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ABSTRACT:

Rifampin and carbamazepine have been recommended in the U.S. Food and Drug Administration draft drug interaction guidance as CYP3A4 inducers for clinical drug-drug interaction (DDI) studies. To optimize the dose regimens of these inducers for use in DDI studies, their effect at various doses and dosing durations on the area under the curve (AUC) of multiple probe substrates was simulated using a population-based simulator. A similar assessment of the inducer phenobarbital was also conducted. CYP3A4 induction by all three inducers was previously determined in hepatocytes, and the results were incorporated into simulations. The pharmacokinetics of the three inducers and their associated CYP3A4 drug interactions were predicted and compared with in vivo observations. The predicted $C_{\text{max}}$ and AUC of all the inducers and substrates correlated closely with those observed clinically. The predicted magnitudes of the DDIs caused by CYP3A4 induction were also in good agreement with the observed clinical results. Comparison of the maximal CYP3A4 induction potential among the three inducers indicated that rifampin is the most potent inducer and is the best choice for clinical CYP3A4 induction DDI studies. Moreover, a near-maximal CYP3A4 DDI was predicted to result from administration of rifampin for approximately 7 days at 450 to 600 mg q.d. or 200 to 300 mg b.i.d. These results suggest optimal dose regimens for clinical trials that maximize the probability of detecting a DDI caused by CYP3A4 induction. The simulation strategy provides the means to predict the induction profiles of compounds in development.

Introduction

Numerous therapeutic compounds are metabolized by cytochrome P450 (P450) enzymes, and many clinically relevant adverse drug-drug or diet-drug interactions are associated with induction or inhibition of P450 enzymes. Drug-drug interactions (DDIs) can cause altered drug exposures that may lead to serious drug toxicity (Honig et al., 1993; Backman et al., 1994, 2002; Gomez et al., 1995) or a reduction in pharmacological effects (Back et al., 1979; Backman et al., 1996). Because of the potential severity of these effects, DDIs remain an important concern in both drug development and clinical practice. Induction of drug-metabolizing enzymes is not as common as inhibition, but it can nonetheless have a profound effect on the pharmacokinetics of drugs that are substrates of the induced enzyme, leading to subtherapeutic drug concentrations and/or an increase in the formation of reactive or active metabolites (Lin, 2006). Although many of the P450 enzymes are known to be inducible, CYP3A4 induction is probably the most important cause of documented induction-based interactions (Lin, 2006). For example, rifampin can precipitate breakthrough bleeding and contraception failure if administered with oral contraceptives (Back et al., 1980) and cause organ rejection if given with cyclosporine (Modry et al., 1985; Hebert et al., 1992).

Rifampin (RIF) and carbamazepine (CBZ) have been recommended as the CYP3A4 inducers to be used when conducting clinical DDI studies. This recommendation was made in the draft U.S. Food and Drug Administration drug interaction guidance published in 2006 (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf). The guidance recommends that clinical DDI studies should be conducted in a manner that maximizes the possibility of finding an interaction. To achieve maximal enzyme induction, multiple doses of an inducer are commonly administered. Ideally a clinical induction DDI study should be conducted as efficiently as possible and avoid unnecessary administration of the inducer to study subjects. This could be achieved if data describing the entire time course of the relationship between the inducer regimen and enzyme induction were available. However, comprehensive in vivo dose-response data of CYP3A4 induction by RIF and other inducers are not readily available, partly because of the limitation in the scope and associated cost of clinical studies.

The importance of CYP3A4 induction has prompted the search for approaches to predict DDIs during the process of drug discovery and development (Chu et al., 2009). Because of major species differences in the degree of response to inducers (Jones et al., 2000; Moore and Kliwer, 2000), there is an increasing demand for the use of human in vitro systems, such as immortalized cell lines (Goodwin et al., 1999), immortalized human hepatocytes (Mills et al., 2004; Ripp et al., 2006), and freshly isolated or cryopreserved human hepatocyte cultures (LeCluyse et al., 2000; Hewitt et al., 2007) for investigating the potential of P450 induction by drug candidates in humans. Using these in vitro systems, various mechanistic approaches have been proposed for deriving quantitative projections of clinical P450 induction-based...
Clinical DDI data between fluconazole (as a CYP3A4 inhibitor) and CBZ (Nair and Morris, 1999). The objectives of the current study were to evaluate and optimize the dosing regimens of clinically used CYP3A4 inducers [rifampin (rifampicin, RIF), carbamazepine (CBZ), and phenobarbital (PB)] for drug interaction studies by modeling and simulation using the population-based clinical trial simulator Simcyp (version 8.01; Simcyp Ltd., Sheffield, UK). Simcyp can simulate the time courses of both perpetrator and victim drug concentrations during sustained administration of the perpetrator. These simulations provide detailed evaluations of enzyme turnover and induction-stimulus response relationships. In the present study, Simcyp was used to model and predict the clinical pharmacokinetics and induction-based DDIs of three CYP3A4 inducers and of four CYP3A4 probe substrates (nifedipine, midazolam, zopidem, and simvastatin) using in vitro CYP3A4 induction kinetic parameters measured previously in human hepatocytes (Shou et al., 2008). The impact of the dosage and dosing duration on the magnitude of DDIs was evaluated for each of the three inducers. Based on the results, recommendations are provided to help guide the efficient design of clinical DDI studies.

### Materials and Methods

**Data Source.** Clinical DDI data described in this report were collected in July 2010 from the Metabolism and Transporter Drug Interaction Database (University of Washington, Seattle, Washington; http://www.druginteractioninfo.org/). Clinical DDI studies involving three CYP3A4 inducers (rifampicin, CBZ, and PB) and four CYP3A4 probe substrates (midazolam, zolpidem, simvastatin, and nifedipine) were chosen for this study. Model input parameters (physicochemical properties, in vitro metabolism data, and pharmacokinetics) for midazolam, zolpidem, simvastatin, and nifedipine and for the inducer RIF were used as supplied in Simcyp. For the other two inducers (CBZ and PB), physicochemical properties, in vitro metabolic stability, competitive inhibition and induction of CYP3A4, and human pharmacokinetic parameters were collected from literature reports and incorporated into Simcyp (Tables 1 and 2). In vitro CYP3A4 induction data (EC₅₀, E_max, and the Hill coefficient, n) for the three inducers from two hepatocyte donors (Table 2) and unbound fractions in plasma (f_u, plasma) and hepatocyte (f_u, hepatocytes) were reported previously (Shou et al., 2008) and were integrated into Simcyp for the simulation. EC₅₀ and E_max were calculated by the Hill equation (eq. 1):

\[
E = \frac{E_{\text{max}} \cdot \text{[Ind]}^{n}}{E_{\text{50}} + \text{[Ind]}^{n}}
\]

where [Ind] is the unbound inducer concentration, E_max is the maximal response (net maximal fold increase), EC₅₀ is the unbound inducer concentration...
at 50% $E_{\text{max}}$, and the Hill coefficient $n$ is the sigmoidicity of the nonlinear curve. $E$ is the induction response at various inducer concentrations for a specific inducer with certain intrinsic induction activity ($E_{\text{max}}$ and $EC_{50}$).

In addition, a third set of in vitro CYP3A4 induction data were generated for CBZ from a fresh human hepatocyte donor (denoted as donor 3) using the method described in Shou et al. (2008) to examine donor variability. Hepatocytes from donor 3 (a 21-year-old white female with no history of smoking) were purchased from CellzDirect (Durham, NC) (body mass index: 27, lot Hu1116, and 90% cell viability).

**Simcyp Simulator for Human DDI Prediction.** The Simcyp population-based absorption, distribution, metabolism, and excretion simulator was used to perform steady-state simulations of clinical drug-drug interactions. Simcyp incorporates dynamic models that account for the time-varying concentration of both victim and perpetrator drugs. The models implemented in Simcyp consider variables such as P450 expression levels, genetic polymorphisms, first-pass intestinal metabolism, and physiological and demographic information in the generation of the virtual populations. The program predicts not only the mean and median effects but also a range and frequency distribution of the magnitude of DDIs (Rostami-Hodjegan and Tucker, 2007).

The algorithm, physiological basis, and differential equations used by the Simcyp software have been described in several publications (Yang et al., 2006, 2007, 2008; Einolf, 2007; Rostami-Hodjegan and Tucker, 2007; Jamei et al., 2009; Xu et al., 2009). The default population mean abundance of CYP3A4 in the liver (137 pmol/mg liver microsomal protein) and gut (66.2 nmol/total gut) was used for the simulations. Predicting the impact of enzyme induction on the in vivo pharmacokinetics of a drug requires knowledge of the turnover rate of the enzyme(s) involved in the clearance of that drug. The Simcyp simulator integrates both liver and gut models (Yang et al., 2007) into the simulations of drug pharmacokinetics and DDIs, with CYP3A4 degradation half-lives of approximately 90 h in the liver and 23 h in the gut (Yang et al., 2008). The simulator uses the intrinsic clearance ($V_{\text{max}}$ and $K_{\text{m}}$) data for all P450s that collectively contribute to the clearance of a particular substrate; consequently, the individual contribution of CYP3A4 ($q_{\text{AUC, CYP3A4}}$) to the total metabolic clearance of each substrate can be determined as shown in Table 3. A northern European white population was used for the subject demographics. Dose, dose interval, and the duration of administration of the inducers and victim drugs were set as described in the figures and legends or corresponded to the regimens of the clinical DDI trials described in the literature. Simulated administration of both the inducers and CYP3A4 substrates was oral, and the fluid intake with each oral dose was 250 ml. The victim drug was dosed as described in literature reports of the clinical DDI trials, or, if not specified, it was dosed simultaneously with the last dose of the inducer. The trials were simulated using a virtual population of healthy volunteers in 10 trials, and in each trial there were 10 subjects aged 18 to 65 years with a female/male ratio of 0.34. The DDIs were determined as the ratios of victim drug AUCs in the presence versus absence of pretreatment with the inducer, and median and population extremes (5th and 95th percentiles) were determined.

**Results**

**Prediction of Multiple Dose Pharmacokinetics of CYP3A4 Inducers.** The in vitro hepatocyte CYP3A4 induction kinetic parameters and the physicochemical properties, intrinsic clearance, competitive inhibition, and in vivo human pharmacokinetic parameters of the three inducers (RIF, CBZ, and PB) are shown in Tables 1 and 2. These values were used for the pharmacokinetic and DDI simulations. When a P450 inducer is used to conduct clinical DDI studies, optimal dosing over multiple days is routinely adopted to maximize the induction potential. Thus, the multiple-dose steady-state pharmacokinetics ($C_{\text{max, ss}}$ and AUC$_{\text{ss}}$) of each CYP3A inducer was simulated with the study designs identical to those in the literature reports, and the predicted versus observed pharmacokinetic values were compared (Table 4). The predicted $C_{\text{max, ss}}$ and AUC$_{\text{ss}}$ values of the three individual inducers were within 2.6-fold of the clinical values reported in the literature (Table 4). Representative simulated mean plasma concentration-time profiles of the three inducers are shown in Fig. 1. CBZ is known to be not only a CYP3A4 inducer but also a substrate; thus, the CBZ concentration-time profile was typical of autoinduction. Because RIF and PB are not metabolized by CYP3A4, their clearance and pharmacokinetics should be independent of induction of CYP3A4 as shown in Fig. 1.

**TABLE 5**

*Predicted and observed pharmacokinetic parameters of CYP3A4 substrates after a single oral dose*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Dose</th>
<th>$C_{\text{max}}$ Predicted</th>
<th>Observed ($\mu g/ml$)</th>
<th>AUC Predicted</th>
<th>Observed ($ng/\text{h/ml}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midazolam</td>
<td>8</td>
<td>28.1</td>
<td>28.3 ± 14.1</td>
<td></td>
<td>175</td>
<td>167 ± 104</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>10</td>
<td>75.2</td>
<td>64.8 ± 34.1</td>
<td></td>
<td>341</td>
<td>216 ± 93.2</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>40</td>
<td>5.54</td>
<td>6.87 ± 3.30</td>
<td></td>
<td>43.4</td>
<td>25.2 ± 16.6</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>10</td>
<td>107</td>
<td>112 ± 50</td>
<td></td>
<td>751</td>
<td>528 ± 337</td>
</tr>
</tbody>
</table>
Prediction of Single Dose Pharmacokinetics of CYP3A4 Substrates. The Simcyp-predicted single oral dose pharmacokinetics ($C_{\text{max}}$ and AUC) of midazolam, zolpidem, simvastatin, and nifedipine are shown in Table 5. The predicted parameters were within 2-fold of the values obtained clinically (Table 5).

Simulation of Rifampin-Nifedipine DDIs. A total of 336 DDI studies using RIF as an inducer have been reported as of July 2010 (Table 6). Of these studies, 600 mg of RIF daily (600 mg q.d.) was the most common dosing regimen (254 of 336, 76%). The next two most common RIF dosing regimens were 450 mg q.d. (7%) and 300 mg b.i.d. (5%). Clinical DDIs after a single oral dose of the inducer RIF pretreatment have also been reported (Ndanasu et al., 1997). In the study, nifedipine (10 mg) was dosed orally 8 h after a single oral treatment of RIF (1200 mg) in healthy volunteers. To test the reliability of Simcyp in predicting induction-based DDIs, the AUC changes in nifedipine after either a single or multiple doses of RIF pretreatment were simulated. In vitro hepatocyte CYP3A4 induction parameters from donor 2 were used for this DDI simulation as well as other simulations in this report unless otherwise specified.

The results of RIF-induced DDIs on nifedipine AUCs are shown in Fig. 2A and B. The median and 5th to 95th percentile DDIs in a population (10 trials) predicted after a single 1200 mg of RIF dose pretreatment are shown in Fig. 2A. The predicted median AUC ratio (0.45) was similar to the observed value of 0.36 reported by Ndanusa et al. (1997). In addition, a 7-day treatment of RIF (600 mg q.d.) followed by a single 10-mg dose of nifedipine (Fig. 2B) resulted in a predicted median AUC ratio of 0.12 (90% central range of 0.031–0.34) that was similar to the clinically observed AUC ratio of 0.082 (Holtbecker et al., 1996). These data indicate that RIF-mediated nifedipine DDIs can be reliably simulated.

Simulation of Rifampin-Midazolam DDIs. A single dose of midazolam (MDZ) ranging from 2 to 15 mg is the most commonly reported dose used in clinical DDI studies with RIF. Therefore, DDIs resulting from single doses of MDZ (2, 5, 8, and 15 mg) after...
repeated treatment with the inducer RIF (600 mg q.d. for 5 days) were simulated. The magnitude of the DDIs was independent of the MDZ doses that were simulated (data not shown). Therefore, 8 mg of MDZ was chosen for the subsequent simulations as described below. MDZ DDIs were simulated with RIF at various dose regimens (50 mg q.d., 100 mg q.d., 450 mg q.d., 600 mg q.d., and 300 mg b.i.d.) and various durations of RIF treatment (1–14 days). The magnitudes of DDIs predicted were compared with results from 11 clinical DDI studies that were performed with the RIF dose regimens of 450 mg q.d., 600 mg q.d., and 300 mg b.i.d. administered for 5 to 15 days (Fig. 3, A–C). The predicted median DDI ratios (0.06–0.12) were similar to the observed MDZ AUC ratios (0.02–0.14), and all of the observed DDI values were within the predicted 5th to 95th percentile ranges (Fig. 3, A–C). The predicted induction DDIs were shown to be dose- and dosing duration-dependent. Each of the three most common dosing regimens simulated (450 mg q.d., 600 mg q.d., and 300 mg b.i.d.) was found to cause profound DDIs, although the dose regimen of 300 mg b.i.d. showed a slightly higher magnitude of DDI (Fig. 3D). An approximate 7-day duration of RIF treatment was required to achieve near maximal induction (Fig. 3, B and D), on the basis of both simulation and clinical results.

The effect of increasing RIF dose levels (daily up to 1600 mg or twice a day up to 800 mg for 7 days) on the MDZ AUC ratio was also evaluated. The results indicated insignificant differences in the predicted magnitude of DDIs when the dose of RIF was greater than 600 mg q.d. or 300 mg b.i.d. (Fig. 4). Thus, it is concluded that 450 to 600 mg q.d. or 200 to 300 mg b.i.d. of RIF administered for approximately 7 days (Figs. 3D and 4) would lead to a near maximal DDI magnitude for the CYP3A4 substrates with a high for approximately 7 days (Figs. 3D and 4). An approximate 7-day duration of RIF treatment was required to achieve near maximal induction (Fig. 3, B and D), on the basis of both simulation and clinical results.

maximal induction, higher doses do not substantially further reduce the 5th to 95th percentile ranges (Fig. 4). Simulation of Rifampin-Zolpidem DDIs. To examine the importance of the fraction of a drug cleared by CYP3A4 (f_m,CYP3A4) on DDIs caused by RIF-mediated induction, a simulation was performed using zolpidem as the victim drug. Zolpidem has an f_m,CYP3A4 of 41% (Table 3). Only one clinical DDI study of zolpidem (20 mg) after pretreatment with RIF (600 mg q.d. for 5 days) has been reported (Villikka et al., 1997). The clinically observed zolpidem AUC ratio (0.28) was lower than the median value predicted in the simulation (0.47) but fell between the 5th and 95th percentiles for the population (Fig. 5). Figure 6 shows the simulated DDI profiles for MDZ and zolpidem after three different RIF dose regimens (450 mg q.d., 600 mg q.d., or 300 mg b.i.d.). This direct comparison shows that the DDI for the substrate with a high f_m,CYP3A4 (MDZ) is greater than that of zolpidem with a low f_m,CYP3A4. It is also noted that ~7 days of RIF treatment at the dose regimens tested is required to achieve near maximal induction for CYP3A4 substrates regardless of f_m,CYP3A4 values.

Simulation of Carbamazepine-Simvastatin DDIs. A total of 68 DDI studies, in which CBZ was used as a CYP3A4 inducer (Table 6), were reported as of July 2010. Of these studies, 18% (n = 12) used a dosing regimen of CBZ of 200 mg b.i.d., 12% used 200 mg q.d., 3% used 300 mg b.i.d., and 7% used 600 mg q.d. Therefore, DDI simulations with CBZ administered at various doses (200 mg q.d., 200 mg b.i.d., 300 mg b.i.d., and 600 mg q.d.) and for various durations (1–21 days) were conducted using simvastatin as the victim drug (Fig. 7A). Prediction of the DDI between simvastatin (80 mg, a commonly used CBZ dose) and CBZ (300 mg b.i.d.) was conducted with the trial design used regimen, was compared with that observed clinically (Fig. 7A). After treatment with 100 mg q.d. of PB for 8 days, the most commonly used regimen, was compared with that observed clinically (Fig. 8A). The predicted median DDI (0.26) was identical to the in vivo observation (0.26) (Fig. 7B). We concluded that 300 mg b.i.d. or 600 mg q.d. of CBZ for ~10 days (Fig. 7A) is needed to achieve the near maximal CYP3A4 induction mediated by CBZ.

Simulation of Phenobarbital-Nifedipine DDIs. A literature search revealed a total of 52 clinical studies in which PB was used as an inducer (Table 6). Various PB dosage regimens were used in these studies (46% with 100 mg q.d., 6% with 60 mg b.i.d., 2% with 60 mg q.d., and 2% with 200 mg q.d.). For the simulation, the dose of the victim drug nifedipine was 20 mg, the most commonly used clinical dose, and in vitro hepatocyte CYP3A4 induction parameters from donor 1 were used. A predicted DDI for a 20-mg dose of nifedipine after treatment with 100 mg q.d. of PB for 8 days, the most commonly used regimen, was compared with that observed clinically (Fig. 8A). The predicted median nifedipine AUC ratio (0.4) was nearly identical to the clinically observed value of 0.39 (Schellens et al., 1989). Other PB dosing regimens were also simulated, and the nifedipine AUC ratios after pretreatment with 200 mg q.d. of PB were clearly lower than those achieved after 60 or 100 mg q.d. of PB dosing regimens (Fig. 8B). These results show that a dose of 200 mg q.d. or higher for...
at least 14 days (Fig. 8B) is required to achieve near maximal PB-mediated CYP3A4 induction.

Impact of CYP3A4 Induction Parameters Obtained from Various Human Hepatocyte Donors on DDI Prediction. Figure 9 shows the time course of DDI profiles (i.e., substrate AUC ratios) at variable inducer doses with the incorporation of in vitro hepatocyte CYP3A4 induction parameters (EC50, Emax, and Hill coefficient) from various donors (two donors for RIF and PB and three for CBZ). Significant donor variability in the magnitude of DDI prediction was observed for CBZ and PB but not for RIF. For the purposes of this simulation, the in vitro CYP3A4 induction data from one of the donors was selected as being more representative of the mean population parameters for the inducers PB (i.e., donor 1) and CBZ (i.e., donor 2), because the selected donor predicted the in vivo DDI better. Estimation of average clinical DDIs for new molecular entities would require the in vitro testing of more donors.

Comparison of Maximal CYP3A4 Induction Potential among the Three Inducers. It was demonstrated in the previous section that the dose regimens of RIF (450–600 mg q.d. or 200–300 mg b.i.d. for ~7 days) exhibited close to the maximal CYP3A4 induction. No further significant increase in CYP3A4 induction was observed when the dose or the duration of RIF was elevated (Figs. 3D and 4). No similarly extensive analysis for CBZ or PB induction was performed, but doses of 1600 mg q.d. for CBZ and 200 mg q.d. for PB are among the highest reported for P450 induction DDI studies (Metabolism and Transporter Drug Interaction Database). Therefore, the maximal CYP3A4 induction potential of 600 mg q.d. of RIF for 7 days was compared with 1600 mg q.d. of CBZ for 14 days and 200 mg q.d. of PB for 14 days. DDIs were simulated for the four CYP3A4 substrates nifedipine, simvastatin, midazolam, and zolpidem with a range of fmCYP3A4 (Fig. 10; Table 3). The substrates with lower fmCYP3A4 were predicted to have smaller observed DDIs. Of the three inducers, RIF was shown to be the most potent, indicating that it is the best choice for clinical DDI studies of CYP3A4 induction.

Discussion

CYP3A4 induction-mediated interaction is a major concern in drug development and clinical practice (Lin and Lu, 1998; Lin, 2006). CYP3A4 induction is not only dose (concentration)-dependent, it is also time-dependent and, therefore, the full extent of an induction-mediated DDI develops more slowly than DDIs due to reversible inhibition (Lin, 2006). The Simcyp simulator can predict from in vitro data the extent of in vivo induction in a virtual population using a concentration-dependent dynamic induction model. In recent studies, Simcyp has been successfully applied by various investigators to quantitatively predict metabolism-based DDIs (Einolf, 2007; Fahmi et al., 2009). It was also used to evaluate the impact of various dosing regimens of ketoconazole on the extent of CYP3A inhibition (Zhao et al., 2009).

The objective of the current simulation study was to evaluate various dosing regimens (dose level and duration) of the three CYP3A4 inducers most commonly used in clinical DDI studies (RIF, CBZ, and PB) with the Simcyp simulator. By simulating complete time- and dose-dependent profiles, the optimal conditions for a DDI study could be evaluated, something not easily accomplished in the clinic. Before generating complete induction profiles, Simcyp was tested for its ability to accurately predict the steady-state pharmacokinetics of the three individual CYP3A4 inducers. The predicted pharmacokinetics (Cmax, ss and AUCss) of all the three inducers cor-
related closely with data obtained clinically (Table 4). The predicted pharmacokinetics (C\text{\textsubscript{max}} and AUC) of the four CYP3A4 probe substrates after a single dose were similarly simulated, and the results agreed well with clinical data (Table 5).

Using the individual inducer and probe substrate kinetics and the in vitro induction parameters, the induction DDIs were simulated. For all of the CYP3A4 inducer-substrate pairs examined, the predicted magnitudes of DDIs were shown to be in good agreement with observed data. The inducers exhibited dose- and dosing duration-dependent effects on the magnitude of victim drug DDIs. This simulation approach was even able to predict a short-term induction DDI when nifedipine was dosed only 8 h after a single dose of 1200 mg of RIF (Fig. 2A). The DDIs predicted by this simulation method were thus validated, indicating that Simcyp could be a useful tool for predicting the induction profiles of compounds in development and assessing the likelihood of DDIs.

The maximal chronic clinical dosage of RIF in most approved indications including tuberculosis is 600 mg q.d. At this simulated dose, the resulting MDZ DDI was near maximal (Fig. 4). Only a minor increase in the magnitude was observed in the simulation as the RIF dose was increased from 600 to 1600 mg q.d. This prediction is consistent with the clinical observation that there was no statistical difference in diazepam DDIs with oral RIF doses between the 600 and 1200 mg (Ohnhaus et al., 1987). After dosing of 600 mg q.d. of RIF for 8 to 10 days in human subjects, the average plasma unbound concentration reached 0.5 to 1.5 \(\mu\text{M}\) (Borin et al., 1997; Swaisland et al., 2005). This concentration is much higher than its in vitro unbound EC\text{\textsubscript{50}} for induction of 0.1 to 0.2 \(\mu\text{M}\), suggesting that at doses of 600 mg and higher CYP3A4 induction is saturated by RIF in vivo. In addition, the true unbound intracellular RIF concentration in hepatocytes in vivo could be underestimated based solely on its unbound plasma concentration because RIF is a substrate for several hepatic uptake transporters (Vavricka et al., 2002; Tirona et al., 2003). Collectively these data indicated that daily doses of RIF higher than 600 mg will not significantly elevate CYP3A4 induction or result in greater DDIs.

The simulations of the various dosing regimens for inducers commonly used in clinical DDI studies revealed some valuable insights into CYP3A4 induction trial design. The results reported here indicate that within the range of common therapeutic doses, RIF dose regimens at 450 to 600 mg q.d. or 200 to 300 mg b.i.d. for 7 days, CBZ at 300 mg b.i.d. or 600 mg q.d. for 10 days, and PB at 200 mg q.d. for 14 days are sufficient for achieving their respective near maximal CYP3A4 induction. Comparison of maximal CYP3A4 induction potentials among the three inducers indicates that RIF is the most potent in vivo inducer and is the best choice for clinical DDI studies of CYP3A4 induction (Fig. 10).

The simulations further indicate that even lower doses of RIF are sufficient to cause near maximal induction and DDIs. RIF dosing regimens as low as 450 mg q.d. or 200 mg b.i.d. for 7 days are sufficient to result in close to the maximal DDI. These doses can, therefore, be considered sufficient for conducting clinical CYP3A4 induction DDI studies (Table 7) and suggest that the recommended RIF dose regimens of 600 mg q.d. (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf) may exceed what is necessary to detect an interaction. In addition, the increase in the median level of induction that can occur at either higher doses or with longer duration is minor compared with the interindividual variability in maximal inducibility (Figs. 3 and 4). Furthermore, the extent of induction...
It is noteworthy that Rif and other commonly used enzyme inducers are often pleiotropic inducers of several P450s (e.g., CYP1A2, 2B6, 2C8/9/19, and 3A4), phase II enzymes, and drug transporters (Rae et al., 2001), all of which play a role in the clearance of drugs. When a drug is metabolized by more than one P450 isofrom, the observed DDI can be greater than predicted solely from the induction of any one of the isofroms. For example, zolpidem is metabolized by not only CYP3A4 but also CYP1A2 and CYP2C9 (Von Moltke et al., 1999). The activities of CYP1A2 and CYP2C9 were reported to be induced by 2- and 3-fold with 20 to 50 µM Rif in human hepatocytes, respectively (Madan et al., 2003). The underestimation of a Rif-zolpidem DDI predicted solely on CYP3A4 induction (Fig. 5) is presumably due to the induction of CYP1A2 and CYP2C9 that was not taken into account. The prediction of the Rif-zolpidem DDI would probably improve upon incorporation of the induction of these isozymes in the simulation. The Rif-zolpidem DDI prediction could also be improved if a shorter hepatic CYP3A4 half-life was used, but the default value was used to maintain consistency with the other simulations.

For inducers with a short half-life (e.g., Rif), the duration of inducer treatment needed for the maximal effect is a function of CYP3A4 half-life and independent of \( f_{\text{u,p,CYP3A4}} \). Therefore, it is critically important to accurately define enzyme half-life to accurately predict the time needed to reach maximal enzyme induction. In our simulations, we used the default Simcyp setting of hepatic and gut CYP3A4 half-lives of 90 and 23 h, respectively. The reported rifampin-midazolam DDIs resulting from a Rif dose of 600 mg q.d. (5–15 days) indicate that the maximal DDI can be reached after as short as 5 days of Rif dosing (Fig. 3B). This finding is consistent with a hepatic CYP3A4 half-life in the range of 24 to 30 h, as well as the 23-h half-life proposed by Wang (2010) that was based on the predictions of drug interactions involving mechanism-based CYP3A inhibitors using both Simcyp and a mathematical model. The literature review by Yang et al. (2008) showed that the estimate of average hepatic CYP3A4 half-life ranges from 10 to 140 h, based on various in vitro and in vivo methods. It is clear that more research is needed to better define the true value of CYP3A4 half-life in vivo. In this regard, our recommendation for the optimal dosing duration of ~7 days for Rif (Table 7) reflects a consideration of both simulation and clinical results (Fig. 3).

Significant variability in the \( E_{\text{max}} \) and \( EC_{50} \) of induction has been reported in the literature (Fahimi et al., 2008) and was also observed for all three inducers among the hepatocytes from different donors in the present study. The magnitude of the DDIs predicted for Rif, the most potent of the inducers tested, varied to only a minor extent among the various in vitro induction parameters used for the predictions (Fig. 9A). As expected, this finding suggests that DDIs caused by potent inducers can be predicted with a high degree of confidence, provided that the elimination route of the victim drug is well described. The approaches can also be used for predicting Rif-mediated DDIs when the substrate is metabolized by multiple inducible P450s (e.g., zolpidem) with the in vitro induction of other P450s by Rif characterized and incorporated into the prediction. In contrast, the predicted magnitude of induction DDIs caused by weak or moderate inducers such as CBZ or PB (for both of these drugs unbound \( C_{\text{max}}/EC_{50} <1 \)) is very sensitive to the in vitro induction parameters used in the simulation (Fig. 9, B and C), and, therefore, DDI predictions for such inducers must be made with considerably more caution. Because of a high degree of interindividual variability in hepatocyte induction, a greater number of donors would be needed to better simulate the average and range of DDIs observed in the clinic.

![Figure 10. Comparison of maximal CYP3A4 induction potentials among the three inducers on four CYP3A4 substrates: nifedipine, simvastatin, midazolam, and zolpidem (with variable \( f_{\text{u,p,CYP3A4}} \) values listed in Table 3). Dose regimens of Rif (600 mg q.d. for 7 days), CBZ (1600 mg q.d. for 14 days), and PB (200 mg q.d. for 14 days) were used, respectively. The CYP3A4 induction parameters used for each inducer were from donor 2 (RIF and CBZ) and donor 1 (PB). The DDI predictions based on the induction parameters from these respective donors were in good agreement with the observed clinical results.](image-url)
In conclusion, the simulation results demonstrate the utility of the simulation tool for prediction of CYP3A4 induction DDIs and provide optimal inducer dosing regimes for determining DDIs. RIF is the most potent in vivo CYP3A4 inducer among the three inducers tested and dose regimes of 450 to 600 mg q.d. or 200 to 300 mg b.i.d. for approximately 7 days were predicted to be sufficient to achieve near maximal CYP3A4 induction and ensure the successful clinical detection of an induction-mediated DDI.

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