Introduction

Erlotinib (Tarceva; Genentech Inc, San Francisco, CA, USA) is an orally available, reversible human epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor\(^1,2\). It received approval from the US Food and Drug Administration in November 2004 for the second-line treatment of locally advanced or metastatic non-small cell lung cancer after the failure of at least 1 previous chemotherapeutic regimen\(^3,4\). Erlotinib was also approved by the United States for the treatment of locally advanced, unresectable or metastatic pancreatic cancer in combination with gemcitabine\(^5\). In addition, clinical trials in a number of other solid tumors are also under-way\(^6-8\).

Erlotinib is considered better tolerated and less toxic than cytotoxic drugs, with the most common adverse reactions in patients being rash and diarrhea\(^6\). However, erlotinib is frequently involved in clinical drug-drug interactions (DDIs). With 45 interactions reported before 2007, the DDI frequency of erlotinib was just second to that of ifosfamide and paclitaxel among all antineoplastic drugs\(^10\). Co-administration of erlotinib has been reported to enhance carboplatin exposure\(^11\) and increase the serum concentration of phenytoin\(^12\). A case of rhabdomyolysis was reported due to the interaction of erlotinib with simvastatin\(^13\). International Normalized Ratio (INR) elevations and bleeding events associated with erlotinib-warfarin co-administration have been reported\(^6\). Because the drugs involved usually had narrow therapeutic indices, DDIs might impair the clinical safety of erlotinib.

One of the major reasons for clinical DDIs has been recognized to be inhibition or induction of drug metabolism enzymes. Erlotinib is extensively metabolized, predominantly by CYP3A4/5 and to a lesser extent by CYP1A2 and the
extrahepatic isozyme CYP1A1[14]. As for the influence of erlotinib on the catalytic activity of CYP3A, conflicting data have been published concerning its clinical consequences. Li et al. found that erlotinib stimulated CYP3A-mediated midazolam metabolism in liver and intestinal microsomes[35]. Nevertheless, in a cell-based CYP3A activity assay, erlotinib was shown to decrease the formation of 1'-hydroxymidazolam, showing the potency to inhibit CYP3A activity[16]. As for the phase II enzymes, erlotinib was shown to exhibit inhibition activity on human UDP-glucuronosyltransferase (UGT) 1A1[17]. The effects of erlotinib on other phase I CYP isoforms are still unknown. Thus, the current data were insufficient to explain the widespread DDI cases. Ascertaining the effect of erlotinib on major CYP isoforms will benefit the clinical safety evaluation of erlotinib in combination with other drugs.

The aim of this study was to ascertain the effect of erlotinib on CYP3A activity and to investigate the amplitude and kinetics of erlotinib-mediated inhibition of seven major CYP isoforms in HLMs. An in vivo magnitude of interaction will be extrapolated from the in vitro inhibition kinetic data to help explain the clinical DDIs associated with erlotinib.

Materials and methods

Chemicals and reagents

Erlotinib (OSI-774, >99%) was purchased from Nanjing Ange Pharmaceutical Co, Ltd (Nanjing, China). D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP⁺, corticosterone, phenacetin, acetaminophen, 7-hydroxycoumarin, 4’-hydroxydiclofenac, sulfaphenazole, 8-methoxypsoralen, clomethiazole, montelukast, nifedipine, oxidized nifedipine, midazolam, 1’-OH-midazolam, troleandomycin (TAO), 6-hydroxychlorzoxazone, 7-hydroxycoumarin, paclitaxel, 6β-hydroxytestosterone and furafylline were purchased from Sigma-Aldrich (St Louis, MO, USA). Tes-}

Enzyme inhibition experiments

Marker assays for each CYP isoform were performed in the presence of 100 μmol/L erlotinib to evaluate its inhibitory effect toward the seven major human CYP isoforms. The concentrations of positive control inhibitors used are as follows[21, 24, 25]: 1 μmol/L ketoconazole for CYP3A, 10 μmol/L furafylline for CYP1A2, 10 μmol/L sulfaphenazole for CYP2C9, 5 μmol/L montelukast for CYP2C8, 2.5 μmol/L 8-methoxypsoralen for CYP2A6, 10 μmol/L quinidine for CYP2D6 and 50 μmol/L clofibrate for CYP2E1. For CYP isoforms that were strongly inhibited, the concentrations at which the enzymes were 50% inhibited (IC₅₀ values) were determined using various concentrations of erlotinib for CYP3A and for CYP2C8. Inhibition constant (Kᵢ) values were determined by incubating various probe substrates (5–50 μmol/L paclitaxel, 30–100 μmol/L testosterone or 5–50 μmol/L nifedipine) in the presence or absence of erlotinib. Ki values were calculated by nonlinear regression using the equations for competitive inhibition (eq 1), noncompetitive inhibition (eq 2), or mixed inhibition (eq 3)[17].
Table 1. Analysis conditions for the relevant P450 isoforms.

| CYPs              | Internal standard concentration (μmol/L) | Mobile phase gradient                                      | Detection          |
|-------------------|-----------------------------------------|------------------------------------------------------------|--------------------|
| 1A2               | 7-Hydroxycoumarin (30 μmol/L)           | Methanol (A): Phosphate buffer (pH=3.0, 50 mmol/L) (B)=34:66| HPLC, UV 245 nm    |
| 2A6               | -                                       | Acetonitrile (A): Acetic acid (0.1%, v/v) (B)=35:65        | HPLC, Fluo E/E_ex  |
| 2C9               | Coumarin (60 μmol/L)                    | Acetonitrile (A): Phosphate buffer (pH=7.4, 100 mmol/L) (B)=32:68, 0–9 min, 68%B–32%B | HPLC, UV 280 nm    |
| 2D6               | -                                       | Acetonitrile (A): Phosphate buffer (pH=3.0, 50 mmol/L ) (B)=25:75 | HPLC, UV 280 nm    |
| 2E1               | Phenacetin (300 μmol/L)                 | Acetonitrile (A): Acetic acid (0.5%, v/v) (B)=22:78, 1–10 min, 78%B–40%B | HPLC, UV 287 nm    |
| 3A4 (Testosterone)| Corticosterone (20 μmol/L)              | Methanol (A): Water (B)=52:48, 0–15 min, 48%B–30%B; 15–22 min, 30%B–20%B | HPLC, UV 254 nm    |
| 3A4 (Nifedipine)  | -                                       | Methanol (A): Water (B)=50:50, 0–20 min, 50%B–45%B       | HPLC, UV 250 nm    |
| 3A4 (Midazolam)   | -                                       | Methanol (A): Acetic acid (0.5%, v/v) (B)=40:60, 0–10 min, 60%B–20%B | UFLC, UV 254 nm    |
| 2C8               | -                                       | Methanol (A): Water (B)=0–2 min, 88%B–44% B; 2–4 min, 44%B–38%; 4–9.5 min, 38%B–32%B | UFLC, UV 230 nm    |

the concentration dependence of midazolam metabolism activation phenomena, various concentrations of erlotinib (1–20 μmol/L) were incubated with midazolam at different concentrations (2–20 μmol/L). 1'-hydroxymidazolam was measured for the concentration dependence of midazolam metabolism activation under conditions of reversible inhibition. For other CYP isoforms, 50 μmol/L of erlotinib was used. Moreover, midazolam was also used as a probe substrate to perform single point inactivation experiments for CYP3A, and 50 μmol/L of erlotinib was used. After incubation, an aliquot (20 μL) was transferred to another incubation tube (final volume 200 μL) containing an NADPH-generating system and probe substrates whose concentrations were proximal to \( K_m \) values. Further incubations were performed to measure residual activity.

Inactivation constant \( (K_i \text{ and } k_{inact}) \) assays

To determine the \( K_i \) and \( k_{inact} \) values for the inactivation of CYP3A, five concentrations of erlotinib (0, 5, 10, 20 and 50 μmol/L) were incubated for 0 to 30 min with pooled HLMs (1 mg/mL) at 37 °C. After preincubation, an aliquot (20 μL) was transferred to another incubation tube (final volume 200 μL) containing an NADPH-generating system and different probe substrates for CYP3A to measure residual activity. Substrate concentrations of four times the \( K_m \) were selected to minimize the reversible inhibition caused by erlotinib. The concentrations used for different probe substrates were as follows: 400 μmol/L testosterone, 20 μmol/L midazolam and 60 μmol/L nifedipine. To determine the \( k_{obs} \) (observed inactivation rate) values, the decrease in natural logarithm of the activity over time was plotted for each erlotinib concentration, and the \( k_{obs} \) values were described as the negative slopes of the lines. Inactivation kinetic parameters were calculated using nonlinear regression of the data according to equation (5):

\[
k_{obs} = \frac{k_{inact} \times [I]}{K_i + [I]} 
\]

where \([I]\) is the initial inhibitor concentration, \( k_{inact} \) is the maximal inactivation rate constant and \( K_i \) is the inhibitor concentration required for half the maximal rate of inactivation. The unbound \( K_i \) (\( K_{i,u} \)) was calculated according to equation (6), where \( f_u \) is the free fraction of erlotinib in the microsomes. \( f_u \) is predicted according to equation (7) as previously reported	extsuperscript{29}. The terms are defined as follows: \( C_{mic} \) is the microsomal protein concentration used in the preincubation, and \( \text{LogP} \) is the log of the octanol-water (pH 7.4) partition coefficient of the erlotinib. A concentration of 1 mg/mL was used for \( C_{mic} \) in this experiment, and \( \text{LogP} \) is approximately...
Quantitative prediction of the DDI potential of erlotinib (AUC/AUC)

The equations (8) and (9) were utilized to predict the interaction potential of erlotinib caused by reversible inhibition and TDI of CYP3A. Equation (10) was used to predict the interaction potential of erlotinib caused by reversible inhibition and competitive inhibition of CYP2C8. All equations were adapted as reported[27, 29].

\[
\frac{\text{AUC}_i}{\text{AUC}} = \frac{1}{1 - f_{m(CYP3A)} + \frac{f_{m(CYP3A)}}{1 + [I]_{i, \text{in vivo}}}}
\]  

(8)

\[
\frac{\text{AUC}_i}{\text{AUC}} = \frac{1}{1 + \frac{f_{m(CYP3A)}}{K_{i, u} \times (1 - [I]_{i, \text{in vivo}})}}
\]  

(9)

\[
\frac{\text{AUC}_i}{\text{AUC}} = \frac{1}{1 - f_{m(CYP2C8)} + \frac{f_{m(CYP2C8)}}{K_{i, u}}}
\]  

(10)

The terms are defined as follows: AUC_i/AUC is the predicted ratio of in vivo exposure of the interacting drug with co-administration of erlotinib versus that in the control situation, \(f_{m(CYP3A)}/f_{m(CYP2C8)}\) is the portion of total clearance of the interacting drug to which CYP3A/CYP2C8 contributes, \(k_{\text{deg(CYP3A)}}\) is the first-order rate constant of in vitro degradation of CYP3A, \(K_{i, u}\) is the maximum inactivation rate constant, \(K_{i, u}\) is the unbound \(K_{i}\), \(K_{\text{deg(CYP3A)}}\) is the in vitro concentration of erlotinib at the enzyme active site. The general assumption is that only unbound drug is available for interaction with the enzyme active site. However, at present, there is no consensus on the in vivo precipitant concentration that should be used. According to a recent publication, the reversible inhibition portion performed the best when the unbound portal vein concentration was used for \([I]_{i, \text{in vivo}}\) while for irreversible inactivation and induction the unbound systemic concentration was the best. Thus, in this research, the unbound portal vein concentration (0.16, 0.18, and 0.31 \(\mu\)mol/L) was used for the reversible inhibition portion (for 50, 100, and 150 mg/d doses, respectively), while the unbound systemic concentration (0.07, 0.19, and 0.13 \(\mu\)mol/L) was adopted to avoid over-prediction of irreversible inactivation[30]. The values for \([I]_{i, \text{in vivo}}\) were derived from references[17, 31]. A \(k_{\text{deg(CYP3A)}}\) of 0.000321 min\(^{-1}\) was adopted, in accordance with Obach et al[27]. The values of \(f_{m(CYP3A)}\) and \(f_{m(CYP2C8)}\) were arbitrarily set to be 0.1–1 to predict the DDI risk for all possible coadministered drugs[17].

Results

Inhibition of major CYP isoforms by erlotinib

Erlotinib with the concentration of 100 \(\mu\)mol/L inhibited the activities of CYP1A2, CYP2C9, CYP2A6, CYP2D6, CYP2C8, and CYP2E1 by 30.0%, 49.0%, 9%, 37%, 76%, and -7%, respectively. For CYP3A, 100 \(\mu\)mol/L erlotinib inhibited 69.3% and 71.6%, respectively, of the enzyme’s testosterone 6β-hydroxylation and nifedipine oxidation activities. However, erlotinib stimulated the midazolam 1’-hydroxy activity by 171%. All positive control inhibitors strongly inhibited the corresponding probe reactions, with less than 20% of control activity remaining upon inhibition. Further kinetic analysis was conducted for CYP3A (testosterone 6β-hydroxylation, nifedipine oxidation) and CYP2C8 (paclitaxel 6α-hydroxylation), whose activities were inhibited by more than 50%. As shown in Figure 1, erlotinib inhibited testosterone 6β-hydroxylation in a concentration-dependent manner with an IC\(_{50}\) of 31.3±8.0 \(\mu\)mol/L. Lineweaver-Burk and Dixon plots showed that the inhibition of CYP3A by erlotinib was well fitted to a competitive model of inhibition. The \(K_i\) value was calculated to be 14.1±4.3 \(\mu\)mol/L using a nonlinear regression equation (eq 1). Erlotinib also inhibited the metabolism of nifedipine in a competitive manner with an IC\(_{50}\) of 20.5±3.5 \(\mu\)mol/L. A \(K_i\) value of 4.3±0.9 \(\mu\)mol/L was obtained by nonlinear fitting. The results demonstrated that erlotinib inhibited paclitaxel 6α-hydroxylation in a concentration-dependent manner, with an IC\(_{50}\) of 6.17±2.0 \(\mu\)mol/L. Lineweaver-Burk and Dixon plots suggested that erlotinib also competitively inhibited CYP2C8. The \(K_i\) value was calculated to be 5.8±1.9 \(\mu\)mol/L using a nonlinear regression equation (eq 1).

Activation of midazolam metabolism

As shown in Figure 2, using different concentrations of midazolam (2–20 \(\mu\)mol/L), erlotinib stimulated the formation of 1’-OH-midazolam. At a constant concentration of midazolam, the formation of 1’-OH-midazolam increased with increasing amounts of erlotinib (1–20 \(\mu\)mol/L). The two-site model fitting results are listed in Table 2. The data fit this model (Table 2) well with an \(\alpha=0.50\) and a \(\beta=1.80\), indicating a decrease in \(K_n\) and an increase in \(V_{max}\) respectively[32]. These results showed the existence of activation.

| Substrate   | Effector  | \(V_{max}\) | \(K_m\) | \(K_i\) | \(\alpha\) | \(\beta\) | \(R^2\) |
|-------------|-----------|--------------|---------|--------|-----------|---------|--------|
| Midazolam   | Erlotinib | 0.34 (0.03)  | 2.63 (0.93) | 7.96 (5.9) | 0.50 (0.33) | 1.80 (0.27) | 0.93 |
probe substrates). When midazolam was used as a probe substrate, interesting results were obtained (Figure 3). When NADPH was not present during the preincubation process, erlotinib stimulated the metabolism of midazolam, but when NADPH was added to the preincubation, erlotinib showed an inhibitory effect on the activity of CYP3A. Inactivation kinetic parameters were obtained using different probe substrates. As calculated from the observed inactivation plots (Figure 4), inactivation parameters ($K_I$ and $k_{inact}$, respectively) for CYP3A were calculated to be 6.3 μmol/L and 0.035 min$^{-1}$, 9.0 μmol/L and 0.045 min$^{-1}$, 10.1 μmol/L and 0.058 min$^{-1}$ for the probe substrates midazolam, testosterone and nifedipine, respectively. The inhibition of other isoforms by erlotinib was not time and NADPH dependent (data not shown).

**Figure 1.** Reversible inhibition of CYP3A and CYP2C8 by erlotinib. A1: Inhibition by erlotinib of testosterone 6β-hydroxylation activity. A2: Dixon plot of the inhibitory effect of erlotinib on testosterone 6β-hydroxylation (TS) activity. A3: Lineweaver-Burk plot of the inhibitory effect of erlotinib on testosterone 6β-hydroxylation activity. B1: Inhibition by erlotinib of paclitaxel 6α-hydroxylation activity. B2: Dixon plot of the inhibitory effect of erlotinib on paclitaxel 6α-hydroxylation activity. B3: Lineweaver-Burk plot of the inhibitory effect of erlotinib on paclitaxel 6α-hydroxylation activity. C1: Inhibition by erlotinib of nifedipine oxidation activity. C2: Dixon plot of the inhibitory effect of erlotinib on nifedipine oxidation activity. C3: Lineweaver-Burk plot of the inhibitory effect of erlotinib on nifedipine oxidation activity.

**In vitro-in vivo extrapolation of DDI magnitudes**

For reversible inhibition of CYP2C8, adopting a $K_I$ of 5.8 μmol/L and unbound portal vein concentrations of 0.16, 0.18, and 0.31 μmol/L for 50, 100, and 150 mg/d doses, respectively, the AUC$_i$/AUCs were predicted to be 1.0027–1.0276, 1.0030–1.0310 and 1.0051–1.0534 for an $f_{im}$ value between 0.1 and 1. For reversible inhibition of CYP3A (with the probe substrate testosterone), using a $K_I$ of 14.1 μmol/L and the same unbound portal vein concentrations as for CYP2C8, the AUC$_i$/AUCs were predicted to be 1.0011–1.0113, 1.0013–1.0128, and 1.0022–1.0220 for an $f_{im}$ value between 0.1 and 1, for 50, 100, and 150 mg/d doses, respectively. For the probe substrate nifedipine, using a $K_I$ of 4.3 μmol/L and the same unbound portal vein concentration, the corresponding results of AUC$_i$/AUCs were...
AUCs were 1.0036–1.0372, 1.0040–1.0419, and 1.0068–1.0721 for an $f_m$ value between 0.1 and 1, for 50, 100, and 150 mg/d doses, respectively. For irreversible inactivation of CYP3A (using different probe substrates), unbound systemic concentrations of 0.07, 0.13, and 0.19 μmol/L were adopted to avoid over-prediction for three oral doses\cite{17}. With a $K_{I, u}$ and a $k_{inact}$ of erlotinib for probe substrates midazolam, testosterone and nifedipine.

Figure 2. Activation of midazolam 1′-hydroxylation by 1–20 μmol/L erlotinib.

Figure 3. Single point inactivation of CYP3A by erlotinib measured using midazolam, testosterone and nifedipine as probe substrates in HLM. The concentrations for erlotinib were 50 μmol/L, 75 μmol/L and 75 μmol/L when using midazolam, testosterone and nifedipine as probe substrates, respectively. The concentration of the positive control inhibitor TAO was 250 μmol/L. Each data point represents the mean±SD of duplicate incubations.

Figure 4. Time- and concentration-dependent inactivation of CYP3A by erlotinib. (A) At the indicated time points, the remaining CYP3A activity was measured by a midazolam 1′-hydroxylation (A1), testosterone 6β-hydroxylation (A2) or nifedipine oxidation (A3) assay. Each point represents the mean of triplicate incubations. The observed inactivation rate constants, $k_{obs}$, were calculated from the slopes of the regression lines in A. (B) The hyperbolic plot of $k_{obs}$ versus erlotinib concentration was used to calculate kinetic constants.
nifedipine being 2.8 μmol/L and 0.035 min⁻¹, 4.0 μmol/L and 0.045 min⁻¹ and 4.5 μmol/L and 0.058 min⁻¹, respectively, the AUC was calculated to increase to 107.9%–372.6%, 109.1%–606.2%, and 109.7%–839.9% for midazolam; 107.7%–345.3%, 108.9%–555.6%, and 109.5%–765.9% for testosterone; 108.0%–381.1%, 109.2%–622.0%, and 109.7%–862.9% for nifedipine.

Discussion

Human CYP3A is one of the most important CYP isoforms involved in drug clearance and metabolized more than 50% of the drugs on the market[33]. Inhibition or stimulation of the catalytic activity of this enzyme could play a key role in clinical DDIs. In previous studies, conflicting data were obtained about the effects of erlotinib on metabolism of the substrate midazolam mediated by CYP3A[34]. Thus, rigorously ascertaining erlotinib’s effect on CYP3A will provide important information that may aid in the prevention of clinical DDIs. In this study, our experimental results showed that DDI patterns via modulation of CYP3A by erlotinib are substrate dependent. Erlotinib stimulated the formation of 1’-hydroxymidazolam in HLMs. However, it inhibited the reactions of testosterone 6β-hydroxylation and nifedipine oxidation. Erlotinib’s time-dependent inhibition of CYP3A was not substrate dependent.

The patterns of interaction between drug compounds and CYP3A were previously shown to be substrate dependent[35]. For example, the flavonoid α-naphthoflavone, although well known to activate CYP3A4[36], may also inhibit the enzyme[37], depending on the CYP3A4 substrate. In a recent report, substrate-dependent phenomena were found for ginsenosides’ effects on CYP3A[38]. Until now, the mechanism of substrate-dependent modulation of CYP3A activity remained unclear. The relatively large active site cavity and the conformational flexibility of CYP3A were considered the major causes of these phenomena[39, 40]. CYP3A can bind multiple ligands simultaneously, resulting in changes in the affinity of the substrate-binding site for different substrates[26]. This multiple ligand-binding property may contribute to the complex substrate-dependent effects, but whether conformational changes occurred simultaneously was unknown. In the case of erlotinib, further investigation was needed to explore the molecular and structural basis of these substrate-dependent effects.

The substrate-dependent effects of erlotinib on CYP3A point to the need for greater attention to the safety of combined medications. In the case of heteroactivation, the clearance of the interacting drug would increase. Alternatively, if erlotinib inhibited the metabolism of the interacting drug, the AUC of the latter drug would increase. In either case, a different DDI might occur and possibly cause harm to the patient. When substrate-dependent effects may be present, it is prudent to employ a testing strategy using several probe substrates to evaluate the DDI potential[41, 42]. Moreover, CYP3A4 and CYP3A5 are the most abundant members of the CYP3A subfamily. A recent study has shown significant differences between the heteroactivation potential of CYP3A4 and CYP3A5 for CYP3A-mediated carbamazepine 10,11-epoxidation[43]. Inhibitors of CYP3A usually have different potencies for inhibition of CYP3A4 and CYP3A5 in terms of both reversible and irreversible inhibition[44, 45]. Thus, the different expression levels of CYP3A4 and CYP3A5 may contribute to interindividual variability in erlotinib interactions.

The time-dependent inhibition of CYP3A was found to be substrate independent. After preincubation, erlotinib showed enhanced inhibition activity for the midazolam 1’-hydroxylation, testosterone 6β-hydroxylation and nifedipine oxidation reactions (Figure 3). The TDI parameters (Ki and k_inact) were 6.3 μmol/L and 0.035 min⁻¹, 9.0 μmol/L and 0.045 min⁻¹ and 10.1 μmol/L and 0.058 min⁻¹, respectively, for the midazolam 1’-hydroxylation, testosterone 6β-hydroxylation and nifedipine oxidation reactions. When midazolam was used as the probe substrate, the following similar inactivation kinetic parameters were reported by Li et al[46]: ki=0.09 min⁻¹ and Ki=22 μmol/L. The discrepancy in parameters may be due to the differences between labs. It should be noted that tyrosine kinase inhibitors such as dasatinib have been reported to inhibit CYPs via generation of reactive intermediates[47, 48]. Recently, the bioactivation of erlotinib was also reported; in that study, reactive epoxide and quinone-imine electrophiles were detected, providing a possible mechanism for the time dependent inhibition of CYP3A[46].

Based on the results shown above, the conflicting data about the effect of erlotinib on CYP3A can be explained as follows. First, without preincubation the action of erlotinib on CYP3A was substrate dependent (Figure 3). Erlotinib stimulated the formation of 1’-hydroxymidazolam[35]. Second, the time-dependent inhibition of CYP3A was actually substrate independent (Figure 3). In the cell-based CYP3A activity assessment method by Harmsen et al, the cells were cultured in medium containing erlotinib for two consecutive days before measurement of the formation of 1’-hydroxymidazolam[36]. During the 2-d culture period, time dependent inhibition of CYP3A could occur, thus decreasing the formation of 1’-hydroxy midazolam.

Using the kinetic information we obtained regarding the reversible and time-dependent inhibition of CYP enzymes, the in vivo DDI magnitude of erlotinib was extrapolated. For the reversible inhibition of CYP3A and 2C8, even using a dose of 150 mg/d and an f_m of 1, the increase in the AUC was predicted to be no more than 10%. On the contrary, the AUC was predicted to increase significantly even with the lower oral dose and the smaller f_m when adopting the TDI prediction equation. The DDI potential of erlotinib on phase II UDP-Glucuronosyltransferases has been evaluated previously[17]. The maximum increase in AUC was estimated to be less than 50% for drugs predominantly cleared by UGT1A1, even at a dose of 150 mg/d. Therefore, time-dependent inhibition of CYP3A might be one of the most important factors leading to clinical DDIs.

Conclusion

In conclusion, our results demonstrate that the action of erlotinib on CYP3A was substrate dependent. It stimulated the metabolism of midazolam and inhibited the formation of
6β-hydroxy testosterone and oxidized nifedipine. In contrast, the time-dependent inhibition of erlotinib on CYP3A was substrate-independent. Moreover, the time-dependent inhibition of CYP3A was a possible reason for clinical DDIs related to erlotinib. Cancer patients often receive multiple concurrent medications and should be carefully monitored for possible DDIs. A better understanding of the modulatory effects of erlotinib on the major CYP isoforms could inform clinical safety evaluations of drug combinations.

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Author contribution
Ling YANG and Pei-pei DONG designed research; Pei-pei DONG, Yu-xi MAO, Liang-liang ZHU, Yan-qing QU, and Wei LI performed research; Ling YANG, Chang-xiao LIU, and Liming WANG contributed new analytical tools and reagents; Pei-pei DONG, Zhong-ze FANG, Yan-yan ZHANG, and Guang-bo GE analyzed data; and Pei-pei DONG wrote the paper.

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