The Role of Efflux and Uptake Transporters in N-[3-Chloro-4-[[3-fluorobenzyl]oxy]phenyl]-6-[5-[[2-(methylsulfonfyl)ethyl]amino]methyl]-2-furyl]-4-quinazolinamine (GW572016, Lapatinib) Disposition and Drug Interactions

Joseph W. Polli, Joan E. Humphreys, Kelly A. Harmon, Stephen Castellino, Michael J. O’Mara, Katie L. Olson, Lisa St. John-Williams, Kevin M. Koch, and Cosette J. Serabjit-Singh


ABSTRACT:

Lapatinib [N-[3-chloro-4-[[3-fluorobenzyl]oxy]phenyl]-6-[5-[[2-(methylsulfonfyl)ethyl]amino]methyl]-2-furyl]-4-quinazolinamine, GW572016, Tykerb] is a tyrosine kinase inhibitor approved for use in combination with capecitabine to treat advanced or metastatic breast cancers overexpressing HER2 (ErbB2). In this work we investigated the role of efflux and uptake transporters in lapatinib disposition and drug interactions. In vitro studies evaluated whether lapatinib is a substrate for efflux transporters or an inhibitor of efflux/uptake transporters. In vivo studies included whole-body autoradiography and an evaluation of the role of efflux transporters on the intestinal absorption and brain penetration of lapatinib using chemical or genetic knockout animals. Lapatinib is a substrate for the efflux transporters P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP). Furthermore, lapatinib is an inhibitor (IC50 values 0.025–5 μM) of Pgp, BCRP, and organic anion transporting polypeptide 1B1 (a hepatic uptake transporter). In contrast, lapatinib yielded little inhibition on renal transporters (organic anion transporters, organic cation transporters, and uric acid transporter). In vivo studies demonstrated that brain concentrations of lapatinib were low and influenced by efflux transporters at the blood-brain barrier. In contrast, systemic exposure of lapatinib after oral dosing was unchanged when efflux by Pgp and BCRP was absent from the gastrointestinal tract. These in vitro and in vivo preclinical investigations provide a mechanistic basis for elucidating clinical drug interactions.

Drug transporters can have a significant impact on the absorption, distribution, metabolism, excretion, and toxicity of a drug (Borst and Oude Elferink, 2002). Transporters can be effective barriers to drug exposure, be the rate determining step in the uptake and/or excretion of a compound or metabolite, and be a cause of drug–drug interactions. Drug efflux transport systems are the most extensively studied family of drug transporters owing to the phenomenon of multidrug resistance, in which cancer cells become cross-resistant to multiple cancer treatments. Drug efflux transportations are pumped out of the cell to result in decreased intracellular accumulation, permitting cancer cells to survive lethal doses of cytotoxic drugs. This phenomenon has been characterized in a number of cancers including breast cancer.

Breast cancer is the most prevalent cancer in the world, affecting more than 4 million women (Parkin et al., 2005). Each year, breast cancer is diagnosed in more than 1 million women, and 450,000 die of this disease. Breast cancer is frequently associated with increased expression and activation of the epidermal growth factor receptor (EGFR; also known as ErbB), a family of transmembrane tyrosine kinase receptors (Rowinsky, 2004; Johnston et al., 2006). These receptors

ABSTRACT:

Lapatinib [N-[3-chloro-4-[[3-fluorobenzyl]oxy]phenyl]-6-[5-[[2-(methylsulfonfyl)ethyl]amino]methyl]-2-furyl]-4-quinazolinamine, GW572016, Tykerb] is a tyrosine kinase inhibitor approved for use in combination with capecitabine to treat advanced or metastatic breast cancers overexpressing HER2 (ErbB2). In this work we investigated the role of efflux and uptake transporters in lapatinib disposition and drug interactions. In vitro studies evaluated whether lapatinib is a substrate for efflux transporters or an inhibitor of efflux/uptake transporters. In vivo studies included whole-body autoradiography and an evaluation of the role of efflux transporters on the intestinal absorption and brain penetration of lapatinib using chemical or genetic knockout animals. Lapatinib is a substrate for the efflux transporters P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP). Furthermore, lapatinib is an inhibitor (IC50 values 0.025–5 μM) of Pgp, BCRP, and organic anion transporting polypeptide 1B1 (a hepatic uptake transporter). In contrast, lapatinib yielded little inhibition on renal transporters (organic anion transporters, organic cation transporters, and uric acid transporter). In vivo studies demonstrated that brain concentrations of lapatinib were low and influenced by efflux transporters at the blood-brain barrier. In contrast, systemic exposure of lapatinib after oral dosing was unchanged when efflux by Pgp and BCRP was absent from the gastrointestinal tract. These in vitro and in vivo preclinical investigations provide a mechanistic basis for elucidating clinical drug interactions.

Drug transporters can have a significant impact on the absorption, distribution, metabolism, excretion, and toxicity of a drug (Borst and Oude Elferink, 2002). Transporters can be effective barriers to drug exposure, be the rate determining step in the uptake and/or excretion of a compound or metabolite, and be a cause of drug–drug interactions. Drug efflux transport systems are the most extensively studied family of drug transporters owing to the phenomenon of multidrug resistance, in which cancer cells become cross-resistant to multiple cancer treatments. Drug efflux transportations are pumped out of the cell to result in decreased intracellular accumulation, permitting cancer cells to survive lethal doses of cytotoxic drugs. This phenomenon has been characterized in a number of cancers including breast cancer.

Breast cancer is the most prevalent cancer in the world, affecting more than 4 million women (Parkin et al., 2005). Each year, breast cancer is diagnosed in more than 1 million women, and 450,000 die of this disease. Breast cancer is frequently associated with increased expression and activation of the epidermal growth factor receptor (EGFR; also known as ErbB), a family of transmembrane tyrosine kinase receptors (Rowinsky, 2004; Johnston et al., 2006). These
signaling proteins regulate cellular growth, proliferation, survival, and differentiation. The EGRF–ErbB family includes four members: EGRF1 (HER1, ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). Upon activation by ligand, these receptors undergo dimerization, resulting in autophosphorylation and initiation of intracellular signal transduction pathways. EGRF1 and HER2 overexpression occurs in 27 to 30 and 20 to 25% of breast cancers, respectively (Moy and Goss, 2006). It has been proposed that increased expression and activation of these receptors are associated with a higher risk for recurrence of breast cancer and a poorer clinical outcome (Rowinsky, 2004; Johnston et al., 2006; Moy and Goss, 2006). Indeed, inhibition of EGFRs has been demonstrated to have important antitumorigenic effects, which has led to the development of several antibody and small molecule treatments (Rowinsky, 2004; Parkin et al., 2005; Johnston et al., 2006).

Lapatinib (Tykerb, GW572016) is a novel member of the 4-anilinoquinazoline class of tyrosine kinase inhibitors (TKIs) (Boyd et al., 2005; Johnston and Leary, 2006; Moy and Goss, 2006). It is a dual inhibitor of both EGFRI and HER2 with IC50 values of approximately 10 nM against the purified receptors in vitro and potently inhibits growth of EGFR and/or HER2 overexpressing tumors both in vitro and in vivo. Evidence of clinical efficacy has been reported, especially in HER2-positive breast cancers, for lapatinib used either alone or in combination with other anticancer agents (Bence et al., 2005; Geyer et al., 2006). An extensive phase II and III program in advanced breast cancer for both refractory disease and as first-line therapy in combination with chemotherapy is in progress (http://www.clinicaltrials.gov).

The objective of this work was to investigate the role of efflux and uptake transporters in the disposition and drug interactions of lapatinib, given that this drug is being used to treat breast cancer and potentially other cancers in which drug transporters can influence therapeutic outcome, disposition, and toxicity. From these in vitro and in vivo investigations, a mechanistic basis for elucidating potential clinical drug interactions has been developed to guide the design of future clinical studies with lapatinib.

Materials and Methods

Materials. GlaxoSmithKline Chemical Registry supplied [14C]lapatinib (55–57 mCi/mmol), GF120918 (Elacridar), and [3H]amprenavir (21 Ci/mmol). [3H]Cimetidine (20 Ci/mmol), [3H]digoxin (5 Ci/mmol), and [3H]estradiol (Piscataway, NJ). [14C]uric acid (52 mCi/mmol) was purchased from Moravek Biochemicals (Brea, CA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO). Transwells (12-well, 11-mm diameter, 0.4 µm pores) were purchased from Corning Costar (Cambridge, MA). 

Monolayer Efflux Studies. Polarized Madin-Darby canine kidney (MDCKII) cells heterologously expressing either human Pgp (MDCKII-MDR1 cell line) or BCRP (MDCKII-BCRP cell line) were used for the in vitro transport studies and were obtained from The Netherlands Cancer Institute (Amsterdam, The Netherlands). Cell culture and transport studies were completed as described previously (Polli et al., 2011). Stock solutions of [14C]lapatinib, GF120918, and the positive control substrates, [3H]amprenavir (Pgp substrate) and [3H]cimetidine (BCRP substrate), were prepared in DMSO. For substrate assessment, donor solutions with and without GF120918 (a Pgp and BCRP inhibitor) were prepared by diluting the stock solutions in transport medium (fasted state simulated intestinal fluid containing 25 mM glucose; 134 mM NaCl, 29 mM NaH2PO4, 1.5 mM l-arginine, and 5 mM sodium taurocholate, pH 7.4 with NaOH, and osmolality of approximately 290 mOsm/kg).

Receiver solutions contained Dulbecco’s modified Eagle medium (without phenol red), 25 mM HEPES, and 1% human serum albumin, with or without GF120918. The transport of [14C]lapatinib and positive control substrates (amprenavir for Pgp and cimetidine for BCRP efflux) was measured in two directions [apical to basolateral (A → B) and basolateral to apical (B → A)]. Drugs were quantified by liquid scintillation counting (LSC) using a TriCarb T2900 liquid scintillation counter and Ultima Gold scintillation cocktail (PerkinElmer Life and Analytical Sciences).

Pgp and BCRP Inhibition Assays. Cell culture and transport studies were completed as described (Rautio et al., 2006). Lapatinib was tested in triplicate at a minimum of six concentrations spanning 0.3 to 50 µM for Pgp and 0.01 to 3 µM for BCRP. Inhibition studies were conducted for 90 min using [3H]digoxin (27 nM) as the probe substrate for Pgp and [3H]cimetidine (80 nM) for BCRP. [3H]Digoxin and [3H]cimetidine were quantified by LSC. GF120918 was used as a positive control inhibitor.

OATP, OAT, OCT, and URAT Inhibition Assays. For OATP1B1 studies, Chinese hamster ovary cell line heterologously expressing the human OATP1B1 transporter (CHO-OATP1B1), obtained from the University of Zurich (Zurich, Switzerland), was used. CHO-OATP1B1 cells were seeded in 24-well assay plates (Becton Dickinson, Franklin Lakes, NJ) at a density of 50,000 cells/cm2 in cell culture medium [Dulbecco’s modified Eagle’s medium with Glutamax, 10% (v/v) fetal bovine serum, 0.5% (v/v) 10,000 units/ml penicillin/streptomycin, 0.1% (v/v) 50 mg/ml L-proline, and 0.7% (v/v) 50 mg/ml Geneticin]. The monolayers were used 2 days after seeding and induced for at least 24 h before use with 5 mM sodium butyrate. For inhibition studies, CHO-OATP1B1 monolayers were preincubated (37°C) for 15 to 30 min in 1 ml of transport medium [Dulbecco’s phosphate buffered saline (DPBS) plus 1% DMSO] with or without lapatinib or rifampicin (positive control inhibitor). After removal of preincubation solution, 400 µl of transport medium containing 20 nM [3H]digoxin 17β-t-glucuronide, with or without inhibitors, was added, and the cells were incubated at 37°C for 5 min. The inhibitor solution was removed, cells were rinsed three times using 800 µl of cold (4°C) DPBS and lysed with 400 µl of 1% (v/v) Triton X-100, and radioactivity was quantified by LSC.

OAT, OCT, and URAT inhibition screening studies were completed at Fuji Biomedix Co. Ltd. (Yamanashi, Japan). For OAT and OCT inhibition assays, transporter-expressing S2 cells were seeded at a cell density of 105 cells/well in 24-well tissue culture plates individually expressing either OAT1, OAT2, OAT3, OCT4, OCT1, OCT2, OCT2-A, or OCT3; the parental S2 cell line is derived from the S2 portion of the renal proximal tubule and carries a temperature-sensitive simian virus 40 large T-antigen gene (Takeda et al., 2002). For URAT inhibition assays, transporter-expressing HEK-293 cells were seeded in 24-well Biocoat plates (BD Biosciences, Franklin Lakes, NJ) at a cell density of 105 cells/well. Transporter-expressing cells were cultured for 2 days at 33°C (S2 cells) or 37°C (HEK293 cells) as described (Takeda et al., 2002). Lapatinib and positive control inhibitors (probenecid, quinidine, and benzhexol) were added to transport medium containing [3H]cimetidine (80 nM) for Pgp, [3H]digoxin and [3H]estradiol for BCRP, and [3H]estradiol in HBSS (Phoenix) for OATs, OCTs, and URAT1, respectively. The transport of [14C]lapatinib and positive control substrates (amprenavir and GF120918) was measured in two directions (A → B and B → A).

Pharmacokinetics. Male Han Wistar rats (Charles Rivers Laboratories, Raleigh, NC) after a single oral dose of 10 mg/kg [14C]lapatinib [formulated in 0.5% (w/v) aqueous hydroxypropyl methyl cellulose containing 0.1% (v/v) Tween 80] was investigated using whole-body autoradiography at 0.5, 4, 8, 24, and 168 h after dose administration. Tissue processing and image analysis were completed as described (Polli et al., 1999). Digital images, obtained by
The passive permeability of lapatinib, probe substrates and Lucifer yellow in the presence of GF120918 was determined using the following equation as described previously (Rautio et al., 2006):

\[
P_{f, j} = -\left(\frac{V_d V_R}{V_d + V_R}\right) \ln \left(1 - \frac{(V_d + V_R) C_d(t)}{(V_d C_d(t) + V_R C_R(t))}\right) \times 10^7 \text{ nm/s}
\]

where \( P_{f, j} \) is the permeability coefficient at pH 7.4, \( V_d \) and \( V_R \) are donor and receiver well volumes, respectively (milliliters), \( A \) is the membrane surface area (square centimeters), \( t \) is the incubation time (seconds), \( C_d(t) \) is the measured concentration in the receiver well at time \( t \) (nanomoles per milliliter), and \( C_R(t) \) is the measured concentration in the donor well at time \( t \) (nanomoles per liter).

For Pgp and BCRP efflux inhibition and OATP uptake inhibition studies, the IC\textsubscript{50} values (the concentration of inhibitor required for 50\% inhibition of the monolayer transport or cellular uptake rates) were calculated with GraFit (version 5.06; Erithacus Software Limited, London, UK) using

\[
y = \frac{\text{range}}{1 + \left(\frac{x}{IC_{50}}\right)^s} + \text{background}
\]

where \( y \) is the rate of transport or uptake of an appropriate probe (expressed as a percentage of the uninhibited control), \( x \) is the slope factor, \( x \) is the inhibitor concentration (micromolar), and \( s \) is the background representing the uninhibited rate of probe transport (expressed as a percentage of the total rate).

**Results**

**Pgp and BCRP Substrate Assays.** To determine whether [\(^{14}\text{C}\)]-lapatinib is a substrate for human Pgp or BCRP, in vitro bidirectional transport across MDCCKI monolayers expressing these transporters was studied. In addition, the passive permeability was determined by inclusion of GF120918, a Pgp and BCRP inhibitor. The efflux ratio for [\(^{14}\text{C}\)]-lapatinib across the MDCCKI-MDR1 monolayers was 15 and decreased to 0.33 in the presence of GF120918, consistent with lapatinib being a substrate for Pgp (Table 1). The in vitro passive membrane permeability of [\(^{14}\text{C}\)]-lapatinib was 11 nm/s (\( P_{f, 4} \) B \( \rightarrow A \) + GF120918). For BCRP, the efflux ratio of [\(^{14}\text{C}\)]-lapatinib across the MDCCKII-BCRP monolayers was 2.6 and decreased to 1.0 in the presence of GF120918, consistent with lapatinib being a substrate for BCRP. The passive membrane permeability was 2.7 nm/s (\( P_{f, 4} \) B \( \rightarrow A \) + GF120918).

**Pgp and BCRP Inhibition Assays.** The inhibition of Pgp (concentration range 0.3–50 \( \mu \text{M} \)) and BCRP (concentration range 0.01–3 \( \mu \text{M} \)) by lapatinib was assessed by determining the B \( \rightarrow A \) transport of [\(^{3}\text{H}\)]digoxin and [\(^{3}\text{H}\)]cimetidine, respectively, across MDCCKI-MDR1 or MDCCKII-BCRP monolayers. Lapatinib decreased the Bgp-mediated transport of [\(^{3}\text{H}\)]digoxin up to 74\%, yielding an IC\textsubscript{50} value of 3.9 \( \mu \text{M} \) (Table 2; Fig. 1A). Similarly, lapatinib decreased the BCRP-mediated transport of [\(^{3}\text{H}\)]cimetidine up to 82\%, yielding an IC\textsubscript{50} value of 0.025 \( \mu \text{M} \) (Table 2; Fig. 1B).

**OATP1B1, OAT, OCT, and URAT Inhibition Assays.** Inhibition of the OATP1B1 uptake transporter by lapatinib (concentration range 0.01–30 \( \mu \text{M} \)) was investigated by determining the uptake of [\(^{3}\text{H}\)]estradiol 17\( \beta \)-d-glucuronide in the CHO-OATP1B1 cell line. Lapatinib reduced the uptake of [\(^{3}\text{H}\)]estradiol 17\( \beta \)-d-glucuronide by OATP1B1 by 70\%, yielding an IC\textsubscript{50} value of 4.0 \( \mu \text{M} \) (Table 2; Fig. 1C).

The inhibitory effect of lapatinib on a panel of human renal transporters was investigated in S2 cells stably expressing OAT1, OAT2, OAT3, or OAT4, OCT1, OCT2 (isoform a), OCT2-A (isoform b), or...
OCT3 and HEK293 cells expressing URAT1. For each transporter, transfected cells were incubated with the radiolabeled substrate in the absence or presence of 30 μM lapatinib (Table 3). Lapatinib reduced the uptake of [3H]estrone sulfate via OAT3 to 40.2% of the control activity, whereas 30 μM probenecid (the positive control inhibitor) reduced the uptake of [3H]estrone sulfate to 12.9% of the control activity. In contrast, the uptake of probe substrates for OAT1, OAT2, OAT4, OCT1, OCT2, OCT2-A, OCT3, and URAT1 were all greater than 62% in the presence of 30 μM lapatinib, demonstrating little to no inhibition (Table 3).

Whole-Body Autoradiography in Rats. The tissue distribution of [14C]lapatinib was determined in male rats by using whole-body autoradiography at 0.5, 4, 8, 24, and 168 h after oral administration. The absorption of radioactivity after a single oral dose of 10 mg/kg [14C]lapatinib yielded low tissue radioactivity concentrations relative to the dose (Fig. 2); high levels of radioactivity were associated with the gastrointestinal tract. However, the radioactivity that was absorbed was widely distributed into tissues with the exception of brain and was cleared from most tissues by 24 h after the dose, mainly by biliary elimination (Table 4). Tissues with the highest radioactivity included the liver, kidney, and hardier gland. Only low levels of radioactivity were detected in the central nervous system (CNS) at any time (brain/plasma ratios <0.13). In contrast with brain parenchyma, cerebrospinal fluid contained measurable levels of radioactivity. Using muscle as a reference tissue (Polli et al., 1999), the brain/muscle ratio was low (0.15–0.2), demonstrating that the low CNS concentrations were not due to an inability of lapatinib to partition into tissues; therefore, another mechanism must account for the limited brain concentrations. Finally, the hardier gland, which expresses high levels of BCRP, had tissue/plasma ratios of 0.31 to 3.71, demonstrating that [14C]lapatinib was able to distribute into this tissue.

Effect of Efflux Transporters on the Systemic Exposure of Lapatinib. To initially evaluate the influence of efflux transporters (e.g., Pgp and BCRP) on the systemic exposure of lapatinib, a GF120918-treated rat model was selected. This model offers several advantages over mouse models such as the ability to inhibit multiple efflux transporters at one time, a serial blood collection design for pharmacokinetic calculation, requirement of very few animals (n = 6), reduced cost, and less time to complete the study. Rats were pretreated with vehicle (0.5% hydroxypropyl methyl cellulose and 0.1% Tween 80) or a single oral dose of 50 mg/kg GF120918 followed 2 h later by a single oral 10 mg/kg dose of lapatinib. The systemic pharmacokinetics (area under the curve, Cmax, and Tmax) of lapatinib in the GF120918-pretreated animals were similar to those of the vehicle-treated animals (Table 5), suggesting that efflux transporters have a limited role in the intestinal absorption of lapatinib.

Effect of Efflux Transporters on the CNS Exposure of Lapatinib. A study was performed to investigate the influence of Pgp on the CNS penetration of [14C]lapatinib in male wild-type (FVB) and Pgp-deficient (mdrla1b−/−) mice. Blood and brain samples were collected at 0.5 and 2 h after either a 1 or 10 mg/kg intravenous dose of [14C]lapatinib. LC radiochromatographic profiling and LC/MS of a pooled sample demonstrated that 73 to 84% (0.5 h) and 77 to 99% (2 h) of radioactivity in plasma was [14C]lapatinib and that 92 to 99% (0.5 h) and 89 to 99% (2 h) of radioactivity in brain was [14C]lapatinib. After intravenous dosing, there were no notable differences in the plasma concentrations of [14C]lapatinib between Pgp-deficient and wild-type FVB mice at either time point or dose level (Table 6); this observation is consistent with the study completed with GF120918 showing no influence of Pgp on systemic exposure. The mean brain/plasma ratios of radioactivity at 0.5 and 2 h in wild-type FVB mice given a 1 mg/kg dose were 0.04 and 0.09, demonstrating low CNS exposure of lapatinib; these results are in agreement with the whole-body autoradiography data (Fig. 2). In contrast, the mean brain/plasma ratios at 0.5 and 2 h in wild-type FVB mice given a 10 mg/kg dose were 0.22 and 0.25, demonstrating that the entry of [14C]lapatinib into the brain after an i.v. bolus was sensitive to dose. In this instance, the 10-fold change in dose yielded a 2.7- to 5.0-fold change in brain/plasma ratios, suggesting inhibition of an efflux pathway(s) with increasing i.v. doses of lapatinib.

In Pgp-deficient mice given a 1 mg/kg dose, the mean brain/plasma ratios of [14C]lapatinib at 0.5 and 2 h were 0.24 and 0.56. At a dose of 10 mg/kg, the mean brain/plasma ratios at 0.5 and 2 h were 0.54 and 0.94. The brain/plasma ratios in Pgp-deficient mice were 2.6- to 6.4-fold greater than the brain/blood ratios in wild-type mice. Furthermore, the brain/plasma ratios of [14C]lapatinib in Pgp-deficient mice remained less than 1.0, suggesting that other efflux pathways or mechanisms influencing lapatinib CNS distribution may be present.

Discussion

Tyrosine kinase inhibitors are a new class of anticancer drugs (Rowinsky, 2004). A number of small molecule TKIs have been approved, including lapatinib (Tykerb, GW572016), imatinib (Gleevec, STI571), sunitinib (Sutent, SU11248), erlotinib (Tarceva, OSI-774), gefitinib (Iressa, ZD1839), dasatinib (Sprycel, BMS-354825), and sorafenib ( Nexavar, Bay 43-9006). Lapatinib is a novel member of the 4-anilinoquinazoline class of TKIs approved for the treatment of breast cancer (Johnston and Leary, 2006). The unique feature of lapatinib is that it is an

TABLE 1

<table>
<thead>
<tr>
<th>Transporter</th>
<th>IC50 μM</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pgp</td>
<td>3.9 (0.6)</td>
<td>[3H]Digoxin</td>
</tr>
<tr>
<td>BCRP</td>
<td>0.025 (0.003)</td>
<td>[3H]Cimetidine</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>4.0 (2.1)</td>
<td>[3H]Estradiol 17β-d-glucuronide</td>
</tr>
</tbody>
</table>

TABLE 2

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Rate A→B</th>
<th>Rate B→A</th>
<th>Apical Efflux Ratio</th>
<th>A→B Mass Balance</th>
<th>B→A Mass Balance</th>
<th>P2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1</td>
<td>0.06 ± 0.01</td>
<td>0.98 ± 0.06</td>
<td>15</td>
<td>85 ± 5.9</td>
<td>88 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>MDR1</td>
<td>0.61 ± 0.03</td>
<td>0.20 ± 0.01</td>
<td>0.33</td>
<td>82 ± 5.3</td>
<td>100 ± 1.0</td>
<td>11 ± 0.2</td>
</tr>
<tr>
<td>BCRP</td>
<td>0.027 ± 0.004</td>
<td>0.071 ± 0.002</td>
<td>2.6</td>
<td>92 ± 4.7</td>
<td>95 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>BCRP</td>
<td>0.046 ± 0.008</td>
<td>0.047 ± 0.001</td>
<td>1.0</td>
<td>90 ± 5.4</td>
<td>97 ± 3.8</td>
<td>2.7 ± 0.2</td>
</tr>
</tbody>
</table>

GF120918 was used in both donor and receiver compartments at 2 μM for Pgp and 5 μM for BCRP.
In addition, as a large (molecular weight of Pgp, BCRP, and OATP (IC50 values of 3.9, 0.025, and 4.0 M), respectively) molecule, lapatinib is typical of drugs that interact with ABC efflux transporters. Moreover, lapatinib undergoes negligible renal elimination (<5% of the dose) in rats, dogs, and humans (unpublished data). Therefore, the potential for pharmacokinetic interactions between lapatinib and drugs that are substrates or inhibitors of OAT, OCT, and URAT transporters is predicted to be low.

Currently, there are few reports describing the interactions of other TKIs with drug transporters. Imatinib and gefitinib are substrates and inhibitors of Pgp and BCRP (Ozvegy-Laczka et al., 2004). Gefitinib inhibited Pgp-mediated transport of calcein-AM with an IC50 of 3.05 M and BCRP-mediated transport of Hoechst 3342 with an IC50 of 0.29 M (Ozvegy-Laczka et al., 2004; Leggas et al., 2006). Imatinib inhibited Pgp-mediated transport of calcein-AM with a Ki value of 18.3 M and BCRP-mediated transport of Hoechst 3342 with a Ki value of 0.9 M (Hamada et al., 2003; Ozvegy-Laczka et al., 2004). Intracellular concentrations of imatinib have also been shown to be influenced by OCT1 (White et al., 2006). Little information on the interactions of other TKIs such as sunitinib, erlotinib, dasatinib, or sorafenib with ABC or SLC transporters has yet been published.

This characterization of the interaction of lapatinib with transporters, together with other preclinical data, is a first step toward understanding 1) the impact of transporters on the efficacy, toxicity, and pharmacokinetics of lapatinib and 2) the involvement of transporters in drug interactions between lapatinib and other anticancer agents. This information is especially relevant in oncology in which tumor penetration and drug combinations are essential for successful therapy. Previous studies in mouse xenografts indicated 5-fold higher concentrations of lapatinib in tumor compared with plasma (Clark et al., 2002). Although the presence and function of transporters were not determined in that experiment, it is possible, based on data presented in this report, that lapatinib could enhance its own distribution into tumors by inhibiting efflux.

Drug transporters have also been implicated in TKI-associated toxicities. The ABCG2 C421A genotype is associated with diarrhea induced by gefitinib, independent of any change in pharmacokinetics (Cusatis et al., 2006). Phase I clinical trials have shown that lapatinib is well tolerated, with diarrhea and rash being the most frequent toxicities (Bence et al., 2005; Johnston and Leary, 2006). To date, no relationship has been clearly demonstrated between ABCB1 or ABCG2 genotype and the pharmacokinetics or toxicity of lapatinib (Zaks et al., 2006).
Intestinal efflux transporters can attenuate the bioavailability of substrates with limited passive permeability, such as lapatinib. In phase I trials, patients receiving concomitant medications capable of inhibiting Pgp (sertraline or paroxetine) or BCRP (omeprazole or folic acid) displayed no alteration in systemic exposure to lapatinib (unpublished data). This finding suggests that lapatinib systemic exposure is not significantly impeded by intestinal efflux transporters, despite its low/moderate permeability, and is consistent with the finding that GF120918 had no effect on lapatinib disposition in rats. However, recent studies have suggested that the food effect on lapatinib bioavailability in cancer patients may involve inhibition of efflux transporters (Reddy et al., 2007). An alternate explanation for the food effect is enhanced solubility of lapatinib with food, leading to increased absorption.

A particular challenge in the treatment of breast cancer, particularly HER2-overexpressing tumors, is CNS metastases. Up to 35% of patients with HER2-positive advanced breast cancer have a relapse because of intracranial disease despite control of the peripheral tumors (Weil et al., 2005). CNS tumors are difficult to treat due to limited brain and/or tumor exposure of most anticancer agents. The preclinical data presented here suggest that brain concentrations of lapatinib are low in normal, healthy animals owing to efflux transporters in the brain and/or tumor exposure of most anticancer agents. The preclinical data imply that lapatinib brain exposure in cancer patients may involve inhibition of efflux transporters (Reddy et al., 2007). An alternate explanation for the food effect is enhanced solubility of lapatinib with food, leading to increased absorption.

A second key aspect of the information presented here is better anticipation of the potential for drug interactions between lapatinib and other anticancer agents, resulting in altered pharmacokinetics, efficacy, or toxicity. The concept of inhibiting efflux transporters to enhance oral delivery is well established (Breedveld et al., 2006). Coadministration with lapatinib could conceivably enhance systemic exposure of other anticancer agents by inhibiting gastrointestinal efflux or biliary/renal excretion. The clinical utility of such interactions is currently under investigation with agents such as topotecan, a BCRP substrate (Molina et al., 2007).

When TKI inhibitors and efflux transporters are used in combination, the therapeutic index of cytotoxic agents can be altered by TKI-mediated inhibition of efflux transporters, which can provide a resistance mechanism in tumor cells and a protective mechanism in sensitive normal cells. This fact is illustrated by studies with docetaxel and paclitaxel, two efflux transporter substrates that are important first-line treatments for breast cancer. In a phase I study (EGF10021) that examined the combination of lapatinib given daily plus docetaxel given once every 3 weeks, neutropenia produced by docetaxel was observed at doses lower than those generally seen when docetaxel is given alone. Despite the potential for lapatinib to inhibit Pgp-mediated transport and CYP3A4-dependent metabolism of docetaxel, no increase in systemic exposure of this taxane was observed. This result is consistent with reports that after i.v. administration, docetaxel pharmacokinetics are not greatly influenced by more potent Pgp inhibitors such as MS209 and zosuquidar (Fracasso et al., 2004; Dieiras et al., 2005). However, potent CYP3A4 inhibitors, such as ketoconazole, reduced docetaxel clearance, requiring a dose adjustment (Engels et al., 2004). Therefore, the enhanced neutropenia of the lapatinib-docetaxel combination is currently hypothesized to be attributed to inhibition of efflux transporters in blood cell progenitors and not the result of a pharmacokinetic drug interaction.

In another phase I study (EGF10009) (Crown et al., 2007) that examined the combination of lapatinib given daily and paclitaxel given every 3 weeks, the duration of peripheral neuropathy produced by paclitaxel appeared to be affected by the concentration of lapatinib. Previous reports have associated this toxicity of paclitaxel with reduced efflux of drug from neurons by Pgp (Sissung et al., 2006). These observations suggest that lapatinib may have inhibited Pgp-mediated efflux of paclitaxel from this sensitive normal cell type. However, combined administration of these agents also produced clinical activity in patients in whom prior treatment with taxanes had failed. The potential for synergy with this combination awaits further examination in larger clinical trials.

In conclusion, lapatinib interacts with a number of ABC and SLC transporters shown to influence treatment outcome by altering tumor resistance and the disposition/toxicity profile for a number of anti-
cancer drugs. These studies, in conjunction with other research, provide a mechanistic basis for the role of efflux and uptake transporters in lapatinib disposition and drug interactions. Studies are ongoing to further clarify the clinical importance of transporters on the efficacy, disposition, toxicity, and drug interactions of lapatinib.

### References


Dr. Joseph W. Polli, Preclinical Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, Inc., P.O. Box 13398, Room 221, Research Triangle Park, NC 27709. E-mail: joseph.w.polli@gsk.com

**Address correspondence to:** Dr. Joseph W. Polli, Preclinical Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, Inc., P.O. Box 13398, Room MAJ2123, Research Triangle Park, NC 27709. E-mail: joseph.w.polli@gsk.com

**TABLE 6**

<table>
<thead>
<tr>
<th>Dose and Time</th>
<th>Wild-Type FVB Mice</th>
<th>mdr1a/b(-/-) Mice</th>
<th>Knockout/Wild-Type Brain/Plasma Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma Concentrations</td>
<td>Brain Concentrations</td>
<td>Average Brain/Plasma Ratio</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>ng/ml</td>
<td>ng/g</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>0.5 h</td>
<td>312 (33)</td>
<td>13.9 (3.7)</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>2 h</td>
<td>68.9 (13.4)</td>
<td>6.26 (2.62)</td>
<td>0.09 (0.02)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>ng/ml</td>
<td>ng/g</td>
<td>0.25 (0.22–0.27)</td>
</tr>
<tr>
<td>0.5 h</td>
<td>4559 (745)</td>
<td>1012 (116)</td>
<td>0.22 (0.06)</td>
</tr>
<tr>
<td>2 h</td>
<td>1869 (1502–2235)</td>
<td>463 (332–594)</td>
<td>0.25 (0.22–0.27)</td>
</tr>
</tbody>
</table>


