Introduction

Breast cancer resistance protein (BCRP, ABCG2) is an efflux transporter that blocks absorption at the apical membrane of enterocytes in the intestine, blood-brain barrier, blood-tissue barrier, mammary gland and other cells. At the apical membrane of hepatocytes and renal proximal tubule cells, BCRP enhances excretion and elimination of xenobiotics. 1 Inhibition of BCRP by drugs or xenobiotics has the potential to cause pharmaceutically significant drug-drug interactions, whereby increasing the exposure of co-administered compounds typically expected to be excreted by cells containing BCRP. For example, inhibition of BCRP in the intestine upon oral administration of GF120918 caused a 24-fold increase in the AUC of topotecan, a BCRP substrate. 2

Therefore, the recent FDA and EMA Guidance recommends evaluation of BCRP inhibition by new drug candidates, 3 which is typically conducted in one of two test systems: (1) bidirectional cell-based transport assays (e.g., MDCKII-BCRP cells) or (2) BCRP-expressing inverted membrane vesicles. In the case of cell-based assays, BCRP function is evaluated by monitoring efflux across the cell monolayer. Inasmuch as the substrate binding site(s) is intracellular or in the cell tadpole, the compound has to cross free membranes to reach the transporter. Conversely, BCRP function in inverted membrane vesicles is evaluated by monitoring the uptake of a probe substrate into the vesicles. Compounds do not have to cross a lipid membrane inasmuch as the substrate binding domain is located on the outside of the vesicle and is exposed to free drug available in the incubation medium. Because of this difference in substrate or inhibitor exposure to the substrate binding domain of BCRP, compounds with various intrinsic permeabilities may cause test system-dependent inhibition of BCRP in vitro.

In this study, we evaluated the system-dependent effects of both a high- and low-permeability compound on BCRP-mediated efflux, namely Ko143 and sulfasalazine, in MDCKII-BCRP cells and BCRP-expressing membrane vesicles. Ko143 is a potent and specific inhibitor of BCRP with moderate permeability (predicted logD7.4 = 3.30 and total polar surface area (TPSA) = 198 Å²) as referenced in Table 1. Sulfasalazine, an anti-inflammatory agent, was reported to cause potent inhibition of BCRP transport (IC50 = 1.87 μM) in BCRP-expressing erythrocytes. 4 However, sulfasalazine, with a predicted logD7.4 = 1.57 and TPSA of 140 Å² (Table 1), is poorly absorbed with limited permeability across physiological membranes. We hypothesized that, due to limited membrane permeability, sulfasalazine would cause significantly less inhibition of BCRP transport in cell-based assays.

Materials and Methods

Table 1. Summary of physicochemical properties and inhibition parameters of Ko143 and Sulfasalazine in vitro tests systems.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical structure</th>
<th>LogD7.4</th>
<th>Molecular Weight</th>
<th>Molar Surface Area</th>
<th>% of control</th>
<th>100</th>
<th>1000</th>
<th>10000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ko143</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>1.57</td>
<td>396.3</td>
<td>98 Å²</td>
<td>98 ± 1%</td>
<td>100</td>
<td>1000</td>
<td>10000</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>3.30</td>
<td>319.5</td>
<td>198 Å²</td>
<td>45 ± 3%</td>
<td>20</td>
<td>50</td>
<td>80</td>
</tr>
</tbody>
</table>

MDCKII-BCRP cells were obtained from The Netherlands Cancer Institute. BCRP-expressing inverted membrane vesicles were obtained from Sino Biotechnologies (Budapest, Hungary). Sulfasalazine, Ko143 and Prasozin were obtained from Sigma (St. Louis, MO, USA). Yttrium-3-3-sulfate (E3S) was obtained from Perkin Elmer Life and Analytical Sciences (Waltham, MA, USA).

The bidirectional permeability of prazosin across MDCK-II-BCRP and control cells was measured in the presence of Ko143 (0.001 - 1 μM) and sulfasalazine (1 - 300 μM) in triplicate in transwell plates (Corning Life Sciences, Lowell, MA, USA). Culture media was removed and incubation media (HBS supplemented with 25 mM HEPES and 25 mM glucose) was added to the cells at 37 ± 2 °C. FEER values were recorded approximately ten min after the incubation media was added and then the cells were washed to 37 ± 2 °C for 30 - 60 minutes. After the preincubation, incubation media with prazosin (1 μM final concentration) containing the solvent control or inhibitor were added to the donor or receiver chambers. Aliquots (30 μL) were taken from the donor chambers at zero minutes and the end of the incubation and from the receiver chambers (100 μL) at the end of the incubation (30 min). Samples containing prazosin were mixed with internal standard and analyzed by LC-MS/MS. E3S efflux ratios and net efflux ratios (efflux ratio on transporter expressing cells - efflux ratio on control cells) were determined for prazosin.

BCRP activity was evaluated in inverted membrane vesicles according to the manufacturer’s protocol. Briefly, the accumulation of E3S in BCRP expressing vesicles was measured in the presence of Ko143 (0.001 - 1 μM) and sulfasalazine (0.01 - 100 μM) with MgATP or MgAMPPNP in duplicate (n = 2). The membrane vesicles were diluted in incubation media and added to the incubation plate (39 μL). The inhibitor or solvent control was added to the membrane vesicles at 1% of final reaction volume and preincubated (37 ± 1 °C) for 15 minutes. The reaction was initiated by the addition of 10.5 μL of incubation media containing the substrate and MgATP (4 mM) or MgAMPPNP (4 mM) and incubated for one minute. Reactions were stopped by the addition of 50-μL ice-cold washing mix (typically 200 μL) and transferred to a filter plate and Blented. The filter plate was washed five times with 200 μL of ice-cold washing mix. After the filter plate was dried, the amount of substrate inside the filtered vesicles was determined by adding an all ofPOINTSMU8 fluid to the plate and counting with a liquid scintillation counter.

Results

Table 1

<table>
<thead>
<tr>
<th>Ko143 Concentration (µM)</th>
<th>0</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1000</th>
<th>10000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin Papp (1x10-6 cm/sec)</td>
<td>60</td>
<td>80</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure 1. Inhibition of Prazosin efflux across BCRP-expressing MDCKII cells in the presence of Ko143 and Sulfasalazine

- Figure 1 (A and B) illustrates the inhibition of prazosin efflux across BCRP-expressing MDCKII cells in the presence of Ko143. The highly permeable compound, Ko143, inhibited BCRP-mediated efflux and increased the exposure of co-administered compounds typically excreted from cells containing BCRP. For example, inhibition of BCRP in the intestine upon oral administration of GF120918 caused a 24-fold increase in the AUC of topotecan, a BCRP substrate. 2

- Figure 1 (C and D) illustrates the inhibition of prazosin efflux across BCRP-expressing MDCKII cells in the presence of sulfasalazine. In this case, the poorly permeable compound did not inhibit BCRP-mediated prazosin efflux (inhibition of BCRP-mediated efflux was limited to 30% (Table 1)). These results were similar to those reported above for inhibition of BCRP in MDCKII cells.

Conclusions

- The highly permeable compound, Ko143, inhibited BCRP-mediated efflux in BCRP-transfected MDCKII cells and membrane vesicles with similar potency.

- Sulfasalazine was identified as a system-dependent inhibitor of BCRP.

References