Comparison of the Drug-Drug Interactions Potential of Erlotinib and Gefitinib via Inhibition of UDP-Glucuronosyltransferases

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ABSTRACT:

We aimed to investigate and compare the effects of erlotinib and gefitinib on UDP-glucuronosyltransferase (UGT) activities and to quantitatively evaluate their drug-drug interaction (DDI) potential due to UGT inhibition. The inhibitory effects of erlotinib and gefitinib on UGTs were determined using high-performance liquid chromatography by measuring the formation rates for 4-methylumbelliferone (4-MU) glucuronide, imipramine *N*-glucuronide, and bilirubin glucuronides using recombinant human UGT isoforms and human liver microsomes (HLMs) in the absence or presence of erlotinib and gefitinib. Inhibition kinetic studies were conducted. Area under the curve (AUC) ratios were used to predict the risk of potential DDI in vivo. Erlotinib exhibited selective potent competi-

tive inhibition against 4-MU glucuronidation by UGT1A1, and gefitinib demonstrated a wide range of inhibition against UGT-mediated 4-MU glucuronidation, particularly against UGT1A1, UGT1A7, UGT1A9, and UGT2B7. Erlotinib also exerted potent mixed inhibition against bilirubin glucuronidation in HLMs. We estimated that coadministration of erlotinib at 100 mg/day or higher doses may result in at least a 30% increase in the AUC of drugs predominantly cleared by UGT1A1. Thus, the coadministration of erlotinib with drugs primarily cleared by UGT1A1 may result in potential DDI. In contrast, gefitinib is unlikely to cause a clinically significant DDI through inhibition of glucuronidation.

Erlotinib and gefitinib are potent, reversible, and selective inhibitors of the tyrosine kinase of the epidermal growth factor receptor. Both drugs have been approved for the treatment of patients with non-small-cell lung cancer, and erlotinib is also indicated for pancreatic cancer. In addition, phase II trials have suggested activity for these agents in a number of other solid tumors (Sequist and Lynch, 2008). Gefitinib and erlotinib share a common chemical backbone structure and exhibit similar oral bioavailability and disposition characteristics in humans after oral administration (Siegel-Lakhai et al., 2005).

Drug-drug interactions (DDI) have received increasing attention over the past few decades. Several DDIs were reported to be associated with erlotinib and gefitinib. Coadministration of erlotinib has been reported to enhance the carboplatin exposure (Patnaik et al., 2006) and increase the serum concentration of phenytoin (Grenader et al., 2007). Drug interactions were also observed in two patients who

received both gefitinib and warfarin simultaneously, which resulted in an enhancement of the warfarin effect (Onoda et al., 2005). Combination of gefitinib with capecitabine and radiation in patients with pancreatic and rectal cancer (Czito et al., 2006), as well as the combination of gefitinib with 5-fluorouracil, leucovorin, and irinotecan in patients with colorectal cancer (Veronese et al., 2005), are associated with excessive toxicity, suggesting an interaction at a pharmacokinetic or pharmacodynamic level.

Metabolizing enzyme-based DDI constitute the major proportion of clinically important DDI. Gefitinib and erlotinib are eliminated predominantly by oxidation by cytochrome P450 enzymes. Both drugs are metabolized primarily by CYP3A4 and CYP3A5. In addition, CYP1A2 is considerably involved in erlotinib metabolism, and CYP2D6 is involved in gefitinib metabolism (Li et al., 2007). Therefore, both drugs may alter the systemic exposure of some cytochromes P450 substrates, implying a potential DDI. However, it is unknown whether DDI by erlotinib and gefitinib are associated with other metabolizing enzymes.

UDP-glucuronosyltransferase (UGT) enzymes catalyze the conjugation of various endogenous substances and exogenous compounds. The human UGT superfamily comprises two families (UGT1 and UGT2). At least 19 human UGTs have been identified to date based on sequence homologies (Mackenzie et al., 2005). UGT-catalyzed glucuronidation reactions are responsible for the metabolism of approximately 35% of all drugs metabolized by phase II enzymes. Approximately one-seventh of the drugs prescribed in

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ABBREVIATIONS: DDI, drug-drug interactions; UGT, UDP-glucuronosyltransferase; HLMs, human liver microsomes; AUC, area under the curve; OSI-774, *N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine; 4-MU, 4-methylumbelliferone; 4-MUG, 4-methylumbelliferone- β -glucuronide; UDPGA, uridine 5'-diphosphoglucuronic acid; HPLC, high-performance liquid chromatography.

the United States in 2002 are cleared by UGTs (Williams et al., 2004). UGT-mediated drug interactions can potentially occur for many drugs. In fact, several significant DDI have been clinically observed. Erlotinib has been suggested to be an inhibitor of UGT1A1 [European Medicines Agency (EMA), <http://www.ema.europa.eu/humandocs/PDFs/EPAR/tarceva/H-618-PI-en.pdf>]. However, the effects of erlotinib and gefitinib on UGT activity have not been fully characterized. Understanding the effects of erlotinib and gefitinib on UGT activities is important to ensure their safe administration and develop new coadministration therapies with both drugs.

The aim of this study was to investigate and compare the effects of erlotinib and gefitinib on the activities of human UGTs. Using a panel of recombinant human UGT isoforms, we found potent inhibition of erlotinib against UGT1A1. On the other hand, gefitinib demonstrated a wide range of inhibition against not only UGT1A1 but also UGT1A7, UGT1A9, and UGT2B7. The effects of erlotinib and gefitinib on bilirubin glucuronidation were investigated in human liver microsomes (HLMs). The potential for DDI in vivo was also quantitatively predicted and compared by using area under the curve (AUC) ratios.

Materials and Methods

Chemicals. Erlotinib (OSI-774) was purchased from BIAFFIN GmbH and Co. KG (Kassel, Germany). Gefitinib and indinavir were purchased from Toronto Research Chemicals, Inc. (North York, ON, Canada). Bilirubin, 4-methylumbelliferone (4-MU), 4-methylumbelliferone- β -D-glucuronide (4-MUG), β -glucuronidase (from *Escherichia coli*), alamethicin (from *Trichoderma viride*), Tris-HCl, imipramine, *p*-nitrophenol, androsterone, diclofenac, phenylbutazone, ascorbic acid, hecogenin, 7-hydroxycoumarin, and uridine 5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of high-performance liquid chromatography (HPLC) grade or of the highest grade commercially available.

Human Liver Microsomes and Recombinant Human UGTs. Pooled HLMs and a panel of recombinant human UGT Supersomes (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17) expressed in baculovirus-infected insect cells were purchased from BD Gentest (Woburn, MA). Pooled HLMs were derived from 22 donors (90% white, 5% Hispanic, and 5% African-American; 15 men and 7 women). The median age was 48 years with a range of 10 to 70.

Inhibition of 4-MU Glucuronidation Assay. 4-MU, a nonselective substrate of UGTs, was used as probe substrate for all UGTs except UGT1A4. Incubations with each individual enzyme were conducted by using conditions described previously (Uchaipichat et al., 2004), with a slight modification. A typical incubation mixture (in a total volume of 200 μ l) contained recombinant UGTs (final concentration: 0.25, 0.15, 0.05, 0.05, 0.05, 0.05, 0.05, 0.5, 0.25, 0.75, and 0.5 mg/ml for UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17, respectively), 5 mM UDPGA, 5 mM MgCl₂, 50 mM Tris-HCl buffer (pH 7.4), 50 μ g/mg protein alamethicin, and 4-MU in the absence or presence of different concentrations of inhibitors. Incubations with 4-MU were performed at the concentration corresponding to the apparent K_m or S_{50} value reported for each isoform (110, 1200, 110, 15, 750, 30, 80, 1200, 350, 250, and 2000 μ M 4-MU for UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17, respectively) (Uchaipichat et al., 2006b). Known UGT inhibitors were used as positive controls: diclofenac for UGT1A1, UGT1A6, UGT1A7, and UGT1A9; androsterone for UGT1A3, UGT2B7, and UGT2B15; and phenylbutazone for UGT1A8 and UGT1A10, respectively (Uchaipichat et al., 2004, 2006a). There is no positive control reported available for UGT2B4 and UGT2B17. The negative controls are incubations without UDPGA. These tested chemicals and inhibitors were dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the incubation system was 1% (v/v). Alamethicin was dissolved in 90:10 incubation buffer/ethanol, and the final concentration of ethanol was less than 0.1%. Microsomes were preincubated with alamethicin on ice for 15 min before incubation. There was a 5-min preincubation step at 37°C before the

reaction was started by addition of UDPGA. Incubation times were 120 min for UGT1A1, UGT1A10, UGT2B4, UGT2B15, and UGT2B17, 75 min for UGT1A3, and 30 min for UGT1A6, UGT1A7, UGT1A8, and UGT1A9. The reactions were quenched by adding 100 μ l of acetonitrile and internal standard (7-hydroxycoumarin, 100 μ M). Rate of product formation for each isoform was linear with respect to protein concentration and incubation time. The incubation mixtures were then centrifuged at 20,500g for 15 min to obtain the supernatant. Aliquots (20 μ l) were then analyzed by HPLC (Hitachi High Technologies America, Schaumburg, IL). Chromatographic separation was achieved by using a C18 column (3.9 $\times$ 300 mm i.d., 10- μ m particle size) (Sigma-Aldrich) at a flow rate of 1 ml/min and UV detection at 316 nm. The mobile phase consisted of 10 mM KH₂PO₄, pH 2.7 (A), and acetonitrile (B). The following gradient was applied at a flow rate of 1 ml/min: 0 to 4 min 80% A and 20% B; 4.1 to 8 min 50% A and 50% B; 8.1 to 12 min, 30% A and 70% B. The metabolites were quantified by using a standard curve made by combining 4-MUG stock and incubation buffer and processing as described above. All experiments were performed in two independent experiments in duplicate.

Inhibition of Imipramine *N*-Glucuronidation Assay. Imipramine was used as probe substrate for UGT1A4. Imipramine *N*-glucuronidation activity was determined as published previously (Nakajima et al., 2002). Imipramine was incubated in the absence or presence of different concentrations of inhibitors. Hecogenin, a known UGT1A4 inhibitor, was used as positive control. Incubation was performed for 90 min by using a protein concentration of 0.5 mg protein/ml recombinant UGT1A4 or pooled HLMs. HPLC separation was achieved by using a C18 column (3.9 $\times$ 300 mm i.d., 10 μ m) (Sigma-Aldrich) at a flow rate of 1 ml/min and UV detection at 254 nm. The mobile phase consisted of 30:70 acetonitrile: 10 mM KH₂KO₄ buffer (pH 2.6) (v:v). For the quantification of imipramine *N*-glucuronide, it was assumed that the glucuronide of imipramine had the same molar absorbance as its aglycone, because no imipramine monoglucuronide standards were available. The quantification of the glucuronide was accomplished by using a standard curve for imipramine. All experiments were performed in two independent experiments in duplicate.

Inhibition of Bilirubin Glucuronidation Assay. Bilirubin glucuronidation activity was determined with a slight modification of a previously published method (Udomuksorn et al., 2007). Bilirubin was incubated in the absence or presence of different concentrations of erlotinib or gefitinib (0–100 μ M). Indinavir, a known inhibitor of bilirubin glucuronidation, was used as positive control. Reactions were performed for 20 min in 0.5 mg/ml pooled HLMs. HPLC separation was achieved by using a SymmetryShield RP8 column (3.0 $\times$ 150 mm i.d., 5- μ m particle size) (Waters, Milford, MA) with a flow rate of 1 ml/min and UV detection at 450 nm. Initial mobile-phase composition was 78% 100 mM ammonium acetate buffer at pH 4.85 (mobile phase A) and 22% acetonitrile (mobile phase B). The proportion of acetonitrile was increased to 44% over 20 min, then further increased to 90% over 1 min, and then held constant for 7 min before returning to the starting composition. Authentic standards of each of the bilirubin glucuronides were not available for use, so a bilirubin standard curve (0.1–5 μ M) was used to quantify glucuronide formation.

Inhibition Kinetics Analysis. Inhibition constant (K_i) values were determined by using various concentrations of 4-MU or bilirubin in the presence or absence of erlotinib and gefitinib. K_i values were calculated by nonlinear regression by using the equations for competitive inhibition (eq. 1), noncompetitive inhibition (eq. 2), or mixed inhibition (eq. 3) (Copeland, 2000):

$$v = (V_{\max}S)/(K_m(1 + I/K_i) + S) \quad (1)$$

$$v = (V_{\max}S)/(K_m + S)(1 + I/K_i) \quad (2)$$

$$v = (V_{\max}S)/(K_m + S)(1 + I/\alpha K_i) \quad (3)$$

where v is the velocity of the reaction; S and I are the substrate and inhibitor concentrations, respectively; K_i is the inhibition constant describing the affinity of the inhibitor for the enzyme; and K_m is the substrate concentration at half of the maximum velocity ($V_{\max}$) of the reaction. The type of inhibition was determined from the fitting of data to the enzyme inhibition models. Goodness of fit to kinetic and inhibition models was assessed from the F statistic, r^2 values, parameter S.E. estimates, and 95% confidence intervals. Kinetic con-

stants are reported as the mean $\pm$ S.E. of the parameter estimate. IC_{50} values (concentration of inhibitor that reduces enzyme activity by 50%) were determined by the Data Analysis Toolbox of Microsoft Excel software (Microsoft Inc., Redmond, WA).

Predicted Concentrations of Erlotinib and Gefitinib at UGT Catalytic Sites. The concentrations of erlotinib and gefitinib in humans were estimated according to the equations for the average systemic plasma concentration after repeated oral administration ($[I]_{av}$) (eq. 4), the maximum systemic plasma concentration after repeated oral administration ($[I]_{max}$) (eq. 5), the maximum unbound systemic plasma concentration after repeated oral administration ($[I]_{max,u}$) (eq. 6), the maximum hepatic input concentration ($[I]_{in}$) (eq. 7), and the maximum unbound hepatic input concentration ($[I]_{in,u}$) (eq. 8) (Ito et al., 2004):

$$[I]_{av} = (D/\tau)/(CL/F) \quad (4)$$

$$[I]_{max} = ([I]_{av} k \tau)/(1 - \exp(-k \tau)) \quad (5)$$

$$[I]_{max,u} = ([I]_{av} k \tau f_u)/(1 - \exp(-k \tau)) \quad (6)$$

$$[I]_{in} = [I]_{av} + k_a F_a D/Q_h \quad (7)$$

$$[I]_{in,u} = f_u ([I]_{av} + k_a F_a D/Q_h) \quad (8)$$

where D and τ represent dose and dosing interval of inhibitors used in the in vivo interaction study, respectively; k is the elimination rate constant; k_a is the absorption rate constant; F_a is the fraction absorbed from the gut into the portal vein; Q_h is the hepatic blood flow rate; and f_u is the unbound fraction. The values of k_a , F_a , and f_u for erlotinib and gefitinib were obtained from the literature (EMEA, <http://www.emea.europa.eu/humandocs/PDFs/EPAR/tarceva/H-618-PI-en.pdf>; Siegel-Lakhai et al., 2005; Frohna et al., 2006; Li et al., 2006; Rudin et al., 2008; Schaiquevich et al., 2008). Q_h was assumed to be 1610 ml/min (Ito et al., 2004).

Calculation of AUC_i/AUC Ratio. The magnitudes of inhibitory interactions of erlotinib and gefitinib were estimated as the ratio of the area under the plasma concentration-time curve in the presence and absence of the inhibitor (AUC_i/AUC). This ratio was predicted by using the formula (eq. 9) for oral administration of a high hepatic clearance drug (Ito et al., 2004):

$$AUC_i/AUC = 1/(f_m/(1 + [I]/K_i) + (1 - f_m)) \quad (9)$$

where AUC_i and AUC are the AUC in the presence and absence of inhibitor, respectively; K_i is inhibitor constant (obtained from in vitro experiments); f_m is the fraction metabolized by the inhibited enzyme; and $[I]$ is the inhibitor concentration at the enzyme active site.

In view of the general assumption that only unbound drug is available for interaction with the enzyme active site, and the consideration that the aim of DDI research is to exclude the highest risk, in this study we used the maximum unbound hepatic input concentration ($[I]_{in,u}$) as the inhibitor concentration at

the active site of the UGTs, with the exception of UGT1A7. Because UGT1A7 is present only in the esophagus, stomach, and lung (Strassburg et al., 1997), we used the maximum unbound systemic plasma concentration after repeated oral administration ($[I]_{max,u}$) as the inhibitor concentration at the enzyme active site in extrahepatic tissues. Because the fraction metabolized by the UGT isoforms inhibited by erlotinib or gefitinib (f_m) of the coadministered drug is unknown, we arbitrarily selected 0.1 to 1 to calculate the AUC_i/AUC ratio.

Results

Inhibition of UGT Activities by Erlotinib and Gefitinib in Recombinant Human UGTs. The IC_{50} values of the inhibitors of each UGT isoform were comparable with previously published data (Uchaipichat et al., 2004, 2006a). No glucuronidation of erlotinib and gefitinib was found in the course of the incubations.

As shown as Fig. 1, erlotinib (100 μ M) inhibited UGT1A1 activity, reducing 4-MU glucuronidation by 88.3% ($P < 0.01$). The inhibition by erlotinib was also observed against UGT1A3, UGT2B7, UGT1A9, UGT1A7, and UGT2B15, reducing 4-MU glucuronidation activities by 42.3, 32.8, 31.9, 27.4, and 18.1% at 100 μ M, respectively.

Likewise, gefitinib had an inhibitory effect against UGT1A1 activity, reducing glucuronidation by 79.1% at 100 μ M. However, it exhibited a slightly broader inhibition profile than erlotinib. At 100 μ M, gefitinib inhibited the activities of UGT1A7, UGT1A9, and UGT2B7 by 61.6, 55.5, and 70.9%, respectively. The inhibition was also observed against UGT2B15 (47.9%), 1A4 (39.8%), and 1A3 (18.8%) at 100 μ M. In addition, erlotinib and gefitinib exhibited a stimulation of UGT1A4 and UGT2B17 catalytic activity by 67.3 and 81.5% at 100 μ M, respectively.

Inhibition Kinetic Analysis in Recombinant UGTs. Kinetic experiments were performed to further characterize the inhibition of UGT activities by erlotinib and gefitinib. Erlotinib and gefitinib strongly inhibited the formation of 4-MUG by UGT1A1. The representative Lineweaver-Burk plots for the inhibition of 4-MUG formation by erlotinib and gefitinib (Figs. 2A and 3A) and analysis of the parameters of the enzyme inhibition model suggested that the inhibition types were competitive. Based on nonlinear regression analysis and Dixon plots presented in Figs. 2B and 3B, erlotinib and gefitinib showed competitive inhibition against the formation of 4-MUG with K_i of 0.64 ± 0.06 and 2.42 ± 0.31 μ M in recombinant UGT1A1, respectively.

Gefitinib was found to be a strong competitive inhibitor of UGT1A7 with a K_i of 5.11 ± 0.43 μ M (Fig. 3, C and D). It also exerted intermediate mixed inhibition against UGT1A9 with K_i of

Fig. 1. The inhibition of erlotinib and gefitinib on recombinant UGT activities. 4-MU or imipramine were incubated with pooled HLMs (0.5 mg protein/ml) or recombinant UGTs (0.5 mg protein/ml) at 37°C in the absence and presence of erlotinib (100 μ M) or gefitinib (100 μ M), respectively. Data represent the mean of triplicate or quadruplicate determination.

FIG. 2. Representative Lineweaver-Burk plots (A) and Dixon plots (B) of the effects of erlotinib on 4-MU glucuronide formation in recombinant UGT1A1. Reactions were performed as described under *Materials and Methods*. All data points shown represent the mean of duplicate measurements.

$1.41 \pm 0.16 \mu\text{M}$ and αK_i of $44.10 \pm 1.55 \mu\text{M}$ (Fig. 3, E and F), as well as intermediate competitive inhibition against UGT2B7 with K_i of $39.48 \pm 4.17 \mu\text{M}$ (Fig. 3, G and H).

Inhibition of Bilirubin Glucuronidation Activity by Erlotinib and Gefitinib in HLMs. The kinetic studies were first performed by using pooled HLMs. The apparent kinetic parameters K_m and V_{max} of bilirubin glucuronidation were estimated to be $1.11 \pm 0.25 \mu\text{M}$ and $460.20 \pm 22.57 \text{ pmol/min/mg protein}$, respectively.

Inhibition experiments were then conducted in HLMs. The IC_{50} value of indinavir was $110.6 \mu\text{M}$, which is comparable with previously published data (Zhang et al., 2005). Erlotinib exhibited potent inhibition against bilirubin glucuronidation with an IC_{50} of $4.19 \pm 0.24 \mu\text{M}$ at a bilirubin concentration of $1 \mu\text{M}$. Further kinetic experiments showed mixed inhibition by erlotinib. The K_i was $2.97 \pm 1.09 \mu\text{M}$, and αK_i , a measure of the affinity of enzyme-substrate complex for I , was $7.78 \mu\text{M}$. However, the effect of gefitinib was surprisingly found to be much weaker than that of erlotinib, and the IC_{50} was more than $100 \mu\text{M}$ (Fig. 4).

The Calculated Concentrations of Erlotinib and Gefitinib in Blood. The oral doses and pharmacokinetic parameters of erlotinib and gefitinib were obtained from previous publications (EMA, <http://www.emea.europa.eu/humandocs/PDFs/EPAR/tarceva/H-618-PI-en.pdf>; Ito et al., 2004; Siegel-Lakhai et al., 2005; Frohna et al., 2006; Li et al., 2006; Rudin et al., 2008; Schaiquevich et al., 2008). The calculated blood concentrations of erlotinib and gefitinib after oral administration are listed in Table 1. Comparison of the calculated $[I]_{max}$ with the reported C_{max} after administration of oral doses of erlotinib or gefitinib showed that the $[I]_{max}$ values fell within the reported concentration range, with the exception of the $[I]_{max}$ at 700 mg/day gefitinib, which is higher than expected.

Quantitative Prediction of DDI Risk (AUC_i/AUC). The magnitudes of the potential inhibitory interactions of erlotinib and gefitinib with UGTs were evaluated by estimating the ratio of the AUC in the presence and absence of the inhibitor (AUC_i/AUC).

The calculated results were shown as AUC ratio isolines plotted against f_m by UGT isoform and oral doses of erlotinib or gefitinib in Fig. 5. When the dose of erlotinib is more than 140 mg/day and the f_m of coadministered drug metabolized by UGT1A1 is more than 0.9, the AUC of coadministered drug will increase more than 40%; when the dose is more than 110 mg/day and the f_m is more than 0.8, or the dose is more than 100 mg/day and the f_m is 1, AUC will increase more than 30%. However, for gefitinib, even when administered at the highest dose (700 mg/day) and an f_m of 1, the AUC ratio is less than 1.3 for the substrates of each UGT isoform inhibited.

In this study, we assumed the f_m of bilirubin is 1, because UGT1A1 is the dominant isoform involved in bilirubin glucuronidation. When the oral dose of erlotinib is 150 mg/day, the AUC of bilirubin will increase more than 10%.

Discussion

Our data offer in vitro evidence that erlotinib is a potent competitive inhibitor of UGT1A1. UGT1A1 inhibition is also the mechanism by which atazanavir and indinavir cause hyperbilirubinemia (Zhang et al., 2005). Reduced glucuronidation rates are associated with the risk for severe toxicity during irinotecan treatment (Innocenti et al., 2004). Because the reduction in UGT1A1 activity varies depending on the substrate and incubation system (Udomuksorn et al., 2007), we also investigated the effects of erlotinib and gefitinib on bilirubin glucuronidation in HLMs in the current study. The inhibition potential is similar to that of atazanavir, but it is much more potent than that of indinavir. It is consistent with the observation that erlotinib can cause hyperbilirubinemia (Jakacki et al., 2008), similar to that observed with atazanavir and indinavir (Zhang et al., 2005). Therefore, inhibition of UGT1A1 activity by erlotinib can decrease the conjugation of bilirubin, which can be clinically significant.

UGT1A1 is also responsible for the metabolism of several other endogenous and exogenous substrates, including 15% drugs that have glucuronidation as a clearance mechanism of the top 200 drugs in the United States in 2002 (Williams et al., 2004). The inhibition of UGT1A1 is particularly important if a drug has a narrow therapeutic index, such as etoposide and irinotecan (Kawato et al., 1991; Wen et al., 2007). It is interesting to note that gefitinib exhibited very weak inhibition against bilirubin glucuronidation in HLMs, although it potentially inhibited 4-MU glucuronidation in recombinant UGT1A1. This finding confirms the observation that the reduction of UGT1A1 activity might vary with the substrate (Udomuksorn et al., 2007), and it also offers new experimental evidence for the opinion that UGT1A1 has two or more binding sites for xenobiotics and endobiotics (Rios and Tephly, 2002).

In contrast with erlotinib, gefitinib exhibited competitive inhibition against not only UGT1A1 but also UGT1A7, UGT1A9, and UGT2B7. The latter enzyme is involved in the metabolism of 35% drugs involved in glucuronidation of the top 200 prescribed drugs in the United States in 2002 (Williams et al., 2004). UGT1A9 and UGT1A7 are also involved in the glucuronidation of a number of drugs (Kiang et al., 2005). In addition, UGT1A1, UGT1A9, and UGT2B7 are expressed in both human liver and some extrahepatic tissues including the gastrointestinal tract, whereas UGT1A7 is present only in the esophagus, stomach, and lung (Kiang et

FIG. 3. Representative Lineweaver-Burk plots and Dixon plots of the effects of gefitinib on 4-MU glucuronide formation in recombinant UGT1A1 (A and B), UGT1A9 (C and D), UGT1A9 (E and F), and UGT2B7 (G and H). Reactions were performed as described under *Materials and Methods*. All data points shown represent the mean of duplicate measurements.

al., 2005). UGTs in the gastrointestinal tract may contribute significantly to the first-pass metabolism of orally administered drugs that undergo glucuronidation. Our results showed that gefitinib might affect the glucuronidation and first-pass metabolism of more orally administered drugs than erlotinib.

We observed an increase of UGT1A4 activity in the presence of

erlotinib and UGT2B17 activity in the presence of gefitinib. Enzyme activation is not an uncommon event in enzymology; however, the underlying mechanisms are unclear. One possibility is the existence of the enzyme in multimeric form, so that the binding of one molecule to one subunit may increase the affinity of the other subunit(s) for another molecule. Williams et al. (2002) found that

FIG. 4. Kinetics of bilirubin glucuronidation in HLMs (A), the inhibition of erlotinib and gefitinib against bilirubin (1 μ M) glucuronidation in HLMs (B), and representative Lineweaver-Burk plots and Dixon plots of the effects of erlotinib on bilirubin glucuronides formation in HLMs (C and D). Reactions were performed as described under *Materials and Methods*. All data points shown represent the mean of duplicate measurements.

TABLE 1
Calculation of possible concentrations of erlotinib and gefitinib

		Calculated Concentrations					
Drugs	Dose	$[I]_{\text{max}}$	$[I]_{\text{max,u}}$	$[I]_{\text{in}}$	$[I]_{\text{in,u}}$	Reported C_{max}	References
	<i>mg/day</i>					<i>μM</i>	
Erlotinib	150	3.72	0.19	6.1	0.31	3.65 (2.44–6.06)	Frohna et al., 2006; Yamamoto et al., 2008
	100	2.64	0.13	3.5	0.18	2.53 (1.45–3.53)	Yamamoto et al., 2008
	50	1.42	0.07	3.6	0.16	1.29 (0.49–2.08)	Yamamoto et al., 2008
Gefitinib	700	6.70	0.228	9.3	0.32	4.8 (3.12–6.48)	Ranson et al., 2002
	500	0.35	0.012	3.7	0.13	0.45 (0.21–1.21)	Swaishland et al., 2006
	250	0.29	0.010	1.9	0.07	0.36 (0.11–0.72)	Swaishland et al., 2005; Bergman et al., 2007
	100	0.15	0.005	0.8	0.03	0.14 (0.07–0.22)	Milton et al., 2007

UGT1A1-catalyzed estradiol-3-glucuronidation is stimulated by 17 α -ethynylestradiol, anthraflavic acid, and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine in human liver microsomes. They also proved that the observed activation is not an artifact of the in vitro systems examined but true behaviors of UGT1A1 at least in vitro (Williams et al., 2002). However, further studies will need to be performed to evaluate whether this in vitro phenomenon also occurs in vivo.

The quantitative prediction of DDI risk indicated that the coadministration of erlotinib at clinical doses could result in a significant increase of AUC of drugs primarily cleared by UGT1A1, suggesting that erlotinib can induce clinically significant DDI with coadministered UGT1A1 substrates. It is noteworthy that our calculation is

based only on the inhibition of hepatic UGT1A1 by erlotinib, and abundant extrahepatic UGT1A1 exists. In addition, the pharmacokinetic parameters used to calculate concentrations are mean values of the parameters reported, but interindividual variability is high. The diplotype of two polymorphic loci in the ABCG2 promoter involving –15622C/T and 1143C/T is associated with higher C_{max} of erlotinib (Rudin et al., 2008). Furthermore, the bioavailability of erlotinib can be increased to almost 100% with the intake of food (Smith, 2005). Moreover, in vitro data tend to underestimate inhibition of drug glucuronidation in vivo (Uchaipichat et al., 2006a). As a result, the actual effects of erlotinib might be more potent than those calculated here, in particular, for some individuals with higher blood concentrations of erlotinib.

FIG. 5. Isolines plots for relationship of AUC ratio against oral dose of erlotinib and f_m by UGT1A1 (A), as well as the relationship of AUC ratio against oral dose of gefitinib and f_m by UGT1A1 (B), UGT1A7 (C), UGT1A9 (D), or UGT2B7 (E) for DDI study.

Compared with erlotinib, gefitinib may significantly affect a drug that is metabolized by a single UGT and is a high-capacity, low-affinity substrate. However, the majority of UGT substrates are glucuronidated by multiple UGT enzymes (Williams et al., 2004), thereby making it difficult to determine whether interactions arising from multiple UGTs inhibition by gefitinib exist when using in vitro inhibition data.

It is noteworthy that although there are a few studies predicting the magnitude of inhibitory drug interactions in vivo (Zhang et al., 2005; Uchaipichat et al., 2006b), the extrapolation from in vitro data to in vivo drug interactions should be taken with caution. Protein binding, active uptake, and efflux transporters in tissues may affect the estimation of unbound drug concentrations at the interaction site. In addition, because the lipid composition varies between membranes from different sources and is a determinant of membrane fluidity, thickness, shape, surface curvature, and the ability to form lipid rafts, it is conceivable that the kinetic properties of single recombinant UGT may be different (Miners et al., 2006). Another potential problem is that the enzymatically generated glucuronidated products are eliminated in vivo, but this does not occur in an in vitro incubation system, and the accumulation of products in the reaction medium may inhibit enzyme activity (Kiang et al., 2005). Furthermore, many studies have indicated

UGTs are oligomeric enzymes. Although there are some evidences and experimental data showing that hetero-oligomerization could not be the major reason or basis for the complex substrate specificity of most UGTs (Finel and Kurkela, 2008), hetero-oligomerization might somewhat affect substrate specificity or the region selectivity of the tested enzyme. Moreover, UGT1A1, UGT1A7, UGT1A9, and UGT2B7 are also expressed in gastrointestinal tract, and drug-drug interactions via inhibition of these UGTs may also take place in the small intestine. The concentrations of substrates in the intestine may differ from the concentrations used here to predict the AUC ratio.

The present findings shed light on the mechanisms underlying clinically significant DDI associated with erlotinib, and they also provide the basis for further clinical studies investigating the DDI potential between tyrosine kinase inhibitors with UGT substrates, such as etoposide and SN-38 (the active metabolite of irinotecan).

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