EXPRESSION, LOCALIZATION, AND FUNCTIONAL CHARACTERISTICS OF BREAST CANCER RESISTANCE PROTEIN IN CACO-2 CELLS

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Received December 21, 2004; accepted February 15, 2005

ABSTRACT:

The function of breast cancer resistance protein (BCRP) and its role in drug absorption, distribution, and elimination has recently been evaluated. The objective of the present study was to examine the expression, localization, and functional characteristics of BCRP in Caco-2 cells, a widely used human intestinal epithelial cell model for investigating intestinal drug absorption. The expression of BCRP in Caco-2 cells was measured by Western blotting using the antibody BXP-21. Localization of BCRP was determined by an immunofluorescence technique using both antibodies BXP-21 and BXP-34. The drug efflux function of BCRP was evaluated via the epithelial transport of methotrexate (MTX) and estrone-3-sulfate (E3S) across Caco-2 cell monolayers in the presence or absence of the BCRP inhibitors Ko143 or GF120918 increased the apical-to-basolateral (A-to-B) transport but decreased the B-to-A transport of MTX and E3S. Caco-2 cells may therefore be used as an in vitro model to study the transport characteristics of BCRP.

Breast cancer resistance protein (BCRP) was originally cloned and sequenced from genomic DNA, from highly mitoxantrone-resistant S1-M1-80 human colon carcinoma cells, and from MCF7 AdVp human breast cancer cells selected in doxorubicin (Doyle et al., 1998; Miyake et al., 1999). BCRP is a member of the ATP-binding cassette transporter G family and is also known as ABCG2 or ABCP or MXR (Ejendal and Hrycyna, 2002; Doyle and Ross, 2003). It is a 655-amino acid polypeptide (72 kDa), containing six putative transmembrane domains and four potential N-glycosylation sites. BCRP is similar to half the duplicated P-glycoprotein (P-gp) or multidrug resistance protein 1 (MRP1) molecule and functions as a homodimer bridged by disulfide bonds (Doyle et al., 1998; Kage et al., 2002).

BCRP is endogenously expressed at a high level in human placenta and to a lesser extent in liver, small intestine and colon, ovary, veins, capillaries, kidney, adrenal, and lung, with little to no expression in brain, heart, stomach, prostate, spleen, and cervix (Doyle et al., 1998; Litman et al., 2001; Maliepaard et al., 2001; Scheffer and Schep, 2002). Importantly, BCRP is expressed in the human jejunum at levels considerably higher than those of many other ABC transporters (Taipalensuu et al., 2001). BCRP has been demonstrated to exist on the apical membrane of intestinal epithelium and has limited the oral absorption of topotecan in mice and humans (Jonker et al., 2000; Kruijtzer et al., 2002a). Given the liver and intestinal localization pattern, BCRP, similar to P-gp, may act as a barrier to uptake and absorption and limit the oral bioavailability of drugs as well as mediating hepatobiliary excretion of drugs (Jonker et al., 2000; Jorritsma et al., 2002; Kruijtzer et al., 2002b).

Caco-2 cells are derived from human colon adenocarcinoma cell line, and exhibit morphological and functional similarities to intestinal enterocytes. It has been widely used as a model of human intestinal epithelium for studies of intestinal drug absorption and metabolism. Many active transport systems such as P-gp (encoded by MDR-1 gene, also named ABCB1) and multidrug resistance-associated protein 2 (MRP2, or ABCC2) have been characterized in Caco-2 cells (Makhey et al., 1998; Doppenschmitt et al., 1999; Gutmann et al., 1999). BCRP mRNA has been detected in Caco-2 cells, although its level is 100-fold lower than that in human jejunum (Taipalensuu et al., 2001).

The present study was aimed at characterization of the protein expression, localization, and efflux function of BCRP in Caco-2 cells. We have used BXP-21 monoclonal antibody (mAb) to determine the expression, localization, and functional characteristics of BCRP in Caco-2 cells.

ABBREVIATIONS: BCRP, breast cancer resistance protein; ABC, ATP-binding cassette transporter; A-to-B, apical-to-basolateral; B-to-A, basolateral-to-apical; E3S, estrone-3-sulfate; FBS, fetal bovine serum; MDR-1, human multidrug resistance gene 1; mAb, monoclonal antibody; MRP, multidrug resistance-associated protein; MTX, methotrexate; Papp, apparent permeability coefficient; P-gp, P-glycoprotein; TEER, trans-epithelial electrical resistance; GF120918, N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isouquinolinyl)ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide; LY335979, zosuquidar trihydrochloride; MK571, 3-[[3-[2-(7-chloroquinolin-2-yl)vinyl]phenyl]-[2-dimethylcarbamoyl-ethylsulfanyl][methylsulfanyl] propionic acid; 2,4-DNP, 2,4-dinitrophenol; FITC, fluorescein isothiocyanate.
BCRP protein expression, and BXP-21 and BXP-34 mAbs to characterize the subcellular distribution of BCRP in Caco-2 cells. In addition, the efflux function of BCRP in Caco-2 cells was determined using estrone-3-sulfate (E3S) and metothrexate (MTX) as substrates. Knowledge of the properties of BCRP in Caco-2 cells is valuable to investigate the absorption mechanism of drug molecules using this in vitro model system.

Materials and Methods

Materials. [3H]Estrone-3-Sulfate (specific activity, 45 Ci/mmol) and [3H]methotrexate (specific activity, 33.5 Ci/mmol) were purchased from Moravek Biochemicals (Brea, CA). GF120918 and LY335979 were synthesized at Millennium Pharmaceuticals, Inc. Ko143 was obtained from the Netherlands Cancer Institute (Amsterdam, Netherlands). MK571 was purchased from Alexis Biochemicals (San Diego, CA). Unlabeled E3S, MTX, prazocin, and 2,4-dinitrophenol (2,4-DNP) were purchased from Sigma-Aldrich (St. Louis, MO). Cell culture media and supplies were obtained from Invitrogen (Carlsbad, CA). BXP-21 and BXP-34 murine monoclonal antibodies were purchased from Signet Laboratories ( Dedham, MA). Horseradish peroxidase-conjugated goat anti-mouse IgG2a, mouse IgG2a, and mouse polyclonal antibody I19 for characterization of BCRP in Caco-2 cells was determined (World Precision Instruments, Inc., Sarasota, FL). Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG was obtained from Abcam Inc. (Cambridge, MA). GF120918, prazocin (100 μM), MK571 (50 μM), Ko143 (1 μM), LY335979 (5 μM) were present in the transport medium of the donor side from the preincubation period throughout the permeability study. At 15, 30, 45, and 60 min, the sample aliquots of receiving solutions were withdrawn from the basolateral side (for A-to-B transport) or from the apical side (for B-to-A transport), and replaced immediately with an equal amount of fresh transport media except at the 60-min time point (the end of the incubation). The samples were mixed with 5 ml of scintillation cocktail and the radioactivity was determined in a liquid scintillation spectrophotometer (Beckman Coulter, Fullerton, CA).

Data Analysis. The cumulative amount of drug (Q) on the receiver side was plotted as a function of time. The steady-state flux J was then estimated from the slope (dQ/dt). The apparent permeability coefficient (Papp) of unidirectional flux for the test compound was estimated by normalizing the flux J (mol/s) against the nominal surface area A (0.33 cm²) and the initial drug concentration in the donor chamber C0 (mol/ml), or Papp = J(A/C0). The B/A ratio was equal to the Papp value for A-to-B transport (Papp A-to-B) divided by the Papp value for B-to-A transport (Papp B-to-A). The kinetic parameters for the E3S transport were estimated by fitting the flux against the donor E3S concentration using a nonlinear regression with a method of least squares fitting (GraphPad Software Inc., San Diego, CA).

Data are expressed as mean ± S.E.M. of three individual monolayers. Tests of significance of differences between mean values were made using a two-tailed unpaired Student’s t test. A probability of less than 0.05 (p < 0.05) was considered to be statistically significant.

Results

Expression and Localization of BCRP. BCRP protein expression was determined in Caco-2 cell lysate samples via Western blot analysis using BXP-21 as the primary antibody. As shown in Fig. 1A, both the half-transporter BCRP (~70 kDa) and its dimer (~140 kDa) were observed in lanes 1 and 4, in which the Caco-2 cell lysates were not treated with reducing agent (7–13% of dithiothreitol). Lanes 2, 3, 5, and 6 are the lysates treated with reducing agent, which showed a strong band of BCRP monomer (~70 kDa). Lane 6 depicts a trace amount of BCRP dimer when a high concentrated unheated sample was loaded.

When Caco-2 cells were cultured in cell culture T-flasks, the expression of BCRP monomer and dimer in Caco-2 cells was more than 3-fold higher in the late passage [passages 56 (p56) or 59 (p59)] than in the early passage [passages 33 (p33) or 36 (p36)] after normalization to the expression of β-actin (Fig. 1, B and C). However, the expression of BCRP dimer when a high concentrated unheated sample was loaded. When Caco-2 cells were cultured in cell culture T-flasks, the expression of BCRP monomer and dimer in Caco-2 cells was more than 3-fold higher in the late passage [passages 56 (p56) or 59 (p59)] than in the early passage [passages 33 (p33) or 36 (p36)] after normalization to the expression of β-actin (Fig. 1, B and C). However, the expression of BCRP dimer when a high concentrated unheated sample was loaded.
membranes but also in the cytoplasm. On the 5-day-old culture, cells reached confluence and showed increased TEER values (indicative of the tight junction formation); BCRP staining was observed only on the apical membrane, and there was no detectable staining in the cytoplasm, indicating the localization of BCRP on the apical membrane. The same phenomena were also noted during the cell differentiation period (i.e., the 12th and 19th days after cell seeding). When the late passage cells (p59) were grown on the Transwell plates for 12 days, multiple layers were observed as demonstrated by the multiple nuclei staining in the x-z and y-z views (Fig. 2B). BCRP staining, however, was still preferentially shown on the apical side of cell membranes (Fig. 2B). The absence of any green staining in the cell monolayers with mouse IgG2a and FITC-conjugated second antibody served as a negative control.

**Efflux Function of BCRP.** The function of BCRP as an efflux transporter in Caco-2 cell monolayers was evaluated using a known substrate, E3S (Suzuki et al., 2002, 2003). As shown in Table 1, the $P_{app}$ value of $[{\text{H}}]$E3S (0.02 M) from B-to-A was $46.1 \times 10^{-6}$ cm/s and was 8-fold higher than the $P_{app}$ value (5.7 $\times 10^{-6}$ cm/s) for A-to-B in the 5-day culture (p33). In 12- and 19-day cultures (p33), the $P_{app}$ values for A-to-B decreased to 4.1 and $4.5 \times 10^{-6}$ cm/s, and the $P_{app}$ value for B-to-A increased to 63.0 and 58.0 $\times 10^{-6}$ cm/s, respectively. In the presence of Ko143 (a BCRP inhibitor), the A-to-B and B-to-A transports of E3S were almost equal, with $P_{app}$ values around 9 to $11 \times 10^{-6}$ cm/s. Figure 3 showed the comparison of BCRP-mediated $[{\text{H}}]$E3S (0.02 M) efflux in both early passage (p36) and late passage (p59) Caco-2 cell monolayers. In p36, the $P_{app}$ value for A-to-B transport of E3S was 2.3 $\times 10^{-6}$ cm/s and was 32-fold less than the $P_{app}$ value for B-to-A transport. The $P_{app}$ value increased to $6.1 \times 10^{-6}$ cm/s in the presence of Ko143. However, $P_{app}$ values of E3S for A-to-B and B-to-A transport in p59 were 16.2 and $37.1 \times 10^{-6}$ cm/s, respectively. The difference in the directional E3S transport was abolished by the BCRP inhibitor Ko143. The TEER value was 4-fold higher in p36 than in p59 in the present cell culture system (Fig. 3).

When $[{\text{H}}]$E3S (0.03 M) was coincubated with Ko143 (1 M), GF120918 (2 M) (an inhibitor for BCRP and P-gp), or prazocin (100 M) (an inhibitor for BCRP and P-gp) in Caco-2 cell monolayers, the $P_{app}$ value of E3S for the A-to-B transport increased and the $P_{app}$ value for B-to-A transport decreased, resulting in the B/A ratio dropping from 27 to almost unity (Fig. 4). In contrast, the P-gp-specific inhibitor LY335979 (5 M) (an inhibitor for BCRP and P-gp) did not significantly change the $P_{app}$ value of E3S (63.0 to 58.0 $\times 10^{-6}$ cm/s) for A-to-B and B-to-A transports of E3S were almost equal, with $P_{app}$ values around 9 to $11 \times 10^{-6}$ cm/s. Figure 3 showed the comparison of BCRP-mediated $[{\text{H}}]$E3S (0.02 M) efflux in both early passage (p36) and late passage (p59) Caco-2 cell monolayers. In p36, the $P_{app}$ value for A-to-B transport of E3S was 2.3 $\times 10^{-6}$ cm/s and was 32-fold less than the $P_{app}$ value for B-to-A transport. The $P_{app}$ value increased to $6.1 \times 10^{-6}$ cm/s in the presence of Ko143. However, $P_{app}$ values of E3S for A-to-B and B-to-A transport in p59 were 16.2 and $37.1 \times 10^{-6}$ cm/s, respectively. The difference in the directional E3S transport was abolished by the BCRP inhibitor Ko143. The TEER value was 4-fold higher in p36 than in p59 in the present cell culture system (Fig. 3).

MTX was chosen to further investigate the BCRP efflux function in the Caco-2 cell monolayers. As shown in Fig. 6, the B-to-A transport of MTX was significantly decreased in the presence of MK571 (a MRP inhibitor; 50 M), Ko143 (1 M), and GF120918 (2 M) ($p < 0.05$). However, neither the $P_{app}$ value for A-to-B nor the $P_{app}$ value for B-to-A of MTX was significantly affected after coincubation with LY335979 (5 M).
The B-to-A efflux of rhodamine 123, which is a substrate for P-gp and mutant BCRP, was significantly decreased in the presence of LY335979 and GF120918 (p < 0.05), but was not changed by coincubating with Ko143 (1 μM) (Fig. 7).

Discussion

Western blot analysis of Caco-2 cell lysates demonstrates the presence of both monomer (∼70 kDa) and homodimer (∼140 kDa) of BCRP in the Caco-2 cells (Fig. 1A). The 140-kDa BCRP complex dissociated to 70-kDa polypeptides in the presence of the reducing agent dithiothreitol, indicating that the BCRP dimer was linked by disulfide bonds. This finding is consistent with the recent discovery by Kage et al. (2002). The same group also demonstrates the necessity of homodimerization for the BCRP function when using a dominant-negative mutation of BCRP with a L554P alteration in the fifth transmembrane domain (Kage et al., 2002).

Immunohistochemical analysis of Caco-2 cells reveals the presence of BCRP on apical and basolateral plasma membranes as well as in the cytoplasm prior to cell confluence (Fig. 2). Upon cell confluence, BCRP was sorted to the “apical membrane” consistent with potential intracellular localization and redistribution of BCRP to the plasma membrane shown in BCRP-overexpressed MCF-7 AdVp3000 and S1-M1-80 drug-resistant cells (Litman et al., 2000). BCRP-transfected MDCK or LLC-PK epithelial cells also exhibited polarized apical localization of BCRP (Jonker et al., 2000; Maliepaard et al., 2001), implying that BCRP may contain a certain endogenous sorting signal, which was recognized by epithelial cells (Jonker et al., 2000; Imai et al., 2003). The polarized apical BCRP distribution in Caco-2 cells continues during the cell differentiation period, which is consistent with the proposed role for BCRP as a secretory detoxifying transporter, contributing to the gastrointestinal epithelial barrier. Results from transport function assays using E3S as the BCRP substrate agreed with immunohistochemical study results. The directional transport of E3S across Caco-2 monolayers was observed immediately after cells reached confluence (5-day culture) and continued throughout the differentiation phase (day 12–19 cell culture) (Table 1). In view of the fact that total BCRP protein expression had not changed over day 5, day 12, and day 19 (Fig. 1B), the higher A-to-B transport and lower B-to-A transport of E3S across Caco-2 cells on day 5, compared with those on day 12 and day 19, indicated that BCRP continuously redistributed to the apical membrane during the differentiation phase.

Similar to P-gp, MRP, and lung-resistant protein (Yu and Sinko, 1997), the expression of BCRP is passage-dependent. The expression
of BCRP increased approximately 3-fold from the early passage to the late passage when Caco-2 cells were cultured in T-flasks, but decreased about 3- to 10-fold for monomer and dimer, respectively, from the early passage to the late passage when Caco-2 cells were grown on Transwell plates (Fig. 1, B and C). Although our findings are novel, they are supported by the previous reports that culture conditions and cell-growing matrices may affect efflux pump protein expression (Yu and Sinko, 1997). The BCRP-mediated E3S efflux in the day 12 p59 Caco-2 cells was much lower than that of day 12 p36 Caco-2 cells, with the B/A ratio of 2 and 30, respectively (Fig. 3), indicating less BCRP expression, particularly by the dimer, which is responsible for decreased efflux pump function. The low efflux function of BCRP was also seen in day 19 culture of passages 56, 59, and 72 (data not shown). The increased A-to-B transport of E3S in p59 was likely due to the decreased tight junctions, reflected by the low TEER value and multiple layers formed as a result of cells grown on Transwell plates (Fig. 2B).

The polarized expression of BCRP in Caco-2 cells is consistent with the net secretory transport (B/A ratio >8) of E3S, a prototypical substrate of BCRP. Although there is a good agreement between the morphological and the functional measurements, the vectorial transport of E3S attributed to the BCRP expression in Caco-2 cells was further supported by the reduction of E3S efflux by the BCRP inhibitors Ko143, GF120918, and prazocin (Fig. 4). Ko143, a specific inhibitor of BCRP (Allen et al., 2002b), totally inhibited the E3S efflux and resulted in almost equal $P_{app}$ values for A-to-B and B-to-A of E3S across Caco-2 cell monolayers at the concentration of 1 μM. Under the same experimental conditions, Ko143 did not change the efflux properties of paclitaxel (a P-gp substrate) and vinblastine (a substrate for P-gp and MRP) in Caco-2 cells (data not shown), indicating that Ko143, at the concentration of 1 μM, did not inhibit P-gp and MRP. P-gp and BCRP inhibitors GF120918 and prazocin (Jonker et al., 2000; Cisternino et al., 2004) completely abolished the efflux of E3S. In contrast, the potent P-gp-specific inhibitor, LY335979 (Shepard et al., 2003), did not change A-to-B or B-to-A transport of E3S. Altogether, the modulation of GF120918 and prazocin on the E3S efflux across Caco-2 cells was due to the inhibition of BCRP. The uncoupling agent 2,4-DNP, increased the A-to-B over B-to-A of E3S from 26.7 to 0.6, suggesting that blocking the mitochondrial ATP synthesis and depleting the cellular energy abolished the BCRP efflux activity. (Data expressed as mean ± S.E.M., n = 3.)

The observed $K_a$ of E3S for BCRP in Caco-2 cells was 13.2 μM, which was close to the $K_a$ of E3S determined by the BCRP vesicle studies. The $V_{max}$ was estimated as 10.8 pmol/s. (Data expressed as mean ± S.E.M., n = 3.)

### Table 1

<table>
<thead>
<tr>
<th>Cell passage No.</th>
<th>P36</th>
<th>P59</th>
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<tr>
<td>TEER (ohm.cm²) (n=6)</td>
<td>461 ± 97</td>
<td>79 ± 12</td>
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### Figure 3

**Fig. 3.** BCRP-mediated efflux of E3S in Caco-2 cells of passages 36 and 59. Ko143 (an inhibitor for BCRP) was used at the concentration of 1 μM. The transport study was conducted on Caco-2 cell monolayers 12 days after seeding on the Transwell plates. Results obtained from the p36 and p59 Caco-2 cells were labeled as p36 and p59, respectively. (Data expressed as mean ± S.E.M., n = 3.)

### Figure 4

**Fig. 4.** The effects of efflux pump inhibitors on the transport of E3S. LY335979 (5 μM), a P-gp-specific inhibitor, did not affect the E3S permeability. However, GF120918 (2 μM) (an inhibitor for P-gp and BCRP), prazocin (100 μM) (an inhibitor for P-gp and BCRP), and Ko143 (1 μM) (a BCRP inhibitor) increased A-to-B transport and decreased B-to-A transport of E3S, indicating that BCRP pumps E3S out of Caco-2 cells. The uncoupling agent 2,4-DNP changed the ratio of B-to-A over B-to-A of E3S from 26.7 to 0.6, suggesting that blocking the mitochondrial ATP synthesis and depleting the cellular energy abolished the BCRP efflux activity. (Data expressed as mean ± S.E.M., n = 3.)

### Figure 5

**Fig. 5.** B-to-A transport rate of E3S in the presence or absence of Ko143 and net flux of E3S across Caco-2 cells. The efflux of E3S was saturable and the $K_m$ of E3S expressed as mean ± S.E.M., n = 3.)
transport of rhodamine 123, indicating that P-gp but not BCRP mediated the transport of MTX (in the presence of MK571 (a MRP inhibitor; Leie et al., 1994) (Fig. 6). Ko143 (a BCRP inhibitor) and GF120918 (an inhibitor for P-gp and BCRP) but not Ko143 (a MRP inhibitor) decreased the B-to-A transport of rhodamine 123, indicating that P-gp but not BCRP mediated the rhodamine efflux across Caco-2 cells. (Data expressed as mean ± S.E.M., n = 3.)

FIG. 6. The effects of efflux pump inhibitors on the transport of MTX. LY335979 (5 μM), a P-gp-specific inhibitor, did not affect the MTX permeability. However, GF120918 (2 μM) (an inhibitor for P-gp and BCRP), Ko143 (1 μM) (a BCRP inhibitor), and MK571 (50 μM) (a MRP inhibitor) decreased the B-to-A transport of MTX, indicating that both BCRP and MRP mediated the MTX efflux across Caco-2 cells. (Data expressed as mean ± S.E.M., n = 3.)

FIG. 7. The effects of efflux pump inhibitors on the transport of rhodamine 123. LY335979 (5 μM) (a P-gp-specific inhibitor) and GF120918 (2 μM) (an inhibitor for P-gp and BCRP) but not Ko143 (1 μM) (a BCRP inhibitor) decreased the B-to-A transport of rhodamine 123, indicating that P-gp but not BCRP mediated the rhodamine efflux across Caco-2 cells. (Data expressed as mean ± S.E.M., n = 3.)
gated using MTX, a substrate for MRP and BCRP (Volk and Schneider, 2003) (Fig. 6). Ko143 (a BCRP inhibitor) and GF120918 (an inhibitor for BCRP and P-gp) but not LY335979 (a P-gp inhibitor) reduced the B-to-A transport of MTX across Caco-2 cell monolayers, implying that BCRP is present in Caco-2 cells. The decreased B-to-A transport of MTX in the presence of MK571 (a MRP inhibitor; Leie et al., 1994) was due to the inhibition of MRP2. All MRP or BCRP inhibitors affected the secretory transport (B-to-A) of MTX or E3S more than the absorptive transport (A-to-B), suggesting the presence of other active transporter(s), such as OAT or OATP, on the basolateral side responsible for the uptake of MTX or E3S (Cha et al., 2001; Nezu et al., 2001; Troutman and Thakker, 2003). Since Ko143 did not inhibit E3S uptake in hOATP2- and hOAT3-expressed xenopus oocytes up to 10 μM (data not shown), Ko143-mediated E3S and MTX efflux in Caco-2 cells could be totally attributed to BCRP.

BCRP mutations have been found in some cancer cell lines (Honjo et al., 2001). The mutation has either threonine (T) or glycine (G), instead of arginine (R), at the amino acid position 482. The R482 of BCRP locates in the third transmembrane domain and may alter the substrate specificity upon mutation. The BCRP-mediated efflux of MTX in our Caco-2 cells indicated that the BCRP expressed on Caco-2 cells might be the wild type because R482T and R482G mutation were unable to transport MTX to any extent (Chen et al., 2003). The conclusion is further demonstrated by the efflux of rhodamine 123, to which BCRP-mediated confinement is observed in the R482T or R482G mutation but not the wild-type expressed cell lines (Allen et al., 2002a). In Caco-2 cells, the efflux of rhodamine 123 could be totally abolished by P-gp inhibitors, LY335979 and GF120918, but not affected by the BCRP inhibitor, Ko143 (Fig. 7). In conclusion, both BCRP monomer and dimer are expressed in the Caco-2 cells. BCRP is polarized at the apical side of Caco-2 cells and can efficiently transport its substrate, such as E3S and MTX, out of cells. Therefore, besides BCRP-transfected cell lines, Caco-2 cells can also be used as an in vitro model to study the transport function of BCRP.

Acknowledgments. We thank Dr. Shimoga Prakash for providing us GF120918 and LY335979. Ko143 was kindly provided by Dr. Alfred Schinkel. We also thank Vilmos Csizmadia and Bei-Ching Chuang for technical support.

References


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