In vitro transport of gimatecan (7-t-butoxyiminomethylcamptothecin) by breast cancer resistance protein, P-glycoprotein, and multidrug resistance protein 2

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Abstract

Lipophilic camptothecin derivatives are considered to have negligible affinity for breast cancer resistance protein (BCRP; ABCG2). Gimatecan, a new orally available 7-t-butoxyiminomethyl–substituted lipophilic camptothecin derivative, has been previously reported to be not a substrate for BCRP. Using a panel of in vitro models, we tested whether gimatecan is a substrate for BCRP as well as for P-glycoprotein (MDR1) or multidrug resistance protein 2 (MRP2; ABCC2). ATP-binding cassette drug efflux transporters involved in anticancer drug resistance, and able to affect the pharmacokinetics of substrate drugs. Cell survival, drug transport, accumulation, and efflux were studied in IGROV1 and (human BCRP overexpressing) T8 cells, Madin-Darby canine kidney II (MDCKII-WT, MDCKII-Bcrp1, MDCKII-MDR1, and MDCKII-MRP2), and LLCPK (LLCPK-WT and LLCPK-MDR1) cells. Competition with methotrexate uptake was studied in Sf9-BCRP membrane vesicles. In vitro, expression of BCRP resulted in 8- to 10-fold resistance to gimatecan. In Transwell experiments, gimatecan was transported by Bcrp1 and transport was inhibited by the BCRP/P-glycoprotein inhibitors elacridar and pantoprazole. Efflux of gimatecan from MDCKII-Bcrp1 cells was faster than in WT cells. In Sf9-BCRP membrane vesicles, gimatecan significantly inhibited BCRP-mediated transport of methotrexate. In contrast, gimatecan was not transported by MDR1 or MRP2. Gimatecan is transported by BCRP/Bcrp1 in vitro, although to a lesser extent than the camptothecin analogue topotecan. Implications of BCRP expression in the gut for the oral development of gimatecan and the interaction between gimatecan and other BCRP substrate drugs and/or inhibitors warrant further clinical investigation. [Mol Cancer Ther 2007;6(12):3307–13]

Introduction

The ATP-binding cassette (ABC) drug efflux transporters breast cancer resistance protein (BCRP; ABCG2; ref. 1), P-glycoprotein (P-gp; MDR1 and ABCB1), and the multidrug resistance proteins (MRP) 1 to 5 (ABCC1-ABCC5) are involved in resistance against anticancer drugs (2, 3). Besides expression in various tumor tissues, these drug transporters are expressed in several normal tissues where they exert partly overlapping physiologic functions. P-gp and BCRP are highly expressed at the luminal side of the intestinal epithelial cells, in the bile canicular membrane, at the synctiotrophoblast, and at the vascular endothelial side of the blood-brain barrier (4, 5). These drug transporters mediate the active (i.e., ATP dependent) efflux of a wide range of chemical compounds with different physicochemical characteristics. In the intestine, blood-brain barrier, and placenta, these transporters have a protective role as they limit uptake from the intestinal lumen into the body, the penetration of compounds into the central nervous system, or the exposure of the fetus by limiting penetration through the placenta (4).

The camptothecin-derived topoisomerase I inhibitors are substrates for BCRP and P-gp (6). The affinity of the camptothecins for BCRP is for most compounds significantly higher than for P-gp. Usually, low cross-resistance is found with classic P-gp substrate drugs such as paclitaxel and docetaxel (7). However, the affinity for BCRP can vary substantially among the different derivatives of camptothecin (6). Topotecan and irinotecan, but especially SN38, the pharmacologically active metabolite of irinotecan, have high affinity for BCRP. They also have moderate affinity for P-gp. We previously reported that the camptothecin derivatives, which are substituted at the 7 position of the planar aromatic five-ring structure resulting in a more lipophilic molecule such as lurtotecan (GI147211, NX211)
and exatecan mesylate (DX-8951f), have less affinity for BCRP than topotecan and SN38 (6). It was suggested by others that the 7-oxyiminomethyl–substituted lipophilic camptothecin derivative gimatecan (ST1481, LBQ707) is not a substrate for BCRP (8, 9). However, based on our experience with a range of other camptothecin derivatives, we hypothesized that gimatecan might also be a substrate for BCRP. We tested this hypothesis in a panel of well-defined \textit{in vitro} models, including the BCRP-overexpressing human ovarian cancer cell line T8 (10), the Madin-Darby canine kidney II (MDCKII) epithelial cells stably expressing mouse Bcrp1 (11), and S9-BCRP membrane vesicles. We used elacridar and pantoprazole as inhibitors of BCRP and topotecan as positive control.

Moreover, we investigated whether gimatecan is a substrate of MRP2 and P-gp (MDR1) \textit{in vitro}.

Affinity of gimatecan for BCRP could be clinically relevant as oral bioavailability may be reduced by BCRP as shown for topotecan (12), and especially after oral administration, drug-drug interactions with other BCRP substrate drugs may take place.

Materials and Methods

\textit{In vitro Studies}

\textbf{Chemicals and Reagents.} [3H]inulin (0.78 Ci/mmol), [14C]inulin carboxylic acid (54 mCi/mmol), [14C]topotecan (SK&F 104864, 48 mCi/mmol), and [3H]methotrexate (5.9 Ci/mmol) were purchased from Amersham Biosciences. Topotecan (Hycamtin) was obtained from GlaxoSmithKline Pharmaceuticals. Gimatecan (ST1481, LBQ707) and [3H]gimatecan (40 Ci/mg) were provided by Novartis Pharmaceuticals, Inc. Pantoprazole (Pantozol, 40 mg; Altana Pharma) was obtained from the pharmacy of The Netherlands Cancer Institute (Amsterdam, the Netherlands). Elacridar (GF120918) was kindly provided by GlaxoSmithKline and zosuquidar (LY335979) was a generous gift from Dr. P. Multani (Kanisa Pharmaceuticals). All other chemicals and reagents were from Sigma and of analytic grade or better.

\textbf{Cell Lines.} Polarized MDCKII cells wild-type (WT) and transfected subclones stably expressing human MRP2 (ABCC2), human MDR1 (P-gp, ABCB1), or mouse Bcrp1 (Abcg2) cDNA were kindly provided by Dr. A.H. Schinkel (The Netherlands Cancer Institute) and described previously (11, 13). They were cultured in DMEM with Glutamax (Life Technologies, Inc.) supplemented with 100 IU/mL penicillin G, 100 μg/mL streptomycin sulfate, and 10% FCS (MP Biochemicals, ICN Biomedicals, Inc.). Bcrp1, MDR1, and MRP2 expression in the various transfected MDCKII sublines was checked by Western blot.

The IGROV1 human ovarian adenocarcinoma and the IGROV1-derived resistant T8 cell lines were cultured in RPMI 1640 supplemented with 25 mM/L HEPES, L-glutamine, 10% FCS, 100 IU/mL penicillin, and 100 μg/mL streptomycin. T8 cells were exposed to 950 nmol/L concentration of topotecan weekly for 1 h, which keeps the resistance level in T8 constant for at least 25 weeks (10, 14).

The polarized porcine kidney epithelial cells LLCPK-WT and LLCPK-MDR1, which were a generous gift from Dr. P. Borst (The Netherlands Cancer Institute), were described previously (15). They were cultured in M199 medium with L-glutamine (Life Technologies) and supplemented with penicillin G (100 IU/mL), streptomycin (100 μg/mL), and 10% (v/v) fetal bovine serum (MP Biochemicals, ICN Biomedicals Inc.).

All cell lines were grown at 37°C with 5% CO2 under humidifying conditions.

\textbf{Cytotoxicity Assays}

Exponentially growing cells were plated (1,000/200 μL per well for the MDCKII-WT, MDCKII-Bcrp1, and MDCKII-MRP2 cells; 1,500/200 μL per well for the MDCKII-MDR1 cells; and 5,000/200 μL per well for the IGROV1 and T8 cells) in 96-well microplates (Costar Corp.) and allowed to attach for 24 h at 37°C under 5% CO2. After this attachment period, 100 μL of drug solution (diluted in culture medium) were added to the wells, and cells were incubated for 72 h at 37°C under 5% CO2. Subsequently, the cytotoxicity was determined using the sulforhodamine B method as described previously (16).

In the combination experiments, elacridar (used as inhibitor of BCRP; however, it is also known as a P-gp inhibitor; ref. 17) was added 30 min before adding gimatecan or topotecan to obtain a final concentration of 500 nmol/L and 2 μmol/L in the MDCKII and IGROV1/T8 cell lines, respectively. The concentration of elacridar was lower than that in the transport experiments (5 μmol/L) to circumvent toxicity but sufficient to inhibit BCRP-mediated and P-gp–mediated transport.

Each agent (and combination) was tested in quadruplicate in at least three independent experiments.

\textbf{Transport across MDCKII and LLCPK Monolayers.} Transepithelial transport assays were done in Costar Transwell plates with 3-μm pore membranes (Transwell 3414, Costar) as described previously (18, 19). In brief, cells (MDCKII-WT, MDCKII-Bcrp1, MDCKII-MRP2, MDCKII-MDR1, LLCPK-WT, and LLCPK-MDR1) were seeded at a density of 1 × 10^6 in 2 mL of complete medium. Cells were grown for 3 days and allowed to form tight monolayers, with medium replacement every day. Two hours before the start of the experiment, complete medium at both sides of the monolayer (apical and basolateral compartments) was replaced by 2.5 mL of (serum-free) Opti-MEM medium (Life Technologies) containing the appropriate concentration of transport modulator (5 μmol/L/200 μmol/L for endogenous P-gp and/or 500 μmol/L pantoprazole or 5 μmol/L elacridar to inhibit endogenous P-gp and BCRP).

At t = 0, 2.5 mL of transport medium supplemented with zosuquidar (5 μmol/L) and without (control) or with elacridar (5 μmol/L) or pantoprazole (500 μmol/L) was applied at both sides of the monolayers, whereas radiolabeled drug ([3H]gimatecan, 1 μmol/L; [14C]topotecan, 5 μmol/L) and radiolabeled inulin ([14C]inulin or [3H]inulin; to check the integrity of the monolayer) were added to the apical or basolateral side of the monolayer in different wells. After 1 and 4 h, samples of 500 μL were taken and the amount of
[\text{[3H]}\text{gimatecan} \text{ or [14C]}\text{topotecan} \text{ appearing in the compartment (apical or basal) opposite to which the labeled drug was added was measured by liquid scintillation counting (Tri-Carb 2100 CA Liquid Scintillation Analyzer, Canberra Packard). Transepithelial transport of the drug and paracellular inulin flux through the monolayer was expressed as percentage of total radioactivity added at the beginning of the experiment. Inulin leakage was tolerated up to 2% of the total radioactivity over 4 h.}

**Accumulation and Efflux Studies.** Intracellular accumulation and efflux of gimatecan were measured in MDCKII-WT and MDCKII-Bcrp1 cell lines. Cells were seeded at a density of $1 \times 10^6$ in cell culturing plates (4.8 cm; Costar) in 5 mL of complete medium and grown to about 80% to 90% confluence. Then, plates were incubated for 30 min at 37°C with 5 mL of complete medium buffered with HEPES (25 mmol/L), adjusted to pH 7.0 and containing 0, 1, 1.5, and 2 μmol/L of [\text{[3H]}\text{gimatecan}]. After incubation, cells were washed twice with ice-cold PBS, scraped immediately, collected in plastic tubes, and centrifuged (2 min, 1,300 rpm, 0°C). Subsequently, the cells were resuspended in 1 mL of acetic acid 0.1% to lyse the cells. Protein concentrations were determined using the Bio-Rad assay based on the Bradford method (20). The concentration of gimatecan in the samples was determined by scintillation counting.

For efflux studies, MDCKII-WT and MDCKII-Bcrp1 cells were loaded with 1.5 and 2 μmol/L of [\text{[3H]}\text{gimatecan}], respectively, for 30 min at 37°C to obtain approximately equal intracellular concentrations of the drug. After loading the cells, medium was removed and replaced by fresh medium. Directly after incubation and at several following time points, intracellular concentrations of gimatecan were determined.

Efflux experiments were also done in the presence of elacridar (5 μmol/L).

Accumulation and efflux of gimatecan were determined in at least three independent experiments.

**Preparation of Membrane Vesicles and Competition Experiments.** Inside-out membrane vesicles from Spodoptera frugiperda (Sf9) cells were prepared as described previously (18). Using Sf9-WT and Sf9-BCRP membrane vesicles, we evaluated the effect of gimatecan on the transport of 0.31 μmol/L methotrexate, a well-known BCRP substrate, in the presence of 4 mmol/L ATP. Sf9-WT and Sf9-BCRP membrane vesicles were incubated with 0.31 μmol/L [\text{[3H]}\text{methotrexate} for 5 min at 37°C in the presence or absence of different concentrations (0.01, 0.1, and 2 μmol/L) of gimatecan. The ATP-dependent transport is plotted as percentage of the control value. Of note, all the experiments were done in the presence and absence of ATP.

**Statistical Analysis.** Statistical analysis was done using Student’s t test (two tailed, unpaired). Differences between two sets of data were considered statistically significant at $P < 0.05$.

### Results

**Reduced Cytotoxicity of Gimatecan by BCRP Expression**

A significant difference in IC50 of gimatecan was found between MDCKII-WT and MDCKII-Bcrp1 cells, with a resistance index (RI) of 8.4 ($P < 0.005$). A significant difference in IC50 with a RI of 10.4 ($P < 0.005$) was also seen in the same assay using the IGROV1 and T8 cell lines, indicating that BCRP expression resulted in resistance to gimatecan. Topotecan was chosen as reference drug (9, 10). The RI of topotecan in MDCKII-Bcrp1 was 83 and, in the T8 cell line, 148, in line with previous publications (10), and substantially higher than the RI of gimatecan in these cell lines.

In the applied cell lines, gimatecan showed a markedly higher cytotoxicity than topotecan (Table 1).

To further show the role of BCRP/Bcrp1 in the resistance to gimatecan, the cytotoxicity assays were repeated in the presence of elacridar, an inhibitor of BCRP as well as of P-gp (6, 17). The cytotoxicity of gimatecan in the MDCKII-WT and IGROV1 was not significantly ($P > 0.05$) affected by coincubation with a nontoxic dose of elacridar (500 nmol/L and 2 μmol/L, respectively).

<table>
<thead>
<tr>
<th></th>
<th>IGROV1</th>
<th>T8</th>
<th>MDCKII-WT</th>
<th>MDCKII-Bcrp1</th>
<th>MDCKII-MDR1</th>
<th>MDCKII-MRP2</th>
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<tr>
<td><strong>IC50 (nmol/L)</strong></td>
<td></td>
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<td><strong>RI</strong></td>
<td></td>
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<tr>
<td>Topotecan</td>
<td>33 ± 5</td>
<td>NA</td>
<td>4,867 ± 337</td>
<td>148 (^{1,1})</td>
<td>122 ± 11</td>
<td>NA</td>
</tr>
<tr>
<td>Gimatecan</td>
<td>3 ± 1</td>
<td>NA</td>
<td>33 ± 4</td>
<td>10.4 (^{1})</td>
<td>8.6 ± 3.6</td>
<td>NA</td>
</tr>
<tr>
<td>Topotecan +</td>
<td>42 ± 11</td>
<td>0.8(^1)</td>
<td>152 ± 21</td>
<td>32 (^{1,1})</td>
<td>143 ± 43</td>
<td>0.8(^{1})</td>
</tr>
<tr>
<td>Gimatecan + elacridar</td>
<td>3.8 ± 1.7</td>
<td>0.8(^1)</td>
<td>10.2 ± 1</td>
<td>3 (^{1,1})</td>
<td>9.2 ± 2</td>
<td>0.9(^1)</td>
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Abbreviation: NA, not applicable.

\(^1\)Assessed by sulforhodamine B cytotoxicity assay after 72 h of drug exposure. Values are the mean ± SD of at least three experiments.

\(^2\)Significant difference ($P < 0.05$).

\(^3\)Ratio between the IC50 values of the resistant and WT cell lines.

\(^4\)Ratio between the IC50 values in the absence and presence of elacridar.
gimatecan in the MDCKII-Bcrp1 and T8 cell lines, yielding an IC50 ratio without/with elacridar of 6.5 and 3, respectively ($P < 0.05$; Table 1).

**Cytotoxicity of Gimatecan Is Not Affected by P-gp and MRP2**

In contrast to the results obtained in BCRP/Bcrp1-overexpressing cells, no significant difference in IC50 of gimatecan was found between MDCKII-WT, MDCKII-MDR1, and MDCKII-MRP2 cell lines ($P > 0.05$; Table 1).

**Transport of Gimatecan across MDCKII Monolayers**

Transport of gimatecan by Bcrp1 was studied in MDCKII-WT and MDCKII-Bcrp1 cell monolayers. To exclude any contribution of P-gp, the P-gp inhibitor zosuquidar (LY335979, 5 μmol/L) was added. An increased transport of gimatecan (1 μmol/L) from the basolateral to the apical compartments compared with the transport from the apical to the basolateral compartments [i.e., active transport (basolateral to apical/apical to basolateral is 3.1 ± 0.46)] was observed in MDCKII-Bcrp1 compared with the WT cell line (basolateral to apical/apical to basolateral is 0.94 ± 0.08; Fig. 1). Moreover, gimatecan transport was completely abolished in MDCKII-Bcrp1 monolayers in the presence of the BCRP/P-gp inhibitors elacridar (5 μmol/L) or pantoprazole (500 μmol/L; refs. 6, 18, 21; Fig. 1).

Transwell experiments using topotecan as control drug have also been done: the results were in line with previous publications (11) and showed active transport of topotecan (data not shown). The magnitude of topotecan transport was of the same order as of gimatecan.

In contrast, no transport was found for gimatecan in Transwell experiments done with MDCKII-MRP2, MDCKII-MDR1, LLCPK-WT, and LLCPK-MDR1 monolayers (data not shown).

**Accumulation and Efflux of Gimatecan in Bcrp1-Overexpressing Cell Lines**

To further elucidate the effect of BCRP/Bcrp1 overexpression on cellular transport of gimatecan, we did accumulation and efflux experiments in MDCKII-WT and MDCKII-Bcrp1 cell lines. The accumulation and efflux of gimatecan could not be tested at concentrations higher than 2 μmol/L due to limited drug solubility.

Accumulation of gimatecan was ~1.5-fold reduced in the MDCKII-Bcrp1 compared with WT cell line (data not shown).

In efflux studies, a significantly increased initial efflux rate of gimatecan was observed in the MDCKII-Bcrp1 cells (~90%) within 1 min compared with WT cells (~30%; Fig. 2).

Cocubination of the cells with elacridar (5 μmol/L) completely restored the intracellular accumulation and efflux of gimatecan in MDCKII-Bcrp1 cells to the intracellular levels observed in the WT cell line. Efflux of gimatecan was not affected by coincubation with elacridar in MDCKII-WT cells (Fig. 2).

**Effect of Gimatecan on BCRP-Mediated Methotrexate Transport in Sf9 Membrane Vesicles**

Using Sf9-BCRP and Sf9-WT membrane vesicles, we studied the effect of different concentrations of gimatecan on the transport of 0.31 μmol/L of [3H]methotrexate. The ATP-dependent transport of methotrexate by human BCRP was inhibited by gimatecan in a concentration-dependent manner, showing competition between gimatecan and methotrexate for BCRP-mediated transport (Fig. 3). Control experiments have been done in Sf9-WT vesicles as well as in Sf9-BCRP and WT vesicles in the presence of pantoprazole, a competitive BCRP transport inhibitor; the results
observed were in line with previous publications (18) and further supported the competition of gimatecan for BCRP-mediated transport of methotrexate (data not shown).

**Discussion**

We tested the hypothesis that gimatecan is a substrate drug for BCRP/Bcrp1, P-gp, and MRP2 in vitro.

The first indication for affinity of BCRP/Bcrp1 for gimatecan was obtained in the cell survival studies using T8 and MDCKII-Bcrp1 cells. Compared with their parental counterparts, the BCRP-expressing cells showed 8.4-fold (T8) and 10.4-fold (MDCKII-Bcrp1) resistance to gimatecan. However, this RI is clearly lower than the resistance factor for topotecan of 148 in T8 and 83 in MDCKII-Bcrp1, respectively. Furthermore, coincubation with a nontoxic concentration of elacridar resulted in a partial reversal of the resistance to gimatecan. This suggests that BCRP/Bcrp1 is involved in the resistance to gimatecan in the two cell systems.

Results obtained in the transport studies with MDCKII-Bcrp1 versus MDCKII-WT cells reveal that there is active Bcrp1-mediated transport of gimatecan. The magnitude of the difference in basolateral to apical versus apical to basolateral transport of gimatecan was in the order of topotecan, which was used as control substrate drug for BCRP. This shows that the difference in the level of resistance to gimatecan and topotecan in the cell survival studies is not the same as the difference in the level of active transport in the MDCKII monolayer experiments. Further proof of active Bcrp1-mediated transport was obtained in the transport studies by coincubation with elacridar or pantoprazole, which collapsed the basolateral to apical/apical to basolateral curves completely.

Similar experiments conducted with LLCPK-MDR1, MDCKII-MDR1, and MDCKII-MRP2 showed that MDR1 and MRP2 do not mediate transport of gimatecan at detectable levels.

In addition, we determined the rate of efflux of gimatecan from loaded MDCKII-Bcrp1 and MDCKII-WT cells. The
results support that Bcrp1 mediates the efflux of gimatecan: the Bcrp1-expressing cells extruded gimatecan significantly faster than the parental cells. Finally, we tested the affinity of gimatecan for human BCRP in competition experiments using Sf9-BCRP vesicles. We could not test gimatecan itself in transport experiments in vesicles because gimatecan is a highly lipophilic drug. Consequently, it sticks to the applied filters in the assay. In competition experiments, we tested the ability of gimatecan to compete with methotrexate by BCRP in a concentration-dependent manner. Therefore, the applied in vitro assays revealed that BCRP is involved in resistance to and transport of gimatecan.

It is of interest that others have not found that gimatecan is transported by BCRP (8). However, in this previous study, another cell system was used, consisting of a human colon carcinoma cell line (HT29/MIT), selected by exposure of the parental (HT29) cell line to increasing concentrations of mitoxantrone, a well-known BCRP substrate. Although the selected HT29/MIT subline was checked for expression, along of BCRP, also of MDR1 and MRPI, expression of other ABC transporters and other mechanisms of resistance could have been induced as well. An overlap in substrate specificities between different ABC transporters induced by mitoxantrone may potentially have affected the reported resistance of the HT29/MIT cell line. For instance, MRP2 and MRP4 (not identified yet at the time that the previous experiments were done) have recently been reported to transport mitoxantrone and several camptothecins (particularly topotecan, irinotecan, and its metabolite SN38), respectively (22, 23). Moreover, in the previous studies, control experiments with BCRP inhibitors to reverse resistance and/or drug transport have not been done. This is relevant considering that recently it has been reported that inhibition of BCRP was not able to restore mitoxantrone sensitivity in irinotecan-selected human leukemia CPT-K5 cells (24). These findings support the hypothesis that induction of other transporters or other mechanisms besides the up-regulation of BCRP may contribute to the multidrug resistance phenotype of resistant cell sublines selected by increased exposure to substrate drugs. In our experiments, we used subclones of MDCKII cell stably transfected with the cDNA of Bcrp1, MDR1, and MRP2, respectively, making the expression of other transporters unlikely. Moreover, we have done control experiments using elacridar and/or pantoprazole as BCRP inhibitors: in the cytotoxicity (applying MDCKII-Bcrp1 and T8 cells) and Transwell (in MDCKII-Bcrp1 monolayers) assays, the BCRP inhibitors (elacridar and pantoprazole) were able to reverse the resistance and transport of gimatecan, respectively.

Moreover, the parental cells in the earlier experiments (8) seem to be much less sensitive to gimatecan than those used in our study and this could explain why in the previous studies the overexpression of BCRP had relatively little effect. Another reason for the discrepancy between our results and the results of other authors can be that the expression level of BCRP in the cell systems used was different. This hypothesis is supported by the relatively higher RI of topotecan observed in our cytotoxicity experiments in Bcrp1/BCRP-overexpressing cells (RI in MDCKII-Bcrp1 cells: 83; RI in T8 cells: 148) compared with the previous study (RI in HT29/MIT: 13.2; ref. 8). A lower BCRP expression in the HT29/MIT cells compared with our cell systems may have contributed to the different results. Finally, the authors in the previous study did not explore the efflux kinetics, which might have shown a significant difference between the resistant and parental cells nor have they studied transport in detail in monolayers of stably Bcrp1-overexpressing cells as the MDCKII cells that we developed and used.

In a subsequent article, Croce et al. (9) evaluated accumulation and efflux of gimatecan from parental and BCRP-overexpressing cells, but the experiments have been conducted at a high concentration (22 μmol/L) that most likely have resulted in precipitation of the drug. Moreover, the authors did not mention at which pH the experiments were done: this is relevant as the transport activity of BCRP has been recently reported to be affected by the pH (25). In our accumulation and efflux experiments, the medium with drug solution used was buffered with HEPES and the pH was adjusted. Finally, as hypothesized also for the other previous studies, a difference in expression of BCRP between the cell systems used may have also contributed to the discrepancy in results.

Conclusions

Our results reveal that in vitro gimatecan is a moderate substrate drug for mouse Bcrp1 as well as for human BCRP. The affinity for BCRP/Bcrp1 seems to be less than for topotecan that was used as control substrate drug.

Implications of BCRP expression in the gut for the oral development of gimatecan may be limited but need to be explored. The interaction between oral gimatecan and other BCRP substrate drugs and/or inhibitors warrants further clinical investigation.

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Molecular Cancer Therapeutics

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