculated for the therapeutic dose to determine risk of DDI as a consequence of intestinal BCRP inhibition. Results are expressed as the mean from a single experimental occasion; where an IC_{50} could not be determined, IC_{50} is expressed as greater than the top concentration tested. IC_{50} was considered equivalent to K_i in both assays as the probe substrate concentration was < K_m.



(a) Concentration-dependent inhibition for lopinavir using E3S (1 µM) as BCRP probe substrate, **(b)** Concentration-dependent inhibition for lopinavir using rosuvastatin (5 µM) as BCRP probe substrate, **(c)** Concentration-dependent inhibition for eltrombopag using E3S (1 µM) as BCRP probe substrate **(d)** Concentration-dependent inhibition for eltrombopag using rosuvastatin (5 µM) as BCRP probe substrate

Figure 2: Comparison of *in vitro* determined IC_{50} values using E3S versus rosuvastatin (RSV) as the BCRP probe substrate

Results

IC_{50} values were determined for all drugs tested using rosuvastatin (5 µM) as the BCRP probe substrate. Using E3S (1 µM) as the BCRP probe substrate, IC_{50} values were determined for all drugs tested with the exception of fenofibrate, for which an IC_{50} was not determined over the concentration range tested and therefore an IC_{50} value of more than top concentration is reported (Table 1).

IC_{50} values determined for perpetrator drugs using rosuvastatin as the BCRP probe substrate were largely in agreement which those reported in the literature [2].

For all drugs included in this study, the experimentally derived IC_{50} values obtained using either E3S or rosuvastatin as the probe substrate were within 2.4 fold of each other (Table 1; Figure 2).

For clopidogrel, a difference in IC_{50} was observed using E3S (IC_{50} 206 µM) as the BCRP probe substrate versus rosuvastatin (IC_{50} 87.4 µM; Figure 2) which correlated to more than a 2-fold under prediction of $[I_2]/IC_{50}$ ratio for the 75 mg dose only. For the higher 300 mg dose that causes the largest AUC change, DDI potential was correctly predicted using E3S as the probe substrate (Table 1).

Conclusions

E3S is a widely used, and recognised BCRP substrate that can be used towards the in vitro identification of BCRP inhibitors.

E3S derived IC_{50} values were within 2.4 fold of rosuvastatin derived IC_{50} values.

$[I_2]$/ IC_{50} ratios generated using E3S as the BCRP probe were in agreement with those determined using rosuvastatin as the BCRP probe substrate giving good concordance of BCRP DDI potential. This suggests that E3S is a suitable surrogate *in vitro* substrate.

These findings should be considered when assessing BCRP inhibition liability of investigational drugs, particularly in target patient populations where co-administration with statins is likely due to comorbidities.

References

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