Accelerated Communication

An Unexpected Synergist Role of P-Glycoprotein and Breast Cancer Resistance Protein on the Central Nervous System Penetration of the Tyrosine Kinase Inhibitor Lapatinib (N-[3-Chloro-4-[[3-fluorobenzyl]oxy]phenyl]-6-[5-[[2-(methylsulfonyl)ethyl]amino]methyl]-2-furyl]-4-quinazolinamine; GW572016)


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ABSTRACT:

Lapatinib is a tyrosine kinase inhibitor approved for use in combination with capecitabine to treat advanced or metastatic breast cancers overexpressing human epidermal receptor 2 (ErbB2). This work investigated the role of P-glycoprotein (Pgp; the protein from the Mdr1a/b gene) and breast cancer resistance protein (Bcrp; the protein from the Bcrp1 gene) in modulating the central nervous system penetration of lapatinib at steady-state conditions in FVBn mice (wild-type), Mdr1a/b(−/−), Bcrp1(−/−), and Mdr1a/b(−/−)/Bcrp1(−/−) knockout mice. After an intravenous infusion of lapatinib for 24 h to a targeted steady-state plasma concentration of 700 ng/ml (0.3 mg/kg/h) or 7000 ng/ml (3 mg/kg/h), lapatinib brain-to-plasma ratios were approximately 3- to 4-fold higher in Mdr1a/b(−/−) knockout mice. After an intravenous infusion of lapatinib for 24 h to a targeted steady-state plasma concentration of 700 ng/ml (0.3 mg/kg/h) or 7000 ng/ml (3 mg/kg/h), lapatinib brain-to-plasma ratios were approximately 3- to 4-fold higher in Mdr1a/b(−/−) knockout mice. There was no difference in the brain-to-plasma ratio in the Bcrp1(−/−) knockout mice. In contrast, Mdr1a/b(−/−)/Bcrp1(−/−) triple knockout mice had a 40-fold higher brain-to-plasma ratio (ratio range from 1.2 to 1.7), suggesting that Pgp and Bcrp work in concert to limit the brain-to-plasma ratio of lapatinib in mice. This finding has important potential consequences for the treatment of brain tumors in breast cancer patients treated with tyrosine kinase inhibitors as well as the basic understanding of ATP binding cassette transporters expressed in the blood-brain barrier on the central nervous system disposition of drugs.

Lapatinib (Tykerb, GW572016) is a novel member of the 4-anilinoquinazoline class of tyrosine kinase inhibitors (TKI) (Boyd et al., 2005; Johnston and Leary, 2006; Moy and Goss, 2006). It is a dual inhibitor of both epidermal growth factor receptor (ErbB) 1 and human epidermal receptor (HER) 2 with IC50 values of approximately 10 nM against the purified receptors in vitro, and it potently inhibits growth of epidermal growth factor receptor and/or HER2-overexpressing tumors both in vitro and in vivo. Lapatinib is approved for use in combination with other anticancer agents for the treatment of HER2-positive breast cancers (Geyer et al., 2006).

A particular challenge to the treatment of breast cancer, particularly HER2-overexpressing tumors, is central nervous system (CNS) metastases. Up to 35% of patients with HER2-positive advanced breast cancer relapse due to 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. Nonclinical data suggest that the brain concentrations of lapatinib and other TKIs are low due to efflux transporters in the blood-brain barrier (Heimberger et al., 2002; Breedveld et al., 2005; Kil et al., 2007; Polli et al., 2008). These nonclinical data imply that TKIs may have limited

ABBREVIATIONS: Lapatinib, GW572016, N-[3-chloro-4-[[3-fluorobenzyl]oxy]phenyl]-6-[5-[[2-(methylsulfonyl)ethyl]amino]methyl]-2-furyl]-4-quinazolinamine; TKI, tyrosine kinase inhibitor; HER, human epidermal receptor; CNS, central nervous system; Pgp, P-glycoprotein; Bcrp and BCRP, breast cancer resistance protein; Mdr1, gene encoding the Pgp protein; Bcrp1, gene encoding Bcrp protein; t1/2, plasma half-life; Cmax, maximum plasma concentration; C0, extrapolated estimate of plasma concentration at end of bolus dose; Css, steady-state plasma concentration; HPLC, high-performance liquid chromatography; Clp, plasma clearance; GF120918, N-[4-{[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide.
utility for the treatment of brain tumors in humans. However, in clinical studies, both lapatinib and gefitinib have been shown to reduce CNS tumor growth (Roggero et al., 2005; Geyer et al., 2006; Lin et al., 2008). It is possible that disruption of the blood-brain barrier by tumors circumvents the usual protective function of transporters or that inhibition of efflux by these agents enhances their own accumulation upon repeat dosing (de Vries et al., 2006).

The objective of this study was to establish the extent of attenuation of CNS penetration of lapatinib by P-glycoprotein (Pgp) and breast cancer resistance protein (Bcrp) 1, which are ATP-dependent efflux transporter proteins localized in the apical membrane of the blood-brain barrier. The mouse is an ideal model, because Mdr1a/(−/−) and Bcrp1/(−/−) knockout mice are well established to study Pgp and Bcrp1-dependent transport at the blood-brain barrier.

Materials and Methods

In Vivo Studies in Mdr1a/(−/−), Bcrp1/(−/−), and Mdr1a/(−/−)/Bcrp1/(−/−) Mice. Procedures in this study were conducted in accordance with the U.S. Animal Welfare Act, as amended in 1990, and the protocol was approved by the Institutional Animal Care and Use Committee. Jugular cannulated male FVBn (wild-type), Mdr1a/(−/−), Bcrp1/(−/−), and Mdr1a/(−/−)/Bcrp1/(−/−) knockout mice were supplied by Taconic Farms (Germantown, NY). The mice weighed between 19 and 26 g at the start of the study. On arrival day, the jugular cannula was exteriorized and connected to a tether system that continuously infused saline (0.06 ml/h) to ensure cannula patency. Mice were fed LabDiet brand Certified Rodent Diet 5002 (PMI Nutrition International, Richmond, IN) ad libitum, with free access to water. Temperature and humidity were maintained at 70 ± 10°F and 55 ± 30%, respectively, and a 12-h light/12-h dark cycle was maintained.

The dose solution of lapatinib was prepared on the day of use in a vehicle of 10% sulfobutyl ether-β-cycloextrin at a concentration of 0.15 or 1.5 mg/ml. Preliminary studies in wild-type mice indicated that the terminal plasma τ1/2 of lapatinib after a single 10 mg/kg i.v. bolus dose ranged from approximately 4 to 6 h, and Cmax and C10 were on average 1000 to 5000 ng/ml. A pilot study in wild-type mice confirmed that after constant rate intravenous infusion of lapatinib, steady-state plasma concentrations of lapatinib were achieved within 16 h (data not shown). Therefore, a standardized study design that includes a 24-h infusion at a rate of 60 μg/h and a dose of 0.3 or 3 mg/kg/h to achieve a targeted steady-state plasma concentration (Css) of 700 or 7000 ng/ml was used for all strains of mice. The dose of formulation administered to each animal was determined by weighing the loaded dose syringe before and the emptied syringe after dose administration. The steady-state concentrations were selected based on the human plasma Cmax values (4.2 μM or 2432 ng/ml) after a 1250-mg daily dose (Polli et al., 2008). This plasma concentration is considered high in mice. Two different plasma concentrations were included to determine whether there is a concentration-dependent effect of lapatinib at the blood-brain barrier (e.g., autoinhibition of transporters by lapatinib resulting in enhanced brain entry). After the 24-h infusion, a single terminal blood sample (1 ml) was drawn from each animal by exsanguination via the vena cava under terminal anesthesia and was transferred into tubes containing EDTA as the anticoagulant. The blood was centrifuged (5000 rpm at 4°C for 10 min) to obtain plasma. Plasma samples were stored at −80°C until analysis. Brains were removed at the time of sacrifice, rinsed with ice-cold saline, frozen (e.g., solid carbon dioxide), and stored at −80°C until analysis.

Liquid Chromatography/Tandem Mass Spectrometry Analysis. The concentration of lapatinib in mouse plasma and brain homogenate was determined by high-performance liquid chromatography (HPLC) with tandem mass spectrometry using validated methods. Before analysis, mouse brains were weighed and homogenized with an equal volume of water. Plasma and brain homogenate samples were then extracted by protein precipitation using a solution of acetonitrile containing stable isotopically labeled lapatinib ([1H3,13C3]JGW572016) as an internal standard. HPLC was performed by using a Shimadzu LC-10A VP HPLC (Shimadzu, Kyoto, Japan). Chromatographic separation was achieved by using a mixture of aqueous 5 or 10 mM ammonium acetate/acetonitrile on a Waters XBridge C18 HPLC column (Waters, Milford, MA). Samples were analyzed in positive mode by turbo ion spray tandem mass spectrometry with an Applied Biosystems/MDS Sciex (Foster City, CA) API 5000 (mouse brain homogenate) or an Applied Biosystems/MDS Sciex API 3000 (mouse plasma). The calibration range was 10 to 10,000 ng/ml in mouse plasma and 1 to 1000 ng/ml in mouse brain homogenate. Data were integrated by using Applied Biosystems/MDS Sciex Analyst Software version 1.4.1. SMS2000 (version 2.0; GlaxoSmithKline, Research Triangle Park, NC) was used to calculate peak area ratios (i.e., analyte/internal standard peak area ratios versus analyte concentrations were constructed, and a weighted 1/x2 linear regression was applied to the data) to construct the calibration lines from which the concentration of lapatinib in the study samples was determined. Descriptive statistics (mean ± S.D.) was performed on the data. Brain homogenate concentrations were converted to brain concentrations for calculations. Brain-to-plasma ratios between the groups were determined and compared.

Pharmacokinetic and Statistical Analysis. Plasma clearance (CLp) of lapatinib was calculated by using the equation CL = R(0)/C10, where R(0) is the infusion rate (nanograms per hour) and C10 is the plasma concentration at terminal blood sample (1 ml) was drawn from each animal by exsanguination via the vena cava under terminal anesthesia and was transferred into tubes containing EDTA as the anticoagulant. The blood was centrifuged (5000 rpm at 4°C for 10 min) to obtain plasma. Plasma samples were stored at −80°C until analysis. Brains were removed at the time of sacrifice, rinsed with ice-cold saline, frozen (e.g., solid carbon dioxide), and stored at −80°C until analysis.

Results and Discussion

We have previously characterized the interaction of lapatinib as a substrate and inhibitor of human Pgp and BCRP transporters (Polli et al., 2008) as part of the effort to understand the impact of transporters on the efficacy, toxicity, and distribution of this drug. Nonclinical studies on lapatinib and other TKIs suggest that brain concentrations are low due to efflux transporters in the blood-brain barrier (Heimberger et al., 2002; Bredveld et al., 2005; Kil et al., 2007; Polli et al., 2008), implying that TKIs may have limited utility for the treatment of brain tumors in humans. However, both lapatinib and gefitinib have been shown in clinical studies to reduce CNS tumor growth (Roggero et al., 2005; Geyer et al., 2006; Lin et al., 2008). This study was completed to assess the importance of the efflux transporters Mdr1a and Bcrp1, individually and together, on the lapatinib brain-to-plasma ratio under steady-state conditions using Mdr1a/(−/−), Bcrp1/(−/−), and Mdr1a/(−/−)/Bcrp1/(−/−) knockout mice.

Lapatinib was delivered i.v. infusion for 24 h at a rate of 60 μg/h and a dose of 0.3 or 3 mg/kg/h to reach a targeted steady-state plasma concentration of 700 or 7000 ng/ml, respectively. The mean plasma Cmax of lapatinib achieved in the wild-type strain was 730 ± 410 and 5097 ± 2729 ng/ml (Table 1). Because these values were similar to the predicted values of 700 and 7000 ng/ml, these infusion conditions were used for the remaining studies with the knockout mice strains. Although this study was not designed to provide an in-depth analysis of the impact of Mdr1a or Bcrp1 deficiency on the plasma pharmacokinetics of lapatinib, analysis of the clearance values across the knockout strains may provide some insight into the possible role of transporters on the CLp of lapatinib. Overall, the mean CLp values were moderate in comparison with hepatic plasma flow and ranged from 13 to 31 ml/min/kg. After the low infusion rate (0.3 mg/kg/h), the CLp of lapatinib was statistically higher (1.5–2.5-fold) in all three knockout strains compared with wild-type mice, and CLp was statistically higher (∼50%) in Mdr1a/(−/−) mice compared with Bcrp1/(−/−) mice. There was no difference in CLp in Mdr1a/(−/−)/Bcrp1/(−/−) triple knockout mice compared with either the Mdr1a/(−/−) or Bcrp1/(−/−) mice. In contrast, after the high infusion rate of 3 mg/kg/h, CLp was not statistically different in any of the knockout strains compared with the wild type. However, CLp was statistically lower (approximately 25–45%) in Mdr1a/(−/−) or Bcrp1/(−/−) mice compared with the Mdr1a/(−/−)/Bcrp1/(−/−) triple knockout mice. Because lapatinib is cleared mainly by metab-
olism in mice (data not shown) and the addition of GF120918 does not change the systemic exposure of an oral dose of lapatinib to rats (Polli et al., 2008), it is not immediately clear why CLp was affected by loss of Pgp and/or Bcrp1 activity. It is possible that the profile of the metabolic enzymes differs across these strains of mice; this has been described previously for these strains (Schuetz et al., 2000). Further characterization of the metabolic and transporter profiles in the knock-out strains relative to the wild-type animal is warranted.

After a constant rate intravenous administration of lapatinib for 24 h, lapatinib brain and plasma levels were measured by liquid chromatography/tandem mass spectrometry. Although there was a statistical difference in the brain-to-plasma ratios in the Bcrp1(−/) mice compared with wild-type mice after the low dose infusion (Table 1), the difference was small. The brain-to-plasma ratios were 0.03 to 0.04 for both mice strains; these ratios are similar to blood contamination of brain tissue. In the Mdr1a/b(−−) mice, there was a modest 3- to 4-fold increase in the brain-to-plasma ratios (0.09 and 0.16 for the 0.3 and 3 mg/kg/h infusion rates, respectively) compared with wild-type mice. Furthermore, there was some evidence of a concentration-dependent effect in the Mdr1a/b(−−) mice with animals receiving the higher infusion concentration of lapatinib having a 1.80-fold larger brain-to-plasma ratio (0.16 versus 0.09) compared with the lower infusion concentration. Even with this increased brain entry of lapatinib in the Mdr1a/b(−−) mice, the brain-to-plasma ratio remained well below that of unity, suggesting that other mechanisms (e.g., permeability, plasma protein binding, other efflux transporters) influence lapatinib entry into the brain.

A recent study by de Vries et al. (2007) using Mdr1a/b(−−)/Bcrp1(−−) knockout mice demonstrated that the two transporters work together to limit the plasma exposure and brain penetration of topotecan, a topoisomerase I inhibitor used in the treatment of ovarian, lung, and cervical cancers. In that study, the brain-to-plasma ratio of topotecan increased 3.7-fold in the triple knockout mice compared with 2.0-fold increase in the Mdr1a/b(−−) or 0.65-fold increase in the Bcrp1(−−) knockout mice. These authors also noted differences in the plasma area under the curve (0.75–2.4-fold) and plasma clearance of topotecan in knockout mice compared with wild-type mice, an observation consistent with the differences in clearance values observed in our study with lapatinib. That report, along with the limited individual effects of Mdr1a/b and Bcrp1 efflux on the lapatinib brain-to-plasma ratio, raised interest as to the possible combined effects of a triple Mdr1a/b and Bcrp1 gene knockout on the brain-to-plasma ratio of lapatinib. In contrast to the modest changes observed in Mdr1a/b(−−) and Bcrp1(−−) mice, the lapatinib steady-state infusion brain-to-plasma ratio in Mdr1a/b(−−)/Bcrp1(−−) mice was 40-fold higher compared with the wild-type mice. The brain-to-plasma ratios were greater than unity (1.2 and 1.7 for the 0.3 and 3 mg/kg/h infusion rates, respectively), suggesting that the CNS penetration of lapatinib is not restricted by protein binding or passive permeability; this agrees with the wide distribution of radioisotope to all tissues except brain in the rat whole body autoradiography study after an oral dose of [14C]lapatinib (Polli et al., 2008). It is noteworthy that there was no concentration-dependent effect in brain-to-plasma ratios observed with the triple knockout animals, unlike that seen in the Mdr1a/b(−−) and Bcrp1(−−) animals (this study; Polli et al., 2008). These results suggest that Mdr1a/b and Bcrp1 have a combined effect to control the brain entry of lapatinib in mice.

The finding that Mdr1a/b and Bcrp1 have a combined effect on the brain entry of drugs has important potential consequences for the treatment of brain tumors in breast cancer patients treated with tyrosine kinase inhibitors as well as the basic understanding of ATP binding cassette transporters in the CNS disposition of drugs. For lapatinib, this information provides insight into possible future strategies to enhance the brain and tumor delivery of this drug. These approaches may include coadministration with a dual Pgp and BCRP inhibitor such as GF120918 (Breedveld et al., 2006) or improved brain penetration of future TKIs by designing out efflux by Pgp and/or Bcrp1 activity. It is also noteworthy that there was no concentration-dependent effect in brain-to-plasma ratios observed with the triple knockout animals, unlike that seen in the Mdr1a/b(−−) and Bcrp1(−−) animals (this study; Polli et al., 2008). These results suggest that Mdr1a/b and Bcrp1 have a combined effect to control the brain entry of lapatinib in mice.

Finally, Pgp is well documented to influence the brain entry of many drugs (Breedveld et al., 2006). In contrast, the role of BCRP/Bcrp1 is still being clarified, with few examples demonstrating that

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mice per Group</th>
<th>Plasma C (ng/ml)</th>
<th>Brain Concentration (ng/ml)</th>
<th>Brain-to-Plasma Ratio</th>
<th>Plasma Clearance (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVBn</td>
<td>5</td>
<td>730 ± 410</td>
<td>18 ± 9.1</td>
<td>0.03 ± 0.01</td>
<td>13 ± 6.1</td>
</tr>
<tr>
<td>Mdr1a/b(−−)</td>
<td>4</td>
<td>239 ± 43ab</td>
<td>21 ± 3.3c</td>
<td>0.09 ± 0.02abc</td>
<td>31 ± 4.2abb</td>
</tr>
<tr>
<td>Bcrp1(−−)</td>
<td>5</td>
<td>394 ± 74</td>
<td>17 ± 3.1d</td>
<td>0.04 ± 0.01cde</td>
<td>20 ± 4.6d</td>
</tr>
<tr>
<td>Mdr1a/b(−−)/Bcrp1(−−)</td>
<td>5</td>
<td>296 ± 150a</td>
<td>319 ± 67d</td>
<td>1.2 ± 0.42</td>
<td>28 ± 13</td>
</tr>
<tr>
<td>FVBn</td>
<td>4</td>
<td>5097 ± 2729</td>
<td>194 ± 65</td>
<td>0.04 ± 0.02</td>
<td>20 ± 13</td>
</tr>
<tr>
<td>Mdr1a/b(−−)</td>
<td>3</td>
<td>4463 ± 811c</td>
<td>732 ± 421abc</td>
<td>0.16 ± 0.06abc</td>
<td>15 ± 2.3</td>
</tr>
<tr>
<td>Bcrp1(−−)</td>
<td>5</td>
<td>6447 ± 2731d</td>
<td>253 ± 23e</td>
<td>0.04 ± 0.01de</td>
<td>13 ± 4.4d</td>
</tr>
<tr>
<td>Mdr1a/b(−−)/Bcrp1(−−)</td>
<td>5</td>
<td>2840 ± 867</td>
<td>4938 ± 1823a</td>
<td>1.7 ± 0.50</td>
<td>23 ± 6.2</td>
</tr>
</tbody>
</table>

*FVBn compared with Mdr1a/b(−−), Bcrp1(−−), or Mdr1a/b(−−)/Bcrp1(−−).

*Mdr1a/b(−−) compared with Bcrp1(−−).

*Mdr1a/b(−−)/Bcrp1(−−) compared with Mdr1a/b(−−).

*Mdr1a/b(−−)/Bcrp1(−−) compared with Bcrp1(−−).
this transporter significantly attenuates the CNS disposition of drugs (Breedveld et al., 2006; de Vries et al., 2007; Polli et al., 2008). The results from this study with lapatinib, along with that for topotecan (de Vries et al., 2007), highlight the need to understand further the role of BCRP/Bcrp1 and other transporters in the blood-brain barrier, and how these transporters interact with Pgp, which seems to be the dominant efflux transporter in the barrier. The triple knockout model will be a useful tool to elucidate these mechanisms.

In conclusion, Mdr1alb and Bcrp1 work in combination to modulate the CNS penetration of lapatinib in mice, resulting in a 40-fold increase in brain-to-plasma ratio compared with wild-type mice. Further research into the interplay between these and other transporters is warranted, because this finding provides unique insight into future cancer treatments with TKIs as well as an increased understanding of ATP binding cassette transporters on the CNS disposition of drugs.

References

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