Substrate-Dependent Breast Cancer Resistance Protein (Bcrp1/Abcg2)-Mediated Interactions: Consideration of Multiple Binding Sites in in Vitro Assay Design

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ABSTRACT:

In vitro assays are frequently used for the screening of substrates and inhibitors of transporter-mediated efflux. Examining directional flux across Madin-Darby canine kidney (MDCK) II cell monolayers that overexpress a transporter protein is particularly useful in identifying whether or not a candidate compound is an inhibitor or substrate for that transport system. Studies that use a single substrate or inhibitor in competition assays can be challenging to interpret because of the possible multiple mechanisms involved in substrate/inhibitor-protein interactions. During our previous studies of substrate-inhibitor-transporter interactions, we observed differences in breast cancer resistance protein (BCRP) inhibition, depending on the substrate and the inhibitor. Therefore, we investigated BCRP-mediated interactions with a 4 × 4 matrix of substrates and inhibitors using monolayers formed from MDCKII cells transfected with murine BCRP (Bcrp1/Abcg2). The selective BCRP inhibitor 3-(6-isobutyl-9-methoxy-1,4-dioxo-1,2,3,4,6,7,12,12a-octahydropyrazino[1′,2′:1,6]pyrido[3,4-b]indol-3-yl)-propionic acid tert-butyl ester (Ko143) effectively inhibited the Bcrp1-mediated transport of all substrates examined. However, N-(4-[[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918), nelfinavir, and Pluronic P85 exhibited differences in inhibition depending on the substrate examined. Our findings support recent reports suggesting that the interactions of substrate molecules with BCRP involve multiple binding regions in the protein. The nucleoside substrates zidovudine and abacavir seem to bind to a region on BCRP that may have little or no overlap with the binding regions of either prazosin or imatinib. In conclusion, the choice of substrate or inhibitor molecules for an in vitro assay system can be crucial for the optimal design of experiments to evaluate transporter-mediated drug-drug interactions.

Multidrug resistance observed in cancer therapy is commonly attributed to ATP-binding cassette (ABC) efflux transporter proteins, such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP). These ABC transport proteins are localized not only in drug-resistant cancer cells, but also in the plasma membranes of epithelial cells in various normal tissues such as intestine, liver, kidney, placenta, and the blood-brain barrier where they can influence absorption, distribution, metabolism, and excretion of their substrate molecules (Maliepaard et al., 2001). The efflux transport protein BCRP is expressed in various organs, and because of its transmembrane orientation, this transporter is able to extrude substrates out of the cell (Maliepaard et al., 2001). BCRP is considered a half-transporter because of the presence of only six transmembrane domains, and it has been shown to exist in a multimeric oligomeric structure that may be critical for its function (Bhatia et al., 2005).

Numerous in vitro studies have characterized substrate-transporter interactions and these effects have also been observed in vivo (Merino et al., 2006; Shaik et al., 2007). Interaction of BCRP with various substrate molecules in vitro and in vivo has provided evidence that BCRP-mediated efflux could play a major role in the absorption, distribution, metabolism, and excretion of its substrate molecules (Staud and Pavek, 2005). Identifying inhibitors that modulate BCRP-mediated efflux of substrates could be valuable in enhancing target exposure to the drug. Various molecules have been shown to have inhibitory effects on BCRP-mediated transport of its substrates. GF120918 is a potent P-gp inhibitor that was shown to also have an inhibitory effect on BCRP-mediated transport. For instance, GF120918 inhibits BCRP-mediated transport of abacavir, zidovudine, STI-571 (imatinib), and prazosin (Breedveld et al., 2005; Ejendal and Hrycyna, 2005; Pan et al., 2007). Fumitremorgin C was identified as the first selective inhibitor of BCRP-mediated transport. Ko143 is a...
fumitremorgin C analog that is reported to be a potent inhibitor of BCRP-mediated transport (Allen et al., 2002). Ko143 has been shown to inhibit BCRP-mediated transport of abacavir, zidovudine, imatinib, and prazosin (Burger et al., 2004; Hori et al., 2004; Pan et al., 2007). The antihuman immunodeficiency virus protease inhibitor, nelfinavir, commonly used in combination therapy with other antihuman immunodeficiency virus agents, was reported to be an effective inhibitor of BCRP-mediated transport of mitoxantrone and pheophorbide A (Gupta et al., 2004; Weiss et al., 2007). Pluronic P85 is a block copolymer that is used as a pharmaceutical excipient. Pluronic P85 has been shown to inhibit both P-gp and MRP-mediated efflux of their substrates (Batrakova et al., 2001b, 2003). Recent studies have indicated that Pluronic P85 inhibits the BCRP-mediated transport of mitoxantrone and topotecan (Yamagata et al., 2007). Use of inhibitor molecules, such as those listed above, to modulate transporter-mediated efflux may be valuable to improve the targeted bioavailability of substrates to the target sites (Fig. 1). These inhibitors also serve as useful pharmacological tools to provide an understanding of potential drug-drug interactions.

In vitro methods such as the ATPase assay, directional flux, and

![Fig. 1. Diverse structures of BCRP substrates imatinib, prazosin, abacavir, and zidovudine and inhibitors Ko143, GF120918, nelfinavir, and Pluronic P85.](image-url)
intracellular accumulation studies are some of commonly used and relatively inexpensive methods to identify substrates and inhibitors of transporter-mediated efflux. Such experiments commonly involve the use of prototypical substrates or inhibitors to identify substrates and inhibitors on the basis of their ability to compete with known substrates and/or inhibitors for the transport protein. The substrate and/or inhibitors may compete for identical binding sites on the transporter or through allosteric modifications.

The existence of multiple binding sites on transport proteins could add to the complexity in identifying substrate and inhibitor molecules in such in vitro assay systems, leading to incorrect interpretation of these substrate-inhibitor interaction studies. The interaction of substrates with human P-gp has been shown to involve multiple binding sites (Ambudkar et al., 2006). P-gp is proposed to have a funnel-shaped drug-binding domain known to accommodate the simultaneous binding of at least two drugs (Loo et al., 2003). Furthermore, P-gp has demonstrated evidence of cooperativity between these multiple binding sites (Senior and Bhagat, 1998; Acharya et al., 2006). Rautio et al. (2006) used probe substrates that are known to bind to different P-gp binding sites to study the potential inhibitory activity of 20 candidate molecules. Variable degrees of affinity (IC50) for an individual inhibitor candidate molecule were observed, depending on the probe substrate used to examine inhibition of P-gp. That study highlighted the need for proper selection of probe substrates, as it can be a crucial consideration when establishing in vitro inhibition assays to examine interaction with P-gp-mediated transport (Rautio et al., 2006).

It is critical to recognize that multiple mechanisms of substrate-protein/inhibitor-protein coupling can cloud predictions about drug-drug interactions. This clouding necessitates the rational design and use of “specific” inhibitors for transporter drug interaction studies. Most studies are directed to examine the inhibitory effects of various candidate molecules on the transport of a single prototypical substrate. In this report, we present observations from the interaction of Bcrp1, the murine ortholog of BCRP, with a matrix of substrate and inhibitor molecules. The ability of four structurally diverse inhibitor molecules, the murine ortholog of BCRP, with a matrix of substrate and inhibitor molecules. The amount of compound transported was calculated using the specific activities of the radiolabeled drugs (0.6 Ci/mmol for abacavir, 53 mCi/mmol for zidovudine, 85 Ci/mmol for prazosin, and 105 mCi/mmol for STI-571) and compared in the wild-type and Bcrp1-transfected cells. Three flux measurements were obtained on three separate occasions to determine the effect of treatment.

**Effect of Inhibitors on Directional Transport.** Directional transport experiments were conducted in Bcrp1-transfected cells to evaluate the effect of inhibitors on the directional transport of substrate molecules as described above in the presence of inhibitors. Assay buffer containing GF120918 (5 μM), Ko143 (200 nM), nelfinavir (25 μM), and Pluronic P85 (0.01% w/w; 22 μM) was used to preincubate the cells and to prepare solutions for assay. The amount of substrate compound transported was calculated using the specific activities of the radiolabeled drugs and compared in the transfected cells in the presence and absence of inhibitors.

**Permeability Calculations and Efflux Ratio.** The effective directional permeabilities (Papp) of the nucleosides were calculated as described previously (Pan et al., 2007). The permeabilities were calculated using eq. 1, where slopes (dQ/dt) were obtained in the initial linear range from the amount transported versus time plots (for up to 90 min). A is the area of the Transwell membrane, and C0 is the initial donor concentration. The efflux ratio was determined as the ratio of the Papp calculated in the B-to-A direction divided by the Papp in the A-to-B direction. An efflux ratio >1.5 was considered to be indicative of Bcrp1-mediated active transport. The effect of an inhibitor was classified as complete if it reduced the efflux ratio in the Bcrp1-transfected cells by at least 85% and as partial if the reduction in efflux ratio was at least 50% but not greater than 85%.

\[
P_{\text{app}} = \frac{dQ/dt}{A/C_0}
\]

**Statistical Analysis.** Statistical analysis was performed using SigmaStat 3.1 (Systat Software, Inc., San Jose, CA). Data were analyzed for statistical significance using Student’s t test. Differences with p < 0.05 were considered statistically significant.

**Results**

**BCRP-Mediated Directional Flux.** MDCKII wild-type and Bcrp1-transfected cells were able to form healthy confluent monolayers in 3 to 4 days with similar transepithelial electrical resistance values in the wild-type and Bcrp1-transfected cells (265 ± 44 and 248 ± 27 ohm·cm², respectively; mean ± S.D.). Paracellular transport was monitored by measuring the permeability of [14C]munitanol across the cell monolayers. Permeability of [14C]munitanol was found to be low in both the wild-type and Bcrp1-transfected cell lines (Table 1). The directional permeability of [14C]munitanol in the A-to-B direction was very similar to the directional permeability in the B-to-A direction for both cell types (11 ± 3 versus 11 ± 1 and 8 ± 3 versus 7 ± 2 × 10−9 cm/s for wild-type and Bcrp1-transfected cells, respectively). The calculated efflux ratios for [14C]munitanol were 1

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**Materials and Methods**

**Chemicals and Reagents.** [14C]Mannitol, [3H]prazosin, [14C]zidovudine, and [3H]abacavir were obtained from Moravek Biochemicals (Brea, CA). [14C]STI-571 (imatinib) was kindly supplied by Novartis (East Hanover, NJ). GF120918 was a gift from GlaxoSmithKline (Research Triangle Park, NC). Ko143, a fumitremorgin C analog, was kindly provided by Dr. Alfred H. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands), and LY335979 was a gift from Eli Lilly and Co. (Indianapolis, IN). Pluronic P85 was a gift from the BASF Corporation (Florham Park, NJ). All other chemicals used were high-performance liquid chromatography or reagent grade.

**Cell Culture.** MDCKII wild-type and Bcrp1-transfected cell lines (Jonker et al., 2000) were kindly provided by Dr. Alfred H. Schinkel (The Netherlands Cancer Institute). Cells used for all our experiments were between passages 7 and 15. The cells were cultured in Dulbecco’s modified Eagle’s medium (Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum (SeraCare Life Sciences, Inc., Oceanside, CA), penicillin (100 U/ml), and streptomycin (100 μg/ml) (Sigma-Aldrich, St. Louis, MO). All other cell culture materials were obtained from BD Biosciences (San Jose, CA). Both wild-type and Bcrp1-transfected cells were grown in 25-mI flasks before seeding onto Transwell permeable supports (Corning Inc., Corning, NY).
and 0.9 in the wild-type and Bcrp1 transfects, respectively, indicating no differences in paracellular transport in these two cell types.

Prazosin, abacavir, and zidovudine permeabilities in the B-to-A direction in the wild-type cells were not significantly different from their A-to-B permeabilities (43 ± 7 versus 33 ± 11, 129 ± 31 versus 100 ± 43, and 12 ± 1 versus 14 ± 1 × 10⁻⁷ cm/s for prazosin, abacavir, and zidovudine, respectively) (Table 1). The efflux ratios calculated from the observed permeabilities for prazosin, abacavir, and zidovudine in the wild-type cells were 1.3, 1.3, and 0.9, respectively, indicating that the influence of active transport systems on the directional flux of these compounds was negligible in the wild-type cells. The permeability of imatinib in the B-to-A direction in the wild-type cells was significantly greater (p < 0.05) than its A-to-B permeability (52 ± 3 versus 29 ± 8 × 10⁻⁷ cm/s) (Fig. 5A). The observed efflux ratio of 1.8 for imatinib in the wild-type cells is indicative of an apically directed endogenous transport system for imatinib in the wild-type MDCKII cells. Observed permeabilities of prazosin, abacavir, zidovudine, and imatinib were significantly greater (p < 0.05) in the B-to-A direction than in the A-to-B direction in the Bcrp1-transfected cells (57 ± 27 versus 5 ± 4, 187 ± 12 versus 7 ± 4, 44 ± 1 versus 5 ± 1, and 181 ± 9 versus 3 ± 1 × 10⁻⁷ cm/s for prazosin, zidovudine, abacavir, and imatinib, respectively) (Figs. 2A, 3A, 4A, and 5A). The calculated efflux ratios in the Bcrp1-transfected cells were 10.5, 27, 10, and 62 for prazosin, abacavir, zidovudine, and imatinib, respectively, indicating that Bcrp1-mediated active efflux plays a prominent role in preferentially transporting these substrates in the B-to-A direction (Table 1).

**Effect of Inhibitors on BCRP-Mediated Directional Flux.** Mean permeabilities of Bcrp1 substrates prazosin, abacavir, zidovudine, and imatinib, in the A-to-B and B-to-A directions, measured in the presence of the inhibitors GF120918, Ko143, nelfinavir, and Pluronic P85 were used to calculate the inhibited efflux ratios. These were compared with those observed in the Bcrp1-transfected cells in the absence of inhibitors (Table 2). Ko143 was a potent inhibitor for all substrates, reducing their monolayer efflux ratios in the Bcrp1-transfected cells to 1.4, 1.1, 0.8, and 2.2 for prazosin, zidovudine, abacavir, and imatinib, respectively (Figs. 2B, 3B, 4B, and 5B). These efflux ratios of substrates in the presence of Ko143 are similar to the efflux ratios of these substrates observed in the wild-type cells (Table 2). GF120918 abolished differences in the directional permeabilities for zidovudine and abacavir in the Bcrp1-transfected cells, reducing their efflux ratios to 1.3 and 1.4, respectively (Figs. 3C and 4C). However, reversal of Bcrp1-mediated transport of prazosin and imatinib by GF120918 was partial, with resultant efflux ratios of 5 and 27.5, respectively (Figs. 2C and 5C). The effect of nelfinavir on Bcrp1-mediated transport was complete for zidovudine and abacavir (Figs. 3D and 4D). Nelfinavir showed partial inhibition of Bcrp1-mediated transport of imatinib (Fig. 5D) but had a negligible effect on the Bcrp1-mediated transport of prazosin (Fig. 2D). Pluronic P85 showed partial reversal of Bcrp1-mediated transport of zidovudine and abacavir (Figs. 2E and 3E) but had no inhibitory effect on the Bcrp1-mediated transport of either prazosin or imatinib (Figs. 2E and 4E).

**Discussion**

ABC efflux transport proteins, such as P-gp, the MRPs, and BCRP/ABCG2, play a critical role in the disposition of a number of clinically used drugs and may be involved in drug-drug interactions (Leslie et al., 2005). For example, Zaher et al. (2006) used Bcrp1-deficient mice to demonstrate that Bcrp1-mediated efflux of sulfasalazine is a major factor for its decreased oral absorption. In the same study, pharmacological inhibition of BCRP by gefitinib also achieved an increase in the plasma exposure of the wild-type mice to sulfasalazine. Therefore, identifying substrates and inhibitors of efflux transport proteins could be useful for drug development and for improving the targeted bioavailability of therapeutic agents to targets such as multidrug-resistant tumors and pharmacological sanctuary sites. However, it is critical to understand the limitations of the in vitro assay systems that are frequently used for characterizing transporter interactions. Inconsistent results in studying interactions of transporter substrates and inhibitors may be due to multiple binding sites on the transport protein. An appreciation of these disparities is essential for the rational design of assays.

MDCKII cells can form a confluent monolayer with tight junctions and can be used as a surrogate for physiological barriers to examine the transport of drug molecules in vitro. Directional flux or permeability studies across such a cell monolayer are useful to screen for substrates and inhibitors of transporter-mediated efflux (Mahar Doan et al., 2002). We examined the vectorial transport of compounds across cell monolayers formed from MDCKII wild-type and MDCKII-Bcrp1 transfected cells. The permeability of mannitol, a marker for paracellular transport, across these cell monolayers was low (7–11 × 10⁻⁷ cm/s) and very similar to that reported previously (4 × 10⁻⁷ cm/s) for the permeability of mannitol across MDCKII cells (Braun et al., 2000). This observation confirmed that the wild-type and Bcrp1 cell monolayers formed tight junctions and that the paracellular transport across both was low with less than 2% of mannitol crossing the cell monolayers over 3 h.

The efflux ratio calculated from the directional permeabilities for prazosin, zidovudine, and abacavir in the wild-type cells was close to unity, suggesting that there was little difference in the directional transport of these compounds in the wild-type cells. The efflux ratio for imatinib in the wild-type cells was 1.8, indicating that there could be some preferential transport in the B-to-A direction in the wild-type monolayers. Imatinib is a substrate for P-gp (Dai et al., 2003), and its affinity for endogenous P-gp (Tang et al., 2002) in the wild-type MDCKII cells may be responsible for such an observation.

**TABLE 1**

Permeabilities of [¹⁴C]mannitol, [³H]prazosin, [¹⁴C]zidovudine, [³H]abacavir, and [¹⁴C]imatinib in MDCK wild-type and MDCK Bcrp1 cells

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MDCK WT</th>
<th>MDCK Bcrp1</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A-to-B P&lt;sub&gt;app&lt;/sub&gt;</td>
<td>B-to-A P&lt;sub&gt;app&lt;/sub&gt;</td>
</tr>
<tr>
<td>Mannitol</td>
<td>11 ± 3</td>
<td>11 ± 1 (N.S.)</td>
</tr>
<tr>
<td>Prazosin</td>
<td>33 ± 11</td>
<td>43 ± 7 (N.S.)</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>14 ± 1</td>
<td>12 ± 1 (N.S.)</td>
</tr>
<tr>
<td>Abacavir</td>
<td>100 ± 43</td>
<td>129 ± 31 (N.S.)</td>
</tr>
<tr>
<td>Imatinib</td>
<td>29 ± 8</td>
<td>52 ± 3*</td>
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N.S., not significantly different from apical-to-basolateral transport.

* Significantly different from apical-to-basolateral transport.
BCRP has been localized on the apical side of plasma membranes and is known to preferentially transport its substrates from the B-to-A direction. Abacavir, zidovudine, prazosin, and imatinib have been reported to be substrates for BCRP in vitro (Burger et al., 2004; Hori et al., 2004; Pan et al., 2007). The B-to-A transport for these substrates was significantly higher than the A-to-B transport across the Bcrp1-
transfected cell monolayers, indicating that these highly diverse structures would be good probes of Bcrp1-mediated transport.

Ko143, a selective BCRP inhibitor, at a concentration of 200 nM completely reversed the Bcrp1-mediated transport of all substrates examined. GF120918 completely inhibited Bcrp1-mediated transport of the nucleoside analogs abacavir and zidovudine; however, it
showed only partial inhibition of imatinib and prazosin transport. Breedveld et al. (2005) previously reported a similar observation, where GF120918 was unable to completely reverse Bcrp1-mediated transport of imatinib in Bcrp1-transfected cells. Both prazosin and imatinib have been reported to be substrates for the efflux transport protein P-gp, which is known to be constitutively expressed in wild-

type MDCKII cells. It should be noted that the inhibitor Ko143 completely abolishes the directional transport of prazosin and imatinib in the Bcrp1-transfected cells, and the efflux ratio in the presence of the dual Pgp-BCRP inhibitor, GF120918, is much greater than the efflux ratio in the wild-type cells. These findings indicate that endogenous P-gp in these cells cannot account for such an observation.
These observations raise the possibility that the substrate-inhibitor interactions could be explained by the existence of multiple binding regions on BCRP. This current study suggests that the nucleoside analogs bind to a similar site where their binding and/or transport by BCRP is completely inhibited by GF120918. In contrast, both prazosin and imatinib may bind to BCRP at a site distinct from the binding site of the nucleoside analogs, where GF120918 is able to only partially inhibit their interaction with BCRP.

Interaction of protease inhibitors with BCRP is of particular interest as they are frequently used in combination active retroviral therapy with nucleoside analogs such as abacavir and zidovudine (Warnke et al., 2007). The protease inhibitor, nelfinavir, has been reported to inhibit BCRP-mediated transport of mitoxantrone and pheophorbide A with IC50 values of 12.5 and 13.5 μM, respectively (Gupta et al., 2004; Weiss et al., 2007). In the present study, nelfinavir (25 μM) completely abolished the Bcrp1-mediated transport of both abacavir and zidovudine. However, nelfinavir had no effect on the Bcrp1-mediated transport of prazosin and was able to only partially reverse the Bcrp1-mediated transport of imatinib. These findings support our earlier suggested hypothesis that the nucleosides interact with a binding region on BCRP that is distinct from the binding region of imatinib or prazosin. Brendel et al. (2007) observed that imatinib inhibited the photolabeling of BCRP with [3H]IAAP, indicating that the binding regions of imatinib and prazosin possibly overlap. Based on our observation that nelfinavir has no effect on the binding of prazosin and only has a partial effect on the binding of imatinib, one can speculate that nelfinavir does not have any effect on the binding region of prazosin but may partially affect the binding region of imatinib.

The maximum plasma concentration of nelfinavir attained with its recommended dosing regimen is approximately 10 μM (Fang et al., 2008). Nelfinavir is reported to be highly bound to plasma proteins, and the free concentrations attained during therapy may be much lower than the IC50 reported for its inhibition of BCRP-mediated transport. However, the concentrations of nelfinavir estimated in the intestinal lumen after an oral dose are significantly higher than its reported IC50 (Gupta et al., 2004) and may alter BCRP-mediated transport of its substrates, such as nucleoside analogs, in the gut lumen.

The block copolymer Pluronic P85 also exhibited differential selectivity for BCRP-mediated transport. Pluronic P85 did not reduce the Bcrp1-mediated transport of either prazosin or imatinib, but partially reversed the Bcrp1-mediated transport of the nucleoside analogs. It is possible that Pluronic P85 mediates its inhibitory effect via changes in the conformation of BCRP to affect the binding of nucleosides but not that of prazosin or imatinib. Another possible explanation for this difference in extent of inhibition of Bcrp1-mediated transport is the existence of multiple binding regions on BCRP for the different substrates as suggested earlier. Batrakova et al. (2001a) have suggested that Pluronic P85 may induce changes in the cell membrane. To investigate the effect of Pluronic P85 on the integrity of cell monolayers, we examined the transport of mannitol across MDCKII wild-type and Bcrp1-transfected cells. The flux of mannitol across both the wild-type and Bcrp1-transfected cells was not affected in the presence of Pluronic P85 (data not shown).

It is possible that the observed discrepancies in inhibitor sensitivity in our study may be concentration dependent. However, we used concentrations of Ko143 and GF120918 that were previously observed to have a maximal effect on BCRP-mediated transport (Pan et al., 2007). The concentration of nelfinavir used in our assay was approximately twice the previously reported IC50 for BCRP-mediated transport (Gupta et al., 2004; Weiss et al., 2007). For Pluronic P85, we used a concentration of 0.01% (w/v), which is less than the reported critical micellar concentration of 0.03% (Batrakova et al., 2001b). However, Pluronic P-85 has been shown to effectively inhibit P-gp-mediated transport and ATPase activity at a concentration of 0.01% (w/v) (Shaik et al., 2008). These previous studies strongly suggest that the observed substrate-dependent discrepancies in the sensitivity of BCRP to various inhibitors in this study may be due to the different affinities of the inhibitor for multiple drug binding sites on the transporter.

The mechanism of interaction of BCRP with its substrates and inhibitors is not yet completely understood. However, there is some information regarding the existence of multiple binding sites on BCRP. Clark et al. (2006) reported that the binding of [3H]daunomycin to the R482G isoform of BCRP was complete, partial, or not significant, depending on the competing BCRP substrate used to examine this binding interaction. These findings suggested the existence of three distinct pharmacological binding sites on BCRP (Clark et al., 2006). Takenaka et al. (2007) observed that purine analog BCRP substrates, such as 9-(2-phosphonylmethoxyethyl) adenosine, did not interfere with the binding of [125I]IAAP, a photoaffinity analog of prazosin, to BCRP, indicating separate binding sites for prazosin and nucleoside analogs (Takenaka et al., 2007). This finding corroborates our current results indicating that the nucleoside binding site is distinct from the binding site for prazosin. Ejendal and Hrycyna (2005) previously observed that the binding of [125I]IAAP, a photoaffinity analog of the BCRP substrate prazosin, to BCRP was not affected in the presence of other BCRP substrates, such as daunomycin, mitoxantrone, rhodamine 123, and methotrexate. Furthermore, Ejendal and Hrycyna (2005) observed that cyclosporin A (CsA) did not inhibit the binding of [125I]IAAP to BCRP leading to the conclusion that CsA is not an inhibitor of BCRP-mediated transport. However, Gupta et al. (2006) observed that the CsA had a potent inhibitory effect on BCRP-mediated transport of pheophorbide A and mitoxantrone. Fur-
nucleoside binding region. These data support existing reports that prazosin and imatinib, which may interact with sites distinct from the nelfinavir, and Pluronic P85 inhibit transport of these nucleoside which in turn may bind to distinct regions on BCRP. GF120918, allosterically blocks transport of all the substrates we examined, that the nucleoside analogs bind to a drug-binding site on BCRP that may be due to the ability of these substrates and inhibitors to each choice of substrate-inhibitor pairs used may influence the interpretation of potential substrate and inhibitor activity. These differences may be due to the ability of these substrates and inhibitors to each interact with distinct binding sites. The data described herein indicate that the nucleoside analogs bind to a drug-binding site on BCRP that is distinct from the binding sites for prazosin and imatinib. The inhibitor Ko143 may bind to a region of the BCRP binding site that allosterically blocks transport of all the substrates we examined, which in turn may bind to distinct regions on BCRP. GF120918, nelfinavir, and Pluronic P85 inhibit transport of these nucleoside analogs by BCRP but have a partial or no effect on the transport of prazosin and imatinib, which may interact with sites distinct from the nucleoside binding region. These data support existing reports that propose multiple binding sites on the binding domain of BCRP. In conclusion, careful selection of substrates and inhibitors is critical for designing definitive studies to identify BCRP-mediated interactions between therapeutic agents.

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References


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