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Coadministration of lapatinib increases exposure to docetaxel but not doxorubicin in the small intestine of mice

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Abstract

Combination therapy is increasingly being utilized for the treatment of metastatic breast cancer. However, coadministration of drugs, particularly agents that are substrates for or inhibitors of p-glycoprotein, can result in increased tissue toxicity. Unfortunately, determination of levels of chemotherapeutics in human tissues is challenging, and plasma drug concentrations are not always indicative of tissue toxicokinetics or toxicodynamics, especially when tissue penetration is altered. The aim of the present work was to determine whether concomitant administration of compounds currently being combined in clinical trials for metastatic breast cancer treatment alters plasma and tissue pharmacokinetics in mice if both agents are p-glycoprotein substrates and/or inhibitors. Accordingly, we investigated the pharmacokinetic interactions of the classic cytotoxics and p-glycoprotein substrates docetaxel and doxorubicin when administered concurrently with the targeted agent and p-glycoprotein inhibitor lapatinib. Our time-course plasma and tissue distribution studies showed that coadministration of lapatinib with doxorubicin did not appreciably alter the pharmacokinetics of this anthracycline in the plasma or six tissues evaluated in mice, presumably because, at doses relevant to human exposure, lapatinib inhibition of p-glycoprotein did not significantly alter doxorubicin transport out of these tissue compartments. However, combining lapatinib with docetaxel significantly increased intestinal exposure to this chemotherapeutic, which has clinical implications for enhancing gastrointestinal toxicity. The significant lapatinib-docetaxel interaction is likely CYP3A4-mediated, suggesting that caution should be exercised when this combination is administered, particularly to patients with compromised CYP3A activity, and recipients should be monitored closely for enhanced toxicity, particularly for adverse effects on the intestine.

Keywords

docetaxel; doxorubicin; lapatinib; pharmacokinetics

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Conflicts of interest

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Introduction

The treatment of metastatic breast cancer is increasingly turning toward the use of combination therapy to optimize clinical outcomes [1–3]. Although additive or synergistic activity of agents is clearly advantageous for enhancing efficacy, a concurrent increase in toxicity may also result from the combination. The latter is particularly likely when the coadministered compounds are substrates for or inhibitors of ATP-binding cassette transporters, which play a critical role in protecting cells from xenobiotics.

One of the best-characterized ATP-binding cassette transporters is p-glycoprotein (PGP), discovered in 1976 [4]. Consistent with its role as a toxin efflux pump, PGP is highly expressed on the apical surface of epithelial cells with excretory roles, such as cells lining the colon, small intestine, pancreatic ductules, bile ductules, kidney proximal tubules, and the adrenal gland [5,6]. The transporter is also located on the endothelial cells of the blood-brain barrier [7], the blood-testis barrier [8], and the blood-mammary tissue barrier [9]. Impairing the ability of PGP to export drugs out of these tissues, either by direct or by competitive inhibition, could result in increased intracellular drug concentrations and thus increased tissue toxicity.

Data on human tissue levels of chemotherapeutics are sparse, and, unfortunately, plasma drug concentrations are not always indicative of the drug's concentration in tissues, especially when tissue penetration is altered. In mice, the disconnect between plasma and tissue pharmacokinetics has been observed when a PGP substrate was administered to *mdrla* (–/–) mice [10] and when two PGP substrates were administered in combination [11]. In terms of the PGP substrate doxorubicin, the latter paper concluded that ‘monitoring of plasma levels of doxorubicin, when used in combination with another drug that is a PGP substrate, will not reflect actual pharmacokinetic changes occurring in other tissues’. Thus, identifying whether the coadministration of compounds will result in increased tissue exposure and consequent enhanced toxicity on the basis of an agent's plasma profile alone is problematic.

To address tissue-specific drug exposure resulting from combination therapy, we carried out studies in mice. The aim of the present work was to determine whether the coadministration of compounds commonly combined for metastatic breast cancer treatment alters plasma and tissue pharmacokinetics if both agents are PGP substrates and/or inhibitors. Accordingly, we investigated the pharmacokinetic interactions of the classic cytotoxics and PGP substrates docetaxel and doxorubicin when administered concomitantly with the targeted agent and PGP inhibitor lapatinib, as both combinations are being explored clinically for the treatment of metastatic breast cancer. There is a precedent to suggest that drug-drug interactions involving PGP could be significant for these combinations; in-vitro studies have shown that lapatinib increased the intracellular accumulation of docetaxel 4.2-fold and doxorubicin 3.6-fold in the ABCB1-over-expressing DLKP-A [12] and MCF7/adr [13] cell lines, respectively. By understanding the plasma and tissue dynamics of these combination therapies in mice, we can then correspondingly perform dose adjustments in humans to mitigate potential increases in toxicity so that the benefit of treatment outweighs the burden.

Materials and methods

Chemicals

Docetaxel (Taxotere; Sanofi, Bridgewater, New Jersey, USA) was obtained from the University of Colorado Hospital Pharmacy. Doxorubicin was obtained from the Colorado State University Veterinary Teaching Hospital Pharmacy. Lapatinib (GW572016) and GW572016AH were generously provided by GlaxoSmithKline (Philadelphia, Pennsylvania, USA). Hydroxypropyl methylcellulose, Tween 80, and daunorubicin were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other reagents were of analytical grade.

Animals

Five-week-old to 6-week-old female FVB mice were purchased from Taconic (Hudson, New York, USA). Animals were housed in polycarbonate cages and kept on a 12 h light/dark cycle. Food and water were provided *ad libitum*. Upon arrival, mice were acclimatized for a minimum of 7 days before any experimentation.

All experimental procedures were approved by Colorado State University's Animal Care and Use Committee and the Department of Defense US Army Medical Research and Material Command (USAMRMC) Animal Care and Use Review Office (ACURO).

Lapatinib pharmacokinetic study

A time-course distribution study of lapatinib was carried out. Lapatinib was formulated as a suspension of 12mg/ml in 0.5% hydroxypropyl methylcellulose: 0.1% Tween 80 in Milli-Q water (Millipore, Billerica, Massachusetts, USA) and administered through an intraperitoneal injection as a bolus dose of 60 mg/kg. Lapatinib was dosed every 3 h for a total of five doses ($q3h \times 5$). Subsequently, three mice were killed at each postdose maximum concentration (C_{max} (determined from previous studies to be 1 h after dose) and minimum concentration (C_{min}) (3 h after dose). For the fifth dose, we only killed mice at the C_{max} . All killing was performed by cardiac stick exsanguination under isoflurane anesthesia. Plasma was immediately collected, frozen in liquid nitrogen, and stored at -80°C until analysis.

Docetaxel pharmacokinetic study

A time-course distribution study of docetaxel with both single-dose and multiple-dose lapatinib was carried out. Docetaxel was obtained as an initial solution of 20 mg/ml in a 50/50 (v/v) ratio polysorbate 80/dehydrated alcohol, further diluted to a solution of 0.6 mg/ml in 0.9% sodium chloride, and administered through an intravenous tail vein injection as a single bolus dose of 3 mg/kg. Lapatinib was formulated as a suspension of 12 mg/ml in 0.5% hydroxypropyl methylcellulose: 0.1% Tween 80 in Milli-Q water and administered through an intraperitoneal injection as a bolus dose of 60 mg/kg. The vehicle was 0.5% hydroxypropyl methylcellulose: 0.1% Tween 80 in Milli-Q water.

For the combination docetaxel and single-dose lapatinib study, docetaxel was injected 1 h after the single lapatinib or vehicle administration. Subsequently, three mice were killed at 1, 2, 4, 8, and 12 h after docetaxel injection. For the combination docetaxel and multiple-

dose lapatinib study, lapatinib or vehicle was dosed $q3h \times 5$. Docetaxel was injected 1 h after the first lapatinib or vehicle dose. Subsequently, three mice were killed at 4, 8, and 12 h after docetaxel injection. All killing was performed by cardiac stick exsanguination under isoflurane anesthesia. Plasma, brain, liver, proximal small intestine, kidney, heart, lung, muscle, and adipose tissue were immediately collected, frozen in liquid nitrogen, and stored at -80°C until analysis.

Doxorubicin pharmacokinetic study

A time-course distribution study of doxorubicin with both single-dose and multiple-dose lapatinib was carried out. Doxorubicin was obtained as an initial solution of 2 mg/ml in 0.9% sodium chloride, further diluted to a solution of 1.2 mg/ml in 0.9% sodium chloride, and administered through an intravenous tail vein injection as a single bolus dose of 6 mg/kg. Lapatinib and vehicle were formulated and administered as for docetaxel studies.

For the combination doxorubicin and single-dose lapatinib study, doxorubicin was injected 1 h after the single lapatinib or vehicle administration. Subsequently, three mice were killed at 1, 2, 4, 8, 12, 24, and 48 h after doxorubicin injection. For the combination doxorubicin and multiple-dose lapatinib study, lapatinib or vehicle was dosed $q3h \times 5$. Doxorubicin was injected 1 h after the first lapatinib or vehicle dose. Subsequently, three mice were killed at 4, 8, 12, 24, and 48 h after doxorubicin injection. Killing, tissue collection, and storage were performed as for docetaxel studies.

Lapatinib high-pressure liquid chromatography-tandem mass spectrometry analysis

Analysis of lapatinib in plasma was carried out using high-pressure liquid chromatography-tandem mass spectrometry (HPLC/MS/MS) analysis on the basis of the method of Bai *et al.* [14], modified as follows: briefly, lapatinib was extracted from plasma by adding 210 μl of acetonitrile and 10 μl of internal standard (17.2 pmol GW572016AH) to 100 μl of unknown sample plasma, vortexing for 10 min, and centrifuging at 18 000g for 10 min at 4°C . An aliquot of 20 μl of the supernatant was injected into the LC/MS/MS system for analysis. Standards and quality control samples were prepared in mouse plasma and analyzed as described above.

The HPLC system consisted of an Agilent 1200 Series binary pump SL, a vacuum degasser, thermostatted column compartment SL (Agilent Technologies, Santa Clara, California, USA), and a CTC Analytics HTC PAL System autosampler (Leap Technologies, Carrboro, North Carolina, USA). The HPLC column was a Waters Sunfire C8 column [4.6×50 mm internal diameter (ID), 2.5 μm bead size; Waters Corporation, Milford, Massachusetts, USA] protected by a SecurityGuard C18 cartridge (4×2.0 mm ID; Phenomenex, Torrance, California, USA) and maintained at room temperature. The mobile phase consisted of an aqueous component (A) of 20 mmol/l ammonium formate in Milli-Q water, pH 2.2 (with formic acid), and an organic component (B) of acetonitrile with 1% formic acid. The 3.5-min run consisted of the following linear gradient elution: 95% A and 5% B at 0 min, 95% A and 5% B at 0.25 min, 25% A and 75% B at 0.35 min, 25% A and 75% B at 3.0 min, 95% A and 5% B at 3.1 min, and 95% A and 5% B at 3.5 min. The system operated at a flow rate of 0.75 ml/min.

Mass spectrometric detection was performed on an API 3200 triple quadrupole instrument (Applied Biosystems Inc., Foster City, California, USA) using multiple reaction monitoring (MRM). Ions were generated in the positive ionization mode using an electrospray interface. Lapatinib compound-dependent parameters were as follows: declustering potential (DP): 60V; entrance potential (EP): 10V; collision cell entrance potential (CEP): 21V; collision energy (CE): 51V; and collision cell exit potential (CXP): 5.8V GW572016AH (internal standard) compound-dependent parameters were as follows: DP: 67V; EP: 7.5 V; CEP: 23 V; CE: 49 V; and CXP: 5.5 V Source-dependent parameters were as follows: nebulizer gas (GS1): 50psi; auxiliary (turbo) gas (GS2): 60psi; turbo gas temperature (TEM): 500°C; curtain gas [15]: 10 psi; collision-activated dissociation gas (nitrogen) (CAD): 6 psi; ionspray voltage (IS): 5000 V; and interface heater (IH): 500°C. Peak area ratios obtained from MRM of lapatinib (m/z 581→365.1) and GW572016AH (m/z 587→367) were used for quantification.

Docetaxel high-pressure liquid chromatography-tandem mass spectrometry analysis

Analysis of docetaxel in plasma and tissues was carried out using HPLC/MS/MS analysis on the basis of a method developed previously in our laboratory [16,17], modified as follows: briefly, docetaxel was extracted from plasma by adding 1000 µl of ethyl acetate to 100 µl of unknown sample plasma, vortexing for 10 min, and centrifuging at 18 000g for 10 min at 4°C. A volume of 800 µl of the organic phase was collected and evaporated to dryness using a rotary evaporator. Dried samples were reconstituted in 200 µl of 80/20 0.1% formic acid in water/acetonitrile, vortexed for 10 min, and centrifuged at 18 000g for 10 min at 4°C. An aliquot of 60 µl of the supernatant was injected into the LC/MS/MS system for analysis. Tissues were homogenized at 100 mg/ml in water and 100 µl of the homogenates was extracted using the method for plasma detailed above. Standards and quality control samples were prepared in the appropriate matrix and analyzed as described above.

The HPLC and autosampler systems were the same as those used with lapatinib. The HPLC column was a Waters Sunfire C8 column (2.1×150 mm ID, 5.0 µm bead size; Waters Corporation) protected by a Security-Guard C18 cartridge (4 × 2.0 mm ID; Phenomenex) and maintained at room temperature. The mobile phase consisted of an aqueous component (A) of 0.1% formic acid in Milli-Q water and an organic component (B) of acetonitrile. The 4.0 min run consisted of the following linear gradient elution: 50% A and 50% B at 0 min, 50% A and 50% B at 0.5 min, 2% A and 98% B at 1.25 min, 2% A and 98% B at 3.0 min, 50% A and 50% B at 3.5 min, and 50% A and 50% B at 4.0 min. The system operated at a flow rate of 0.5 ml/min.

The mass spectrometric system was the same as that used with lapatinib. Docetaxel compound-dependent parameters were as follows: DP: 21V; EP: 4.5 V; CEP: 71V; CE: 23V; and CXP: 3.5 V Source-dependent parameters were as follows: GS1: 40 psi; GS2: 60 psi; TEM: 400°C; CUR: 30 psi; CAD: 2 psi; IS: 4500V; and IH: 500°C. Peak area ratios obtained from MRM of docetaxel (m/z 808.5→226) were used for quantification.

Doxorubicin high-pressure liquid chromatography-fluorescence analysis

Analysis of doxorubicin in plasma and tissues was carried out using HPLC-fluorescence analysis on the basis of a method developed previously in our laboratory [18,19], modified as follows: briefly, doxorubicin was extracted from plasma by adding 600 μ l of methanol and 10 μ l of internal standard (100 ng/ml daunorubicin) to 100 μ l of unknown sample plasma, vortexing for 10 min, adding 250 μ l of 12 mmol/l phosphoric acid, vortexing for 10 min, and centrifuging at 18 000g for 10 min at 4°C. An aliquot of 100 μ l of the supernatant was injected into the HPLC system for analysis. Tissues were homogenized at 100 mg/ml in water and 100 μ l of the homogenates were extracted using the method for plasma detailed above. Standards and quality control samples were prepared in the appropriate matrix and analyzed as described above.

The HPLC system consisted of a Shimadzu prominence LC-20AD binary pump, a prominence DGU-20A₃ vacuum degasser, a prominence CTO-20A column oven, a prominence SIL-20AC autosampler, a prominence CBM-20A communications bus module, and an RF-10A_{XL} fluorescence detector with excitation and emission wavelengths set at 480 and 580 nm, respectively (Shimadzu, Columbia, Maryland, USA). The HPLC column was a Waters Sunfire C18 column (4.6 $\times$ 50 mm ID, 2.5 μ m bead size; Waters Corporation) protected by a SecurityGuard C18 cartridge (4 $\times$ 2.0 mm ID; Phenomenex) and maintained at room temperature. The mobile phase consisted of an aqueous component (A) of 15 mmol/l sodium phosphate in Milli-Q water, pH 2.2 (with orthophosphoric acid), and an organic component (B) of acetonitrile. The 7.5 min run consisted of the following linear gradient elution: 80% A and 20% B at 0 min, 80% A and 20% B at 1.5 min, 50% A and 50% B at 6.5 min, 80% A and 20% B at 7.0 min, and 80% A and 20% B at 7.5 min. The system operated at a flow rate of 0.75 ml/min.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using noncompartmental modeling performed using Microsoft Excel and standard equations for noncompartmental analysis.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism v5.01 (GraphPad Software, San Diego, California, USA). For the comparison of concentration means, two-tailed unpaired *t*-tests were used.

Results

Combination lapatinib and chemotherapy clinical trials

To determine the effect of lapatinib on the pharmacokinetics of multiple classes of chemotherapeutics in humans, we reviewed all phase I clinical trials to date that involved drugs administered in combination with lapatinib and included pharmacokinetic data. In these eight clinical trials [15,20–26], the plasma pharmacokinetics of eleven drugs and metabolites were reported; only three of these compounds showed statistically significant alterations in pharmacokinetic parameters upon concomitant administration with lapatinib (Table 1). When dosed with lapatinib, the plasma area under the concentration-time curve

(AUC) [27] of SN-38 and topotecan increased by 45 and 18%, respectively. The authors of both studies suggested that the decreased clearance was likely because of the interaction of lapatinib with efflux transporters, particularly PGP. Lapatinib has been shown to be both a substrate for PGP and breast cancer resistance protein and an inhibitor of PGP, BCRP, and organic anion transporting polypeptide 1B1 (OATP1B1) [28]. As an inhibitor, lapatinib could prevent PGP from transporting xenobiotics out of the cell, thus increasing exposure to compounds that are PGP substrates. As a substrate, lapatinib could act as a competitor for PGP efflux.

To determine whether the other chemotherapeutics used in the clinical trials are PGP substrates, we utilized Althotas Virtual Laboratory [29]. The support vector machine method predicted that 4/10 compounds assessed are substrates of PGP (Table 1). Of these, two drugs (SN-38 and topotecan) showed an increase in exposure when administered with lapatinib, whereas two (irinotecan and docetaxel) did not.

To further investigate the relationship with PGP, we also used Althotas Virtual Laboratory [29] to calculate the docking energies of human PGP-ligand interactions. The lowest free energy of docking to PGP for each compound is presented in Table 1. In comparison, the lowest free energy of docking to PGP for lapatinib is -10.3 kcal/mol. The significance of these energies is unclear. The geometries of the human PGP-ligand interactions are shown in Supplementary Figs 1 and 2 (Supplemental digital content 1, <http://links.lww.com/ACD/A15>).

In the human clinical trials, although the plasma pharmacokinetics were altered for only 27.3% of the compounds evaluated, all combination regimens caused an increase in toxicity. In six of the trials, dose reduction [15,21,22,24,25] or the addition of pegfilgrastim [23] was warranted. Thus, the plasma pharmacokinetic data were not indicative of tissue pharmacokinetics or toxicodynamics.

Currently, there are 114 breast cancer clinical trials involving concomitant lapatinib (<http://www.clinicaltrials.gov>). Of all trials, 69% ($n = 79$) involve another drug that is a PGP substrate (as determined by Althotas Virtual Laboratory [29]). Of these, 57% ($n = 45$) include a taxane (docetaxel or paclitaxel) and/or an anthracycline (doxorubicin or epirubicin) (Supplementary spreadsheet 1, Supplemental digital content 2, <http://links.lww.com/ACD/A16>). Hence, taxanes and anthracyclines are commonly administered with lapatinib for the treatment of breast cancer and are also PGP substrates. Therefore, we chose to further explore the plasma and tissue pharmacokinetics of docetaxel and doxorubicin when administered in combination with lapatinib in mice.

Human equivalent dosing of lapatinib in mice

For the subsequent combination studies, our aim was to administer a dose of lapatinib to mice that would result in plasma exposure equivalent to the steady-state plasma exposure in humans when administered the recommended dose of lapatinib (1250 mg/day). We determined that dosing mice intraperitoneally with 60 mg/kg lapatinib $q3h \times 5$ resulted in C_{max} and C_{min} of lapatinib that were similar to human peak (2430 ng/ml at 4 h) and trough levels (1000ng/ml) (Fig. 1a). Extrapolating the mouse steady-state concentrations (achieved

after five doses) out to 24 h, this dosing regimen resulted in an AUC of 39.9 $\mu\text{g/ml h}$, which is comparable with both the calculated human AUC_t of 41.2 $\mu\text{g/ml h}$ (Fig. 1b) and the observed human geometric mean AUC_t of 36.2 $\mu\text{g/ml h}$ [30]. Accordingly, we used this mouse dosing regimen for the following combination studies.

Combination lapatinib and docetaxel studies in mice

Two time-course plasma and tissue distribution studies of combination lapatinib and docetaxel were carried out in female FVB mice, which were administered either a single or multiple ($q3h \times 5$) intraperitoneal 60 mg/kg doses of lapatinib. In both experiments, a single intravenous injection of 3 mg/kg docetaxel was administered 1 h after the first lapatinib dose. Samples were collected at 1, 2, 4, 8, and 12 h after docetaxel administration.

After a single dose of lapatinib, there was a statistically significant increase in the concentration of docetaxel in kidney at 1h (10.6%) and 12h (18.3%), intestine at 2h (72.4%), and adipose tissue at 8h (41.1%) versus docetaxel following vehicle. After multiple doses of lapatinib, there was a statistically significant increase (vs. vehicle) in the concentration of docetaxel in the kidney at 8h (25.5%), intestine at 4h (19.4%) and 8h (89.7%), muscle at 8 h (23.4%), and plasma at 8 h (21.8%) (Fig. 2).

In terms of exposure, combination therapy resulted in more than 25% increases in intestine and adipose tissue (Table 2). In the intestine, there was a 32.8 and 44.6% increase after single-dose and multiple-dose lapatinib, respectively. In adipose tissue, there was a 35.4 and a 25.2% increase after single-dose and multiple-dose lapatinib, respectively.

In addition to exposure, we also evaluated the effect of lapatinib on docetaxel concentration-time curve shape parameters by comparing half-life and $C_{\max}$ values (Supplementary Tables 1 and 2, Supplemental digital content 3, <http://links.lww.com/ACD/A17> and Supplemental digital content 4, <http://links.lww.com/ACD/A18>). For the former, half-lives differed by $\pm 25\%$ after multiple-dose lapatinib in muscle (-42.5%) and brain ($+144.0\%$). However, for terminal half-life calculations using nonlinear regression, our curve comprised only three time points (4, 8, and 12 h) and for the brain and muscle multiple-dose lapatinib curves, the r^2 (weighted) good-ness-of-fit values for the regression lines were suboptimal. Specifically, in muscle, the r^2 (weighted) values were 0.5740 and 0.6994 for docetaxel alone and combination lapatinib and docetaxel, respectively. In the brain, the r^2 (weighted) values were 0.8515 and 0.5249 for docetaxel alone and combination lapatinib and docetaxel, respectively. Thus, the half-life calculations from these curves are flawed and, as such, the differences are likely misrepresentations. With respect to $C_{\max}$ values, there were no statistically significant differences.

Plasma concentrations of lapatinib in the combination single-dose lapatinib and docetaxel study and the multiple-dose lapatinib and docetaxel study are shown in Fig. 1c and d, respectively. In the single-dose lapatinib and docetaxel study, the $C_{\max}$ (700 ng/ml) was below the human trough concentration. In the multiple-dose lapatinib and docetaxel study, all three lapatinib concentrations measured were within the targeted range [between the human steady-state $C_{\max}$ and $C_{\min}$ following the recommended dose of lapatinib (1250 mg/day)].

Combination lapatinib and doxorubicin studies in mice

Two time-course plasma and tissue distribution studies of combination lapatinib and doxorubicin were carried out in female FVB mice, which were administered either a single or multiple (q3h $\times$ 5) intraperitoneal 60 mg/kg doses of lapatinib. In both experiments, a single intravenous injection of 6 mg/kg doxorubicin was administered 1 h after the first lapatinib dose. Samples were collected at 1, 2, 4, 8, 12, 24, and 48 h after doxorubicin administration.

After a single dose of lapatinib, there was a statistically significant increase in the concentration of doxorubicin in adipose tissue at 4h (65.5%) and a statistically significant decrease at 24 h (40.4%) versus doxorubicin following vehicle (Fig. 3). There were no statistically significant differences in doxorubicin concentrations in plasma or tissues after multiple-dose lapatinib versus vehicle. Doxorubicin levels in the brain could not be evaluated because all sample peaks were below our lower limit of quantification (50 ng/g).

In terms of exposure, the only change greater than $\pm 25\%$ was a decrease in adipose tissue (26.0%) after multiple-dose lapatinib (Table 3). There was also a 46.2% decrease and a 28.6% increase in adipose tissue terminal half-life (calculated from the 12, 24, and 48 h time points) after single-dose and multiple-dose lapatinib, respectively. Half-life values for plasma and tissues are shown in Supplementary Table 3 (Supplemental digital content 5, <http://links.lww.com/ACD/A19>). As with docetaxel, these half-lives are likely distorted as the r^2 (weighted) values for these regression lines were substandard. As for $C_{\max}$ values, we found no statistically significant differences (Supplementary Table 4, Supplemental digital content 6, <http://links.lww.com/ACD/A20>).

Plasma concentrations of lapatinib in the combination single-dose lapatinib and doxorubicin study and the multiple-dose lapatinib and doxorubicin study are shown in Fig. 1e and f, respectively. In the single-dose lapatinib and doxorubicin study, the $C_{\max}$ (553 ng/ml) was below the human trough concentration. In the multiple-dose lapatinib and doxorubicin study, all three lapatinib concentrations measured during the multiple dosing period were within the targeted range [between the human steady-state $C_{\max}$ and $C_{\min}$ following the recommended dose of lapatinib (1250mg/day)].

Discussion

Cytotoxic and biologic combinations for the treatment of metastatic breast cancer have been approved by the US Food and Drug Administration and several investigational drug combinations are currently undergoing evaluation in clinical trials [1]. Although there are clear advantages to combining therapies, there is also the potential disadvantage of increasing the toxicity burden to the patient, with only moderate improvements in efficacy and benefit [2]. In our evaluation of eight clinical trials involving coadministration of lapatinib with cytotoxic agents, all combinations caused an increase in toxicity versus the regimen without lapatinib, indicating that concomitant administration increased tissue drug exposure beyond a tolerable level. However, plasma pharmacokinetics were altered for only 27.3% of the compounds evaluated, showing that chemotherapeutic concentrations in plasma alone were not indicative of adverse drug-drug interactions in tissues.

Drug–drug interactions are often mediated by competition for or inhibition of efflux proteins. As lapatinib is both a substrate for and inhibitor of PGP [28], we combined this drug with cytotoxic agents that are PGP substrates and used clinically in conjunction with lapatinib for the treatment of metastatic breast cancer. Our study of lapatinib and docetaxel in mice showed that coadministration resulted in intestinal docetaxel exposure increases of 32.8 and 44.6% after single-dose and multiple-dose lapatinib, respectively. Although we did not evaluate toxicodynamics because of the short duration of our pharmacokinetic studies (12 h), this amplified intestinal exposure likely would have clinical ramifications, as the gastrointestinal tract is a major site of reported docetaxel-related adverse events. In patients treated with docetaxel as a single agent for various tumor types ($n=2045$), nausea (39%), diarrhea (39%), and vomiting (22%) were observed; other gastrointestinal events included anorexia, taste perversion, constipation, abdominal pain, gastrointestinal bleeding, and esophagitis [31].

In terms of the increased docetaxel exposure in adipose tissue (35.4% and a 25.2% after single-dose and multiple-dose lapatinib, respectively), this may also have clinically significant consequences, given that adipose tissue could theoretically serve as a reservoir of docetaxel (as many lipid-soluble drugs are stored in fat) and thereby contribute toward the significant increases in plasma, kidney, muscle, and intestine docetaxel concentrations at later time points (8 and 12 h).

In a phase I study of lapatinib and docetaxel in patients with advanced cancer, the plasma pharmacokinetics of both compounds in combination were not significantly different from the drug profiles when administered separately; however, there was an increase in toxicity [23]. Specifically, the drug-related adverse events reported by most patients were diarrhea (56%), rash (52%), fatigue (27%), and nausea (25%). The authors could not characterize the diarrhea, nausea, or rash as specific to either lapatinib or docetaxel but, in light of the data from our mouse study, we can conjecture that the gastrointestinal toxicities were likely because of an increase in docetaxel exposure in the enterocytes. Neutropenia, a frequent toxicity associated with docetaxel, also occurred during the phase I trial and necessitated the addition of pegfilgrastim to the dosing regimen. The authors suggest that lapatinib increased the sensitivity to this toxicity, possibly by inhibiting PGP-mediated efflux of docetaxel from bone marrow stem cells [23].

In contrast to docetaxel, lapatinib did not significantly alter the pharmacokinetics of doxorubicin in plasma or tissues associated commonly with doxorubicin-related toxicity, such as the heart, intestine, and liver [32]. However, increases in doxorubicin $AUC_{0\rightarrow 24h}$ in these tissues (24% in the heart, 65% in the intestine, and 339% in the liver) were observed in mice lacking *mdrla* versus wild-type mice, implicating PGP as a causative factor in the alteration of doxorubicin pharmacokinetics in these tissues. This proposition is further support by additional rodent combination studies of doxorubicin with PGP inhibitors cyclosporin A [33,34] and SDZ PSC 833 [35], in which coadministration resulted in significant increases in tissue levels of doxorubicin. Thus, our study suggests that lapatinib is a weaker inhibitor of PGP than cyclosporin A and SDZ PSC 833. MDCKII-MDR1 monolayer efflux studies using 3H-digoxin as a probe substrate reported half-maximal inhibitory concentrations of 3.9 [28] and 1.6 $\mu\text{mol/l}$ [36] for lapatinib and cyclosporin A,

respectively, indicating that cyclosporin A is ~2.5 times more potent than lapatinib as a PGP inhibitor.

In addition to altering the pharmacokinetics of doxorubicin, cyclosporin A has also been shown to increase the plasma exposure of oral docetaxel nine-fold [37]. However, only a three-fold increase was observed when docetaxel was administered per os to *mdr1a/1b* (–/–) mice compared with wild-type mice [38], suggesting that PGP inhibition was not the major factor accountable for the magnified systemic AUC when docetaxel was administered in combination with cyclosporin A. Alternatively, the increase in exposure was likely more resultant of competitive inhibition of cytochrome P450 enzymes by cyclosporin A, as both this immunosuppressant and docetaxel are substrates for CYP3A4 [39–41]. In mice, this is evidenced by a 12-fold plasma docetaxel exposure increase in *cyp3a* (–/–) versus wild-type mice after oral dosing [38]. Moreover, after docetaxel dosing, the *cyp3a* (–/–) mice showed moderate toxicity in the small intestine, whereas this was only mild in *mdr1a/b* (–/–) mice [38].

Further evidence that CYP3A metabolism plays a more important role than PGP-mediated efflux in docetaxel elimination comes from studies of an intravenous injection of docetaxel in wild-type and *mdr1a/b* (–/–) mice, which resulted in no difference in systemic exposure to docetaxel [37]. Coadministration of cyclosporin A, however, increased plasma docetaxel AUC by three-fold in both wild-type and *mdr1a/b* (–/–) mice [37], presumably because of the effect of cyclosporin A on docetaxel metabolism by CYP3A4.

Similar to cyclosporin A, lapatinib is not only an inhibitor of PGP but this targeted agent is also a CYP3A4 substrate and inhibitor [30,42]. As the latter, we propose that lapatinib competitively inhibits docetaxel intestinal metabolism by CYP3A4 and, consequently, is responsible for the considerable increase in docetaxel exposure that we observed in the small intestine of mice. A similar escalation was not observed in the liver because hepatic CYP3A is much more abundant than intestinal CYP3A, which is only ~2% of that in the liver [43–45]. Thus, these metabolic enzymes in the liver are not as susceptible to saturation as those in the small intestine. However, the importance of intestinal CYP3A metabolism of docetaxel should not be understated and is illustrated by a 16.6-fold versus a 2.2-fold decrease in docetaxel plasma exposure after oral administration to *cyp3a* (–/–) mice with human CYP3A4 in only the intestine or only the liver, respectively [46]. In contrast to docetaxel, a CYP3A4-mediated effect of lapatinib on doxorubicin exposure was not noted because this anthracycline is primarily metabolized to doxorubicinol by cytoplasmic aldo-keto and carbonyl reductases [47,48].

In conclusion, coadministration of lapatinib with doxorubicin did not appreciably alter the pharmacokinetics of this cytotoxic in the plasma or six tissues evaluated in mice, presumably because, at doses relevant to human exposure, lapatinib inhibition of PGP did not significantly alter doxorubicin export from these compartments and lapatinib inhibition of CYP3A4 was inconsequential for doxorubicin metabolism to doxorubicinol. However, combining lapatinib with docetaxel markedly increased intestinal exposure to this chemotherapeutic, which has clinical implications for enhancing gastrointestinal toxicity. The significant lapatinib-docetaxel interaction is likely CYP3A4-mediated and thus, our

study suggests that caution should be exercised when this combination is administered, particularly to patients with compromised CYP3A activity. As coadministration of these two agents is protocol for clinical trials that are either recruiting or active, we recommend closely monitoring the recipients of combined lapatinib and docetaxel for enhanced toxicity, particularly for adverse effects on the intestine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Lapatinib concentrations in mouse plasma. (a) Time course of maximum and minimum lapatinib concentrations after five 60 mg/kg intraperitoneal doses (at times 0, 3, 6, 9, and 12 h). Maximum and minimum concentrations were achieved 1 and 3 h after dose, respectively. Filled black diamonds represent the mean concentrations and error bars represent SDs ($n = 3$). (b) Filled black diamonds and error bars as in (a). Open gray diamonds represent extrapolated maximum and minimum lapatinib concentrations with continued every 3h (q3h) dosing after achievement of steady state. Filled black circles represent human steady-state maximum (2430 ng/ml) and minimum (1000 ng/ml) concentrations (achieved 4 and 24 h after dose, respectively). (c) Time course of lapatinib concentrations after a single dose of 60 mg/kg intraperitoneal lapatinib administered at time -1 h (arrow), followed by a single dose of 3 mg/kg intravenous docetaxel administered at time 0 h. Filled black diamonds represent the mean concentrations and error bars represent SDs ($n = 3$). (d) Time course of lapatinib concentrations after five 60 mg/kg intraperitoneal doses [at times -1 , 2, 5, 8, and 11 h (arrows)], followed by a single dose of 3 mg/kg intravenous docetaxel administered at time 0 h. Filled black diamonds represent the mean concentrations and error bars represent SDs ($n = 3$). (e) Time course of lapatinib concentrations after a single dose of 60 mg/kg intraperitoneal lapatinib administered at time -1 h (arrow), followed by a single dose of 6 mg/kg intravenous doxorubicin administered at time 0 h. Filled black diamonds represent

the mean concentrations and error bars represent SDs ($n = 3$). (f) Time course of lapatinib concentrations after five 60 mg/kg intraperitoneal doses [at times – 1, 2, 5, 8, and 11 h (arrows)], followed by a single dose of 6 mg/kg intravenous doxorubicin administered at time 0 h. Filled black diamonds represent the mean concentrations and error bars represent SDs ($n = 3$). In all graphs, dashed lines represent the human steady-state maximum concentration (2430 ng/ml) and dotted lines represent the human steady-state minimum concentration (1000 ng/ml) after administration of the recommended dose of lapatinib (1250 mg/day).

Fig. 2.

(a–i) Time courses of docetaxel concentrations in mouse plasma and tissues after a single dose of 3 mg/kg intravenous docetaxel administered at time 0 h. For the single-dose lapatinib study, 1 h before docetaxel administration (at time – 1 h), mice were administered either single-dose intraperitoneal vehicle (solid white bars) or single-dose 60 mg/kg intraperitoneal lapatinib (horizontally striped bars). For the multiple-dose lapatinib study, 1 h before docetaxel administration (at time – 1 h) and again at times 2, 5, 8, and 11 h, mice were administered either intraperitoneal vehicle (solid black bars) or 60 mg/kg intraperitoneal lapatinib (diagonally striped bars). All bars represent the mean concentrations and error bars represent SDs ($n = 3$). Asterisks represent statistically significant differences ($P < 0.05$).

Fig. 3. (a–g) Time courses of doxorubicin concentrations in mouse plasma and tissues after a single dose of 6 mg/kg intravenous doxorubicin administered at time 0 h. For the single-dose lapatinib study, 1 h before doxorubicin administration (at time – 1 h), mice were administered either single-dose intraperitoneal vehicle (solid white bars) or single-dose 60 mg/kg intraperitoneal lapatinib (horizontally striped bars). For the multiple-dose lapatinib study, 1 h before doxorubicin administration (at time – 1 h) and again at times 2, 5, 8, and 11 h, mice were administered either intraperitoneal vehicle (solid black bars) or 60 mg/kg intraperitoneal lapatinib (diagonally striped bars). All bars represent the mean concentrations and error bars represent SDs ($n = 3$). Asterisks represent statistically significant differences ($P < 0.05$).

Table 1

Clinical, pharmacokinetic, and p-glycoprotein evaluation of drugs administered in combination with lapatinib in phase I clinical trials

Clinical trials	Drug	Number of patients	Lapatinib dose (mg/day)	Increased toxicity	PK parameter	Statistically significant % change ^d	PGP substrate ^b	Docking energy (kcal/mol) ^c	References
Lapatinib + FOLFIRI	Irinotecan	12	1250	Yes	AUC _∞ , CL, V _{SS}	None	Yes	-12.0	24
Lapatinib + FOLFIRI	SN-38	12	1250	Yes	AUC _{0-24h}	+44.9	Yes	-10.0	24
Lapatinib + FOLFIRI	SN-38	12	1250	Yes	C _{max}	+27.0	Yes	-10.0	24
Lapatinib + topotecan	Topotecan	9	1250	Yes	AUC _{0-24h}	+18.1	Yes	-9.4	20
Lapatinib + topotecan	Topotecan	9	1250	Yes	CL	-15.7	Yes	-9.4	20
Lapatinib + topotecan	Topotecan	9	1250	Yes	C _{max} t _{1/2} , V _{SS}	None	Yes	-9.4	20
Lapatinib + docetaxel	Docetaxel	8	1250	Yes	AUC _∞ , CL, V _{SS}	None	Yes	-9.2	22
Lapatinib + letrozole	Letrozole	8	1500	Yes	AUC _{0-24h} , C _{max} , T _{max} , C _t	None	No	NA	19
Lapatinib + capecitabine	Capecitabine	19	1250	Yes	AUC _{0-24h} , C _{max} , T _{max}	None	No	NA	25
Lapatinib + FOLFOX4	Unbound platinum	17	1500	Yes	AUC, C _{max} , T _{max} , t _{1/2} , CL, V _{SS}	None	No	NA	26
Lapatinib + FOLFIRI	5-Fluorouracil	12	1250	Yes	C _{ss}	None	No	NA	24
Lapatinib + capecitabine	5-Fluorouracil	19	1250	Yes	AUC, T _{max}	None	No	NA	25
Lapatinib + capecitabine	5-Fluorouracil	19	1250	Yes	C _{max}	-20.6	No	NA	25
Lapatinib + FOLFOX4	5-Fluorouracil	17	1500	Yes	C _{ave} , CL	None	No	NA	26
Lapatinib + carboplatin	Carboplatin	10	750	Yes	AUC	None	No	NA	21
Lapatinib + capecitabine	α-Fluoro-β-alanine	19	1250	Yes	AUC, C _{max} , T _{max}	None	No	NA	25
Lapatinib + trastuzumab	Trastuzumab	27	1000	Yes	AUC _{0-24h} , C _{max}	None	NA ^d	NA ^d	23

AUC, area under the concentration-time curve; AUC_{0-24h}, area under the concentration-time curve from 0 to 24 h; AUC_∞, area under the concentration-time curve from 0 to infinity; AUC_t, area under the concentration-time curve within a steady-state dosing interval; C_{ave}, time-averaged concentration at steady state; CL, clearance; C_{max}, maximum concentration; C_{ss}, concentration at steady state; C_t, concentration at the end of a dosing interval; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; FOLFOX4, oxaliplatin, leucovorin, and 5-fluorouracil; PGP, p-glycoprotein; t_{1/2}, half-life; T_{max}, time of maximum concentration; V_{SS}, volume of distribution at steady state; NA, not applicable.

^aPercentage change was calculated as 100×[(combinationAUC) - (single agent AUC)/single agent AUC].

^bPGP substrate determination of the ligand in the column 2 was calculated using the support vector machine method at <http://pgp.althotas.com> (21991360).

^cDocking energy of ligand in the column 2 with human PGP was calculated at <http://pgp.althotas.com> (21 991360).

Althotas (21991360) did not have information available on trastuzumab.^p

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Table 2
Comparison of AUCs from combination lapatinib and docetaxel pharmacokinetic studies in mice

Samples	Single-dose vehicle + docetaxel AUC _{0-12h}	Single-dose lapatinib + docetaxel AUC _{0-12h}	% Change ^d	Multiple-dose vehicle + docetaxel AUC _{4-12h}	Multiple-dose lapatinib + docetaxel AUC _{4-12h}	% Change ^d
Lung ^b	15085	15442	+2.4	9211	9987	+8.4
Kidney ^b	12303	12974	+5.5	5930	6964	+17.4
Heart ^b	9989	9481	-5.1	5601	5548	-0.9
Muscle ^b	5283	4878	-7.7	3084	3546	+15.0
Intestine ^b	3511	4664	+32.8	1957	2830	+44.6
Liver ^b	3096	3471	+12.1	1517	1668	+10.0
Adipose ^b	1911	2587	+35.4	1351	1691	+25.2
Plasma ^c	378.6	423.2	+11.8	179.5	185.3	+3.2
Brain ^b	167.9	162	-3.5	91.45	88.01	-3.8

AUC_{0-12h}, area under the concentration-time curve from 0 to 12 h; AUC_{4-12h}, area under the concentration-time curve from 4 to 12 h.

^a Percentage change was calculated as $100 \times [(\text{lapatinib} + \text{docetaxel AUC}) - (\text{vehicle} + \text{docetaxel AUC}) / (\text{vehicle} + \text{docetaxel AUC})]$.

^b Tissue AUC values are ng/g h.

^c Plasma AUC values are ng/ml h.

Table 3
Comparison of AUCs from combination lapatinib and doxorubicin pharmacokinetic studies in mice

Samples	Single-dose vehicle + doxorubicin AUC _{0-48h}	Single-dose lapatinib + doxorubicin AUC _{0-48h}	% Change ^d	Multiple-dose vehicle + doxorubicin AUC _{4-48h}	Multiple-dose lapatinib + doxorubicin AUC _{4-48h}	% Change ^d
Kidney ^b	238066	225903	-5.1	194490	204623	+5.2
Lung ^b	222640	211633	-4.9	169515	175490	+3.5
Liver ^b	126709	130778	+3.2	78636	87168	+10.8
Heart ^b	74829	71633	-4.3	52493	52137	-0.7
Intestine ^b	68936	62982	-8.6	50136	58130	+15.9
Adipose ^b	21058	17796	-15.5	15555	11506	-26.0
Plasma ^c	490.4	543.9	+10.9	343.3	369.0	+7.5

AUC_{0-48h}, area under the concentration-time curve from 0 to 48 h; AUC_{4-48h}, area under the concentration-time curve from 4 to 48 h.

^dPercentage change was calculated as $100 \times [(lapatinib+doxorubicinAUC) - (vehicle+doxorubicinAUC) / (vehicle+doxorubicinAUC)]$.

^bTissue AUC values are ng/g h.

^cPlasma AUC values are ng/ml h.