Role of Breast Cancer Resistance Protein in the Bioavailability and Fetal Penetration of Topotecan


Background and Methods: Breast cancer resistance protein (BCRP/MXR/ABCP) is a multidrug-resistance protein that is a member of the adenosine triphosphate-binding cassette family of drug transporters. BCRP can render tumor cells resistant to the anticancer drugs topotecan, mitoxantrone, doxorubicin, and dactinomycin. To investigate the physiologic role of BCRP, we used polarized mammalian cell lines to determine the direction of BCRP drug transport. We also used the BCRP inhibitor GF120918 to assess the role of BCRP in protecting mice against xenobiotic drugs. Bcrp1, the murine homologue of BCRP, was expressed in the polarized mammalian cell lines LLC-PK1 and MDCK-II, and the direction of Bcrp1-mediated transport of topotecan and mitoxantrone was determined. To avoid the confounding drug transport provided by P-glycoprotein (P-gp), the roles of Bcrp1 in the bioavailability of topotecan and the effect of GF120918 were studied in both wild-type and P-gp-deficient mice and their fetuses. Results: Bcrp1 mediated apically directed transport of drugs in polarized cell lines. When both topotecan and GF120918 were administered orally, the bioavailability (i.e., the extent to which a drug becomes available to a target tissue after administration) of topotecan in plasma was dramatically increased in P-gp-deficient mice (greater than sixfold) and wild-type mice (greater than ninefold), compared with the control (i.e., vehicle-treated) mice. Furthermore, treatment with GF120918 decreased plasma clearance and hepatobiliary excretion of topotecan and increased (re-)uptake by the small intestine. In pregnant GF120918-treated, P-gp-deficient mice, relative fetal penetration of topotecan was twofold higher than that in pregnant vehicle-treated mice, suggesting a function for BCRP in the maternal–fetal barrier of the placenta. Conclusions: Bcrp1 mediates apically directed drug transport, appears to reduce drug bioavailability, and protects fetuses against drugs. We propose that strategic application of BCRP inhibitors may thus lead to more effective oral chemotherapy with topotecan or other BCRP substrate drugs. [J Natl Cancer Inst 2000;92:1651–6]

After a period of treatment with a single cytotoxic drug, breast cancer cells can become resistant to multiple drugs, a phenomenon known as multidrug resistance. Several mechanisms of multidrug resistance have been identified, including the overexpression of P-glycoprotein (P-gp) and MRP1, which are members of the adenosine triphosphate-binding cassette (ABC) superfamily of transport proteins that are situated in the plasma membrane and can actively transport drugs out of the cell (1–3). Recently, breast cancer resistance protein (BCRP) (also known as mitoxantrone resistance protein [MXR] and placenta-specific ABC transporter [ABCP]), a new member of this superfamily involved in multidrug resistance, was identified in an MCF-7 breast cancer cell subline that was selected for resistance to doxorubicin. This BCRP-overexpressing cell line was markedly cross-resistant to mitoxantrone and daunorubicin (4,5). Subsequently, several other groups (6–8) have shown overexpression of BCRP or its murine homologue, Bcrp1, in cell lines selected for resistance to the anticancer agents mitoxantrone, doxorubicin, and topotecan. BCRP-mediated drug resistance could be effectively reversed by GF120918 (a P-gp inhibitor) in human (9) and murine (7) cell lines.

In this study, we investigate the direction of BCRP-mediated drug transport in various polarized cell lines and determine the role of BCRP in protecting mice against xenobiotic drugs (10), by using the efficient BCRP inhibitor GF120918.

Materials and Methods

Animals

The mice were housed and handled according to institutional guidelines and Dutch laws. For all experiments, the animals used were male mdr1a/1b(−/−) or wild-type mice of a 99% FVB genetic background; they were 9–14 weeks of age. The mice were kept in a temperature-controlled environment with a 12-hour light–12-hour dark cycle and were given a standard diet (AM-III; Hope Farms, Woerden, The Netherlands) and acidified water ad libitum.

Reagents

Topotecan (Hycamtin®) and [14C]topotecan (56 Ci/mol) were from SmithKline Beecham Pharmaceuticals (King of Prussia, PA). GF120918 was from Glaxo Wellcome (Research Triangle Park, NC). Ketamine (Ketalar®) was from Parke-Davis (Hoofddorp, The Netherlands). Xylazine was from Sigma Chemical Co. (St. Louis, MO). Methoxyflurane (Metofane®) was from Mallinckrodt Veterinary, Inc. (Mundelein, IL). All other compounds used were reagent grade.

Drug Preparation, Administration, and Analysis

GF120918 was suspended at 5 mg/mL in a mixture of hydroxypropyl methylcellulose (10 g/L)/2% (vol/vol) Tween 80/H2O (0.5:1:98.5 [vol/vol]) for oral administration. Animals, lightly anesthetized with methoxyflurane, were administered GF120918 (50 mg/kg; 10 μL of drug solution/g body weight) or a corresponding amount of vehicle by gavage into the stomach. Topotecan (0.2 mg/mL) (freshly prepared in 5% [wt/vol] d-glucose; 5 μL/g body weight) was administered orally at a dose of 1.0 mg/kg body weight. For intravenous administration, topotecan or, where indicated, [14C]topotecan at 5 μL of drug solution/g body weight was injected into the tail vein of mice lightly anesthetized with methoxyflurane. Animals were killed by cardiac puncture or axillary bleeding after being anesthetized with methoxyflurane, and their blood was collected. Heparinized plasma was dramatically increased in P-gp-deficient mice (greater than sixfold) and wild-type mice (greater than ninefold), compared with the control (i.e., vehicle-treated) mice. Furthermore, treatment with GF120918 decreased plasma clearance and hepatobiliary excretion of topotecan and increased (re-)uptake by the small intestine. In pregnant GF120918-treated, P-gp-deficient mice, relative fetal penetration of topotecan was twofold higher than that in pregnant vehicle-treated mice, suggesting a function for BCRP in the maternal–fetal barrier of the placenta. Conclusions: Bcrp1 mediated apically directed drug transport, appears to reduce drug bioavailability, and protects fetuses against drugs. We propose that strategic application of BCRP inhibitors may thus lead to more effective oral chemotherapy with topotecan or other BCRP substrate drugs.

After a period of treatment with a single cytotoxic drug, cancer cells can become resistant to multiple drugs, a phenomenon known as multidrug resistance. Several mechanisms of multidrug resistance have been identified, including the overexpression of P-glycoprotein (P-gp) and MRP1, which are members of the adenosine triphosphate-binding cassette (ABC) superfamily of transport proteins that are situated in the plasma membrane and can actively transport drugs out of the cell (1–3). Recently, breast cancer resistance protein (BCRP) (also known as mitoxantrone resistance protein [MXR] and placenta-specific ABC transporter [ABCP]), a new member of this superfamily involved in multidrug resistance, was identified in an MCF-7...
plasma was mixed with three volumes of ice-cold methanol (−20 °C). Their organs were removed and subsequently homogenized in 4% (wt/vol) bovine serum albumin. Where applicable, the intestinal content was separated from the intestinal tissue before homogenization. Radioactivity in homogenates was determined as described previously (11). Because topotecan is hardly metabolized in vivo, the amounts of 14C reflect total unchanged levels of topotecan (12). The total topotecan levels (lactone plus carboxylate form) in plasma were determined by high-pressure liquid chromatography as described earlier (13). The area under the plasma concentration–time curve (AUC) was calculated (from 0 to 4 hours for oral administration and from 1 minute to 4 hours for intravenous administration) by use of the linear trapezoidal rule. Plasma bioavailability (i.e., the extent to which a drug becomes available in plasma after administration) of administered drug was determined as the ratio of the AUC after oral and intravenous administrations. For gallbladder cannulation experiments, mice were anesthetized and cannulated as described previously (14). Anesthetics, a combination of ketamine (100 mg/kg) and xylazine (6.7 mg/kg), were injected intraperitoneally into the mice at 2.33 μL/g body weight.

Ribonuclease Protection Analysis

Total RNA was isolated from mouse tissues by use of the TRIZol® reagent (Life Technologies, Inc. [GIBCO BRL], Rockville, MD), according to the manufacturer’s instructions. Ribonuclease (RNase) protection assays were performed, as described previously (15), with 10 μg of total RNA per sample. A mouse probe for bcrp1 was made by cloning a 405-nucleotide (nt) polymerase chain reaction fragment (positions 1554–1959 relative to the translation start) into the pGEM-T vector (Promega Corp., Madison, WI). After the vector was linearized with restriction endonuclease EcoRI, a 280-nt antisense RNA probe was generated by transcription with SP6 RNA polymerase, yielding a protected probe fragment of 205 nt.

Expression of Full-Length Mouse bcrp1 Complementary DNA in LLC-PK1 and MDCK-II Cells

The full-length mouse bcrp1 complementary DNA (cDNA) (7) was excised from pBluescript KS with Smal and NotI and was cloned into the LZRS-MSIRES-GFP expression vector between the SmalI and NotI sites (16). The resulting vector was a monocistronic construct containing bcrp1 followed by sequences for an internal ribosome entry site and the enhanced green fluorescent protein. This construct was transfected into the amphotropic Phoenix producer cell line (17) by use of the calcium phosphate precipitation method. Viral supernatants from these transfected cells were used to transduce LLC-PK1 or MDCK-II cells. Transduced clones were selected first for expression of the enhanced green fluorescent protein and then for the reduced accumulation of mitoxantrone by flow cytometry. The expression of bcrp1 cDNA in selected clones was determined by northern blot analysis.

Transport Assay

Transport assays were carried out as described earlier (14), with minor modifications. M199 medium containing t-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL), and fetal calf serum (10%) was used throughout. Cells were seeded on microporous polycarbonate membrane filters (3.0-μm pore size, 24.5-mm diameter, Transwell® [14C]topotecan (7 Ci/mmol) and 192 nM [3H]mitoxantrone when mouse bcrp1 was expressed in the polarized canine kidney cell line MDCK-II (data not shown). Thus, Bcrp1-mediated drug transport is apically directed in various polarized cells, which suggests that Bcrp1 is located apically in polarized epithelia, and can be effectively inhibited by GF120918.

Expression of bcrp1 Messenger RNA in Mouse Tissues

P-gp mediates apically directed drug transport in polarized cultured cells and the elimination of drugs by organs such as liver and intestine. P-gp also reduces the uptake of drugs from the intestine and prevents the accumulation of drugs in certain critical tissues and the fetus (10,19–21). To assess a possible pharmacologic role of Bcrp1, we first determined the tissue distribution of mouse bcrp1 by using RNase protection assays. Fig. 2, a shows that mouse bcrp1 is highly expressed in kidney and expressed more moderately in liver, colon, heart, spleen, and placenta. The moderate levels of bcrp1 expression in the mouse placenta contrast with the very high levels of BCRP expression previously observed in human placenta (4,22).

Effect of GF120918-Mediated Inhibition of Bcrp1 on the Pharmacokinetics of Topotecan in Mice

We studied the pharmacologic role of Bcrp1 in vivo in liver, intestine, kidney, and placenta by analyzing the effects of the Bcrp1 inhibitor GF120918, which is well tolerated by both
mice and humans ([23]; unpublished data), on the pharmacokinetics of topotecan. Because GF120918 inhibits both P-gp and Bcrp1, we used P-gp-deficient mdrla/1b(−/−) mice (10) to exclude any confounding effects of P-gp inhibition. Comparison of the expression of bcrp1 in several organs between wild-type and mdrla/1b(−/−) mice established that expression of bcrp1 was not increased by the loss of P-gp (Fig. 2, b). To study topotecan bioavailability, we administered GF120918 or vehicle orally to mdrla/1b(−/−) mice 15 minutes before oral or intravenous administration of topotecan, and we determined the plasma concentration of topotecan as a function of time (Fig. 3, a and c). In GF120918-treated animals, the bioavailability of topotecan given orally, as measured by the AUC, was more than sixfold higher than that in vehicle-treated animals (596 ± 62 versus 96 ± 18 hours · mg/L; *P* < .001; Fig. 3, a). In GF120918-treated animals, the bioavailability of topotecan given intravenously increased about twofold (406 ± 25 versus 200 ± 29 hours · mg/L; *P* < .001; Fig. 3, c). Taking the bioavailability of intravenously administered topotecan in vehicle-treated mice as 100%, the bioavailability of topotecan administered orally was 48% ± 9% in vehicle-treated mice and 299% ± 31% in GF120918-treated mice. Thus, Bcrp1 appears to be a major determinant for the bioavailability of topotecan that is administered orally.

Topotecan is a weak to moderate substrate for P-gp (24). Because the bioavailability of topotecan administered orally to

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**Fig. 1.** Transepithelial transport of [14C]topotecan (10 μM) in LLC-PK1 (a and c) and L-Bcrp1.1 (a representative clone) (b and d) monolayers. The experiment was started with the addition of [14C]topotecan to one compartment (basal or apical) of the culture dish. After 0.5, 2, 4, and 6 hours, the percentage of radioactivity appearing in the opposite compartment was measured and plotted. The P-glycoprotein (P-gp) inhibitor PSC 833 (a and b) or the breast cancer resistance protein (BCRP)/P-gp inhibitor GF120918 (c and d) was present as indicated. Results are the means; the error bars indicate the standard deviations (n = 3). Transport from the apical to the basal sides and from the basal to the apical sides (at 6 hours) was only significantly different for L-Bcrp1.1 cells treated with 10 μM PSC 833 (*P* = .002; two-sided Student’s *t* test).

**Fig. 2.** Expression of the murine breast cancer resistance protein (bcrp1) messenger RNA in mouse tissues. Ribonuclease protection analysis was performed with 10 μg of total RNA/sample. a) bcrp1 expression in mdrla/1b(−/−) mouse tissues: colon (lane 1), cecum (lane 2), heart (lane 3), lung (lane 4), skeletal muscle (lane 5), thymus (lane 6), stomach (lane 7), epididymis (lane 8), brain (lane 9), liver (lane 10), kidney (lane 11), spleen (lane 12), placenta (lane 13), and uterus (lane 14). b) Comparison of bcrp1 expression between wild-type (wt) and mdrla/1b(−/−) (ko) mice: wt brain (lane 15), ko brain (lane 16), wt liver (lane 17), ko liver (lane 18), wt kidney (lane 19), ko kidney (lane 20), wt spleen (lane 21), ko spleen (lane 22), wt small intestine (lane 23), and ko small intestine (lane 24). Positions of bcrp1- and Gapdh (i.e., glyceraldehyde-3-phosphate dehydrogenase)-protected RNA fragments are indicated. The Gapdh expression differs between tissues and is used to compare amounts of RNA from the same tissues from mice with different genotypes.
vehicle-treated wild-type mice (41 ± 7 hours /H11080 mg/L) is two-fold lower than that in vehicle-treated P-gp-deficient mdr1a/1b−/− mice (96 ± 18 hours /H11080 mg/L) (P < .001; compare lower curves in Fig. 3, a and b), P-gp also appears to have a role in the bioavailability of topotecan. When wild-type mice, clinically the most relevant model, were treated with GF120918, the bioavailability of topotecan given orally increased ninefold (381 ± 41 versus 41 ± 7 hours /H11080 mg/L; P < .001). This result indicates that inhibition of both Bcrp1 and P-gp by GF120918 has a strong effect on uptake of topotecan administered orally, although the resulting availability did not quite reach the level observed in GF120918-treated mdr1a/1b−/− mice (596 ± 62 hours /H11080 mg/L).

We next determined how GF120918 given orally affected the levels of topotecan excreted in the small intestine. GF120918 was administered orally to mdr1a/1b−/− mice; 15 minutes later, [14C]topotecan was administered intravenously; then 15 and 60 minutes later, the amount of [14C]topotecan excreted into the small intestine was measured. Fifteen and 60 minutes after [14C]topotecan was administered to GF120918-treated animals, the percentage of total [14C]topotecan in the small intestinal lumen was about twofold and threefold lower, respectively, and the plasma levels were about 1.5-fold and 2.5-fold higher compared with vehicle-treated animals (Table 1). These observations could reflect diminished excretion of topotecan into the small intestine and/or increased (re-)uptake from the small intestine, both caused by GF120918. To analyze this effect further, we separately determined the hepatobiliary, direct intestinal, and renal excretion of [14C]topotecan. For the measurement of hepatobiliary excretion, anesthetized mdr1a/1b−/− mice were given GF120918 or vehicle orally 15 minutes before [14C]topotecan administration and the amount of [14C]topotecan excreted in bile was determined at 10, 20, 30, 40, 50, and 60 minutes. Results are the means ± standard deviation (n = 3).

We next determined how GF120918 given orally affected the plasma levels of topotecan. Mdr1a/1b−/− (a) or wild-type (b) mice were given an oral dose of GF120918 (50 mg/kg) or vehicle 15 minutes before an oral dose of topotecan (1 mg/kg). Plasma levels of unchanged topotecan were determined by high-pressure liquid chromatography at 15, 30, 60, 120, and 240 minutes. Results are the means ± standard deviation (n > 3). e) Plasma topotecan concentration versus time curves for intravenously administered topotecan in mdr1a/1b−/− mice treated with GF120918 or vehicle. Mdr1a/1b mice received an oral dose of GF120918 (50 mg/kg) or vehicle 15 minutes before intravenously administered topotecan (1 mg/kg). Plasma levels of topotecan were determined at 1, 5, 15, 30, 60, 120, and 240 minutes. Results are the means ± standard deviation (n ≥ 3). f) Cumulative biliary excretion of topotecan. Bile was collected through a cannula in the gallbladder of mdr1a/1b−/− mice with a ligated common bile duct. Mice received an oral dose of GF120918 (50 mg/kg) or vehicle 15 minutes before topotecan (1 mg/kg) was administered intravenously. Levels of unchanged topotecan in bile were determined at 10, 20, 30, 40, 50, and 60 minutes. Results are the means ± standard deviation (n = 3).
null–fetal barrier. Our data strongly suggest that Bcrp1 is present and functional in the apical membrane of the intestinal epithelium, in the bile canalicular membrane, and in the membrane of placental trophoblasts that is in contact with the maternal circulation.

The highest levels of bcrp1 mRNA were found in the kidney, suggesting that Bcrp1 might play an important pharmacologic role in the renal excretion of substrate drugs. Our experiments measuring this renal excretion gave highly variable results between individual mice and were essentially not influenced by GF120918 (12.6% ± 7.9% with GF120918 and 18.0% ± 10.4% with vehicle). Studies in patients also have found high variability in renal elimination of topotecan (mean = 40%; range = 26%–80%) (25). A possible explanation for the high variability in renal excretion is that human and murine kidneys could have several transport mechanisms for topotecan that vary extensively among individuals.

Oral administration of drugs is highly preferred for its convenience and potential use on an outpatient basis. However, the therapeutic use of orally administered drugs is frequently limited by the poor and (consequently) highly variable drug bioavailability, factors that are largely determined by the extent to which the drugs are absorbed from the gut, metabolized, and excreted. The narrow therapeutic index of most anticancer drugs implies that this variability will frequently result in excessive toxicity or, conversely, in inadequate efficacy. For instance, for topotecan administered orally, the bioavailability in humans is moderate, with a high inter-patient variation (30% ± 7.7%) (26), and current chemotherapeutic schedules for topotecan are, therefore, mainly based on intravenous administration (27). Our findings suggest that, by combining topotecan administered orally with an effective BCRP (and P-gp) inhibitor, such as GF120918, the bioavailability of topotecan and thus its clinical usefulness might be dramatically improved. We should note that, based on these data, no conclusions can be made about whether the therapeutic index of topotecan (i.e., toxicity of topotecan for a tumor as opposed to its overall toxicity to the organism) is improved by GF120918. However, the ability to inhibit placental Bcrp1 with orally administered GF120918 suggests that a BCRP component of multidrug resistance in clinical tumors could also be blocked with GF120918 administered orally because the systemic exposure to GF120918 is apparently high enough.

Although we cannot exclude the possibility that other, as yet unidentified, GF120918-sensitive topotecan transporters are also contributing to the in vivo pharmacologic effects that we observed, the potential clinical application of GF120918 to improve the bioavailability of topotecan administered orally to patients should be pursued. In fact, we have started clinical trials to test whether it is feasible to increase the bioavailability of topotecan administered orally to patients by blocking BCRP with GF120918. If this procedure is successful in patients as well, it may prove to be applicable to other drugs transported by BCRP.

**REFERENCES**


**NOTES**

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