

Simulation of Clinical Drug-Drug Interactions from Hepatocyte CYP3A4 Induction Data and Its Potential Utility in Trial Designs

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Abbreviations: DDI, drug-drug interaction; CYP, cytochrome P450; PK, pharmacokinetics; RIF, rifampin; CBZ, carbamazepine; PB, phenobarbital; QD, once a day; BID, twice a day; $f_{m,CYP}$, fraction of a drug cleared by a CYP; AUC, area under the curve.

ABSTRACT

Rifampin and carbamazepine have been recommended in the US 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 AUC of multiple probe substrates was simulated using a population-based simulator. A similar assessment of the inducer phenobarbital was also conducted. In vitro CYP3A4 induction by all three inducers was determined in hepatocytes (Shou et al., 2008) 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_{\max} and AUC of all the inducers and substrates correlated closely with those clinically observed. The predicted magnitudes of the DDIs caused by CYP3A4 induction were also in good agreement with the observed clinical results. Comparison of the maximum 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 - 600 mg QD, or 200 - 300 mg BID. 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 (CYP) enzymes and many clinically relevant adverse drug-drug or diet-drug interactions are associated with induction or inhibition of CYP enzymes. Drug-drug interactions (DDIs) can cause altered drug exposures that may lead to serious drug toxicity (Backman et al., 1994; Backman et al., 2002; Gomez et al., 1995; Honig et al., 1993) 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 sub-therapeutic drug concentrations, and/or an increase in the formation of reactive or active metabolites (Lin, 2006). Although many of the CYP 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 drug-drug interaction (DDI) studies. This recommendation was made in the draft US Food and Drug Administration (FDA) drug interaction guidance published in 2006 (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guid>

ances/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. Unfortunately, comprehensive *in vivo* dose-response data of CYP3A4 induction by RIF and other inducers are not readily available, partly due to 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 Kliever, 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 (Hewitt et al., 2007; LeCluyse et al., 2000) 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 CYP induction-based DDIs, applying either mathematic prediction approaches (Fahmi et al., 2008; Kato et al., 2005; Kozawa et al., 2009; Shou et al., 2008) or calibration curve approaches (Ripp et al., 2006; Kanebratt and Andersson, 2008). Although some success has been achieved by these approaches, these

methods focus on prediction of mean changes in the exposure of a victim drug in the presence of an inducer at a steady-state concentration (Almond et al., 2009; Chu et al., 2009). These static models lack the ability to predict induction DDIs in a population because they do not consider inter-individual variability. Furthermore, they do not provide time course-based dynamics of the pharmacokinetic and DDI profiles with various dose regimens.

The objectives of the current study are to evaluate and optimize the dosing regimens of clinically used CYP3A4 inducers [RIF, CBZ, and phenobarbital (PB)] for drug interaction studies by modeling and simulation using a population-based clinical trial simulator Simcyp (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, zolpidem 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 (RIF, 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, 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 (Table 1 and Table 2). In vitro CYP3A4 induction data (EC_{50} , E_{max} and the Hill coefficient, n) for the three inducers from two hepatocyte donors (Table 2), and unbound fractions in plasma protein ($f_{u,p}$) and hepatocyte ($f_{u,hept}$) were reported previously (Shou M et. al, 2008) and were integrated into Simcyp for the simulation. EC_{50} and E_{max} were calculated by the Hill equation below (Eqn. 1):

$$E = \frac{E_{max} \cdot [Ind]^n}{EC_{50}^n + [Ind]^n} \quad [1]$$

where [Ind] is the unbound inducer concentration, E_{max} is the maximum response (net maximum fold increase), EC_{50} is the unbound inducer concentration at 50% E_{max} , and the Hill coefficient n is the sigmoidicity of the non-linear curve. E is the induction response at various inducer concentrations for a specific inducer with certain intrinsic induction activity (E_{max} and EC_{50}).

In addition, a third set of *in vitro* CYP3A4 induction data was 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 (21-year-old Caucasian female with no history of smoking) were purchased from CellzDirect (BMI 27, lot Hu1116 and 90% cell viability).

Simcyp Simulator for Human DDI Prediction. The Simcyp population-based ADME simulator (version 8.01; Simcyp Ltd., Sheffield, UK) 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 CYP expression levels, genetic polymorphisms, first-pass intestinal metabolism, 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 software have been described in several publications (Yang et al., 2006; Yang et al., 2007; Rostami-Hodjegan and Tucker, 2007; Yang et al., 2008; Einolf, 2007; Xu et al., 2009; Jamei 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) were 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 hours in the liver and 23 hours in the gut (Yang et al., 2008). The simulator uses the intrinsic clearance (or V_{\max} and K_m) data for all CYPs that collectively contribute to the clearance of a particular substrate; consequently, the individual contribution of CYP3A4 ($f_{m,CYP3A4}$) 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 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 were 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 18-65 years with a female to 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 Table 1 and Table 2. These values were used for the pharmacokinetic and DDI simulations. When a P450 inducer is used to conduct clinical DDI studies, the optimal dosing over multiple days is routinely adopted to maximize the induction potential. Accordingly, the multiple-dose steady-state pharmacokinetics ($C_{\max, ss}$ and AUC_{ss}) of each CYP3A inducer was simulated with the study designs identical to the literature reports, and the predicted versus observed pharmacokinetic values were compared (Table 4). The predicted $C_{\max, ss}$ and AUC_{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 auto-induction. Because RIF and PB are not metabolized by CYP3A4, their clearance and pharmacokinetics should be independent of induction of CYP3A4 as shown in Figure 1.

Prediction of single dose pharmacokinetics of CYP3A4 substrates. The Simcyp-predicted single oral dose pharmacokinetics (C_{\max} and AUC) of midazolam, zolpidem, simvastatin, and nifedipine are shown in Table 5. The predicted parameters were within 2-fold of the clinically obtained values (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, RIF 600 mg daily (600 mg QD) was the most common dosing regimen (254 out of 336, 76%). The next two most common RIF dosing regimens were 450 mg QD (7%) and 300 mg BID (5%). Clinical DDIs after a single oral dose of the inducer RIF pretreatment have also been reported (Ndanusa et al., 1997). In the study, nifedipine (10 mg) was dosed orally 8 hr after a single oral treatment of RIF (1200 mg) in healthy volunteers. To test the reliability of Simcyp in predicting induction-based DDI, 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 Figures 2A and 2B. The median and 5th - 95th percentile DDIs in a population (10 trials) predicted after a single 1200 mg 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, 7-day treatment of RIF (600 mg QD) 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 to 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. Accordingly, DDIs resulting with single doses of MDZ (2, 5, 8, and 15 mg) after repeated treatment with the inducer RIF (600 mg QD 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 QD, 100 mg QD, 450 mg QD, 600 mg QD and 300 mg BID), and various durations of RIF treatment (1 to 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 QD, 600 mg QD, and 300 mg BID administered for 5 to 15 days (Fig. 3A, B and 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 -95th percentile ranges (Fig. 3A, B and 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 QD, 600 mg QD and 300 mg BID) were found to cause profound DDIs, albeit the dose regimen of 300 mg BID showed slightly higher magnitude of DDI (Fig. 3D). Approximate 7 day duration of RIF treatment was required to achieve near maximal induction (Fig. 3B and D), based on both simulation and clinical results.

The effect of increasing RIF dose levels (QD up to 1600 mg or BID up to 800 mg for 7 days) on 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 QD or 300 mg BID (Fig. 4). Thus, it is concluded that 450 - 600 mg QD or 200 - 300 mg BID of RIF administered for approximately 7 days (Fig. 3D, Fig. 4) would lead to a near maximal DDI magnitude for the CYP3A4 substrates with a high $f_{m, CYP3A4}$ [e.g., $f_{m, CYP3A4}$ of MDZ = 84% (Table 3)]. It is worth noting that BID dosing appears to provide narrower 90% central ranges for the predicted DDI than QD dosing (Fig. 3 and Fig. 4). In addition, at doses above those necessary to achieve the maximum median induction, higher doses do not substantially further reduce the 5th - 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 DDI 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 QD 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). Fig. 6 shows the simulated DDI profiles for MDZ and zolpidem after three different RIF dose regimens (450 mg QD, 600 mg QD, or 300 mg BID). 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 have been reported as of July 2010 where CBZ was used as a CYP3A4 inducer (Table 6). Of these studies, 18% of them (n = 12) used a dosing regimen of CBZ 200 mg BID, 12% used 200 mg QD, 3% used 300 mg BID, and 7% used 600 mg QD. Accordingly, DDI simulations with CBZ administered at various doses (200 mg QD, 200 mg BID, 300 mg BID, and 600 mg QD) and for various durations (1 to 21 days) were conducted using simvastatin as the victim drug (Fig. 7A). Prediction of the DDI between simvastatin (80 mg, a commonly used dose) and CBZ (300 mg BID) was conducted with the trial design identical to the clinical report (Ucar et al., 2004). As a result, the predicted median DDI (0.26) was identical to the in vivo observation (0.26) (Fig. 7B). We concluded that 300 mg BID or 600 mg QD 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 where PB was used as an inducer (Table 6). Various PB dosage regimens were used in these studies (46% with 100 mg QD; 6% with 60 mg BID, 2% with 60 mg QD and 2% with 200 mg QD). 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 PB QD 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 QD of PB were clearly lower than those achieved after 60 mg or 100 mg QD of PB dosing regimens (Fig. 8B). These results show that a dose of 200 mg QD 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. Fig. 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 (EC_{50} , E_{max} and Hill coefficient) from various donors (2 donors for RIF and PB, and 3 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* CYP3A 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), since 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 maximum CYP3A4 induction potential among the three inducers.

It was demonstrated in the previous section that the dose regimens of RIF (450 - 600 mg QD or 200 - 300 mg BID for ~7 days) exhibited close to the maximum CYP3A4 induction. No further significant increase in CYP3A4 induction was observed when the dose or the duration of RIF is elevated (Fig. 3D, Fig. 4). No similarly extensive analysis for CBZ or PB induction was performed, but doses of 1600 mg QD for CBZ and 200 mg

QD for PB are among the highest reported for CYP induction DDI studies (Metabolism and Transporter Drug Interaction Database®, University of Washington). Accordingly, the maximum CYP3A4 induction potential of 600 mg RIF QD for 7 days was compared with 1600 mg CBZ QD for 14 days and 200 mg PB QD for 14 days. DDIs were simulated for the four CYP3A4 substrates nifedipine, simvastatin, midazolam and zolpidem with a range of $f_{m, CYP3A4}$ (Fig. 10, Table 3). The substrates with lower $f_{m, CYP3A4}$ 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. Recently, Simcyp has been successfully applied by various investigators to quantitatively predict metabolism-based DDIs (Fahmi et al., 2009; Einolf, 2007). It was also recently 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) using 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. Prior to 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 ($C_{\max, ss}$ and AUC_{ss}) of all the three inducers correlated closely with data obtained clinically (Table 4). The predicted pharmacokinetics (C_{\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 hrs after a single dose of 1200 mg 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 maximum chronic clinical dosage of RIF in most approved indications including tuberculosis is 600 mg QD. 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 mg QD to 1600 mg QD. 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 600 mg QD of RIF for 8 to 10 days in human subjects the average plasma unbound concentration reached 0.5 – 1.5 μM (Borin et al., 1997; Swaisland et al., 2005). This concentration is much higher than its in vitro unbound EC_{50} for induction of 0.1 - 0.2 μM 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 during in vitro evaluation could be underestimated based solely on its unbound plasma concentration since 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 RIF dose regimens at 450 - 600 mg QD or 200 - 300 mg BID for 7 days, CBZ at 300 mg BID or 600 mg QD for 10 days, and PB at 200 mg QD for 14 days are sufficient for achieving their respective near maximal CYP3A4 induction. Comparison of maximum CYP3A4 induction potential among the three inducers indicate 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 QD or 200 mg BID for 7 days are sufficient to result in close to the maximum DDI. These doses can, therefore, be considered as sufficient for conducting clinical CYP3A4 induction DDI studies (Table 7) and suggest that recommended RIF dose regimens of 600 mg QD (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf>) may exceed what is necessary to detect an interaction. Additionally, the increase in the median level of induction that can occur at either higher doses or with longer duration is minor compared to the inter-individual variability in maximum inducibility (Fig. 3 and Fig. 4). Furthermore, the extent of induction among subjects after BID dosing is predicted to be less variable than after QD dosing (Fig. 3). Thus, the BID dosing may be preferable to QD dosing to reduce the variability in the DDIs observed clinically.

The effect of the fraction of a drug that is metabolized by CYP3A4 ($f_{m, \text{CYP3A4}}$) on an induction-mediated DDI was also simulated (Fig. 6 and Fig. 10). Substrates with

various $f_{m, CYP3A4}$ values were examined and as expected the results show that the induction DDI worsens as the $f_{m, CYP3A4}$ of a substrate increases. These findings are consistent with clinical observations and a mathematic model (Shou et al., 2008). The magnitude of DDI is, therefore, not only dependent on the potency of an inducer but also the fraction of the victim drug's clearance that occurs via CYP3A4. While the magnitude of the DDI may be less for a drug with a low $f_{m, CYP3A4}$, there is no difference in the dosing regimen necessary to achieve the maximum DDI (Fig. 6).

It has been established that to detect a significant inhibition-based DDI, the affected drug should have an appreciable fraction of its clearance mediated by the target enzyme (i.e., $f_m > 70\%$) (Yao and Levy, 2002). For DDIs due to induction, however, low $f_{m, CYP3A4}$ drugs (e.g., zolpidem $f_{m, CYP3A4} = 41\%$) can still be significantly affected by a potent enzyme inducer. This is because $f_{m, CYP3A4}$ of the victim drug increases as a result of CYP3A4 induction. Calculations based on the mathematic model (Eqn. 2) developed by Shou et al. (2008) were performed using the induction kinetics (Table 2), unbound fraction in hepatocytes (0.419) and in vivo unbound C_{max} (2.5 μ M) of RIF (Shou et al., 2008). These calculations revealed that the AUC of a victim drug with $f_{m, CYP3A4}$ of $\geq 8\%$ can be reduced by 50% or more after chronic RIF treatment. Therefore, substrates with a low $f_{m, CYP3A4}$ cannot be assumed to be free of a DDI risk due to CYP3A4 induction.

$$\frac{AUC'}{AUC} = \frac{1}{f_{m, CYP3A4} \cdot \left(1 + \frac{E_{max} \cdot [f_{u,p} \cdot Ind]^n}{(EC_{50} \cdot f_{u,hept})^n + [f_{u,p} \cdot Ind]^n}\right) + (1 - f_{m, CYP3A4})} \quad [2]$$

It is noteworthy that RIF and other commonly used enzyme inducers are often pleiotropic inducers of several CYPs (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 CYP isoform the observed DDI can be greater than predicted solely from the induction of any one of the isoforms. For example, zolpidem is metabolized by not only CYP3A4 but also CYP1A2 and CYP2C9 (Von Moltke et al., 1999). The activities of CYP1A2 and 2C9 were reported to be induced by 2- and 3- fold with 20-50 μ M of 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 maximum effect is a function of CYP3A4 half-life and independent of f_m , CYP_{3A4} . It is, therefore, critically important to accurately define enzyme half-life in order to accurately predict the time needed to reach maximum enzyme induction. In our simulations, we used the default Simcyp setting of hepatic and gut CYP3A4 half-lives of 90 and 23 hrs, respectively. The reported rifampin-midazolam DDIs resulting from a RIF dose of 600 mg QD (5 to 15 days) indicate that the maximum 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 24~30 hrs, as well as the 23 hr 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 mathematic model.

Significant variability in the E_{\max} and EC_{50} of induction has been reported in the literature (Fahmi 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 suggests that DDIs caused by potent inducers can be predicted with a high degree of confidence providing the elimination route of the victim drug is well described. The approaches can also be employed for predicting RIF-mediated DDI when the substrate is metabolized by multiple inducible CYPs (e.g., zolpidem) with the in vitro induction of other CYPs 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 $C_{\max, fu}:EC_{50} < 1$) is very sensitive to the in vitro induction parameters used in the simulation (Fig. 9B and C) and, therefore, DDI predictions for such inducers must be made with considerably more caution. Due to a high degree of inter-individual 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.

In conclusion, the simulation results demonstrate the utility of the simulation tool for prediction of CYP3A4 induction DDIs and provide optimal inducer dosing regimens for determining DDIs. RIF is the most potent in vivo CYP3A4 inducer among the three inducers tested and dose regimens of 450 - 600 mg QD, or 200 - 300 mg BID 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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Authorship Contributions

Participated in research design: Xu and Shou.

Conducted experiments: Hayashi.

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Legends for figures

Figure 1. Simcyp-simulated systemic plasma concentration versus time profiles for three inducers. PB: 100 mg QD dosed for 38 days; CBZ: 200 mg BID dosed for 32 days; and RIF: 300 mg BID dosed for 14 days. Refer to Table 4 for detailed pharmacokinetic parameters.

Figure 2. Comparison of Simcyp-predicted versus observed RIF-mediated DDIs on nifedipine. **(A)** Effect of nifedipine dosing time (hours after a single pretreatment of 1200 mg of RIF) on the magnitude of RIF-nifedipine DDI, expressed as nifedipine AUC ratio. The observed DDI data was the mean of 6 subjects (Ndanusa et al., 1997). **(B)** Effect of RIF (600 mg QD) dosing days (1-14 days) on the magnitude of RIF-nifedipine DDI, expressed as nifedipine AUC ratio. The observed DDI data was the mean of 6 subjects (Holtbecker et al., 1996).

Figure 3. Effect of RIF dosing days (1-14 days) at various RIF dose levels on the predicted magnitude of RIF-MDZ DDI (expressed as MDZ AUC ratio). The observed clinical DDI data are also shown in **A**, **B** and **C** for comparison. **(A)** Dose of RIF: 450 mg QD. The observed DDI data was the mean of 4 subjects (Eap et al., 2004). **(B)** Dose of RIF: 600 mg QD. The observed DDI data were the means of: 9 or 10 subjects (Kharasch et al., 2004; Backman et al., 1998; Backman et al., 1996) for RIF dosed for 5 days; 8 subjects (Link et al., 2008) for RIF dosed for 6 days; 14 subjects (Gorski et al., 2003) for RIF dosed for 7 days; 19 subjects (Chung et al., 2006) for RIF dosed for 9 days; 16 subjects (Adams et al., 2005) for RIF dosed for 14 days and 57 subjects (Floyd et al.,

2003) for RIF dosed for 15 days, respectively. (C) Dose of RIF: 300 mg BID. The observed DDI data was the mean of 19 or 16 subjects (Gurley et al., 2006; Gurley et al., 2008) for RIF dosed for 7 days. (D) The simulated effect of different dose regimens of RIF on RIF-MDZ DDI (median AUC ratio). The 5th and 95th percentiles are omitted for clarity.

Figure 4. Effect of RIF dosage (A: BID from 50 to 800 mg for 7 days; B: QD from 50 to 1600 mg for 7 days) on the predicted magnitude of RIF-MDZ DDI (expressed as MDZ AUC ratio). The observed clinical DDI data are also shown in A and B for comparison. (A) RIF dosed as BID. The observed DDI data was the mean of 19 or 16 subjects (Gurley et al., 2006; Gurley et al., 2008). (B) RIF dosed as QD. The observed DDI data was the mean of 14 subjects (Gorski et al., 2003).

Figure 5. Effect of RIF (600 mg QD) dosing days (1-21 days) on the magnitude of RIF-zolpidem DDI (expressed as zolpidem AUC ratio). The observed DDI data was the mean of 8 subjects (Villikka et al., 1997).

Figure 6. Effect of $f_{m, CYP3A4}$ of probe substrates on the magnitude of RIF-mediated DDIs (expressed as substrate AUC ratio) at various RIF dose levels. RIF doses: (A) 450 mg QD; (B) 600 mg QD; (C) 300 mg BID. Predicted DDIs of RIF-zolpidem (RIF dosed at 600 mg QD) were also presented in Fig. 5. The purpose of Fig. 6 is to illustrate the importance of $f_{m, CYP3A4}$ on the magnitude of induction DDI. The mean $f_{m, CYP3A4}$ values of zolpidem (41%) and MDZ (84%) were calculated from Simcyp (Table 3).

Figure 7. Effect of CBZ dosing days (1-21 days) on the magnitude of CBZ-simvastatin DDI (expressed as simvastatin AUC ratio). **(A)** The four dose regimens of CBZ chosen for simulation were commonly used in DDI studies: 200 mg QD or BID, 300 mg BID, and 600 mg QD. **(B)** Simulation results of CBZ dosed at 300 mg BID were extracted from **A** to show the 5th -95th percentile ranges and compare with clinical data. The observed DDI data was the mean of 12 subjects (Ucar et al., 2004).

Figure 8. Effect of PB dosing days (1-21 days) on the magnitude of PB-nifedipine DDI (expressed as nifedipine AUC ratio). **(A)** PB was dosed at 100 mg QD. The observed DDI data was the mean of 15 subjects (Schellens et al., 1989). **(B)** The three dose levels of PB simulated were commonly used in DDI studies: 60 mg, 100 mg and 200 mg QD.

Figure 9. Effect of human hepatocyte donor variability (donor 1, 2 and 3) on DDI prediction. **(A)** Effect of RIF dosing days (1-21 days) on RIF-MDZ DDI (RIF dosed at 450 mg or 600 mg QD) with induction parameters from donor 1 and 2, respectively (Table 2). **(B)** Effect of CBZ dosing days (1-21 days) on CBZ-simvastatin DDI (CBZ dosed at 200 mg QD or 300 mg BID) with induction parameters from donor 1, 2 and 3, respectively (Table 2). **(C)** Effect of PB dosing days (1-21 days) on PB-nifedipine DDI (PB dosed at 60 mg or 200 mg QD) with induction parameters from donor 1 and 2, respectively (Table 2).

Figure 10. Comparison of maximum CYP3A4 induction potential among the three inducers on four CYP3A4 substrates: nifedipine, simvastatin, midazolam and zolpidem (with variable $f_{m, CYP3A4}$ values listed in Table 3). Dose regimens of RIF (600 mg QD for 7 days), CBZ (1600 mg QD for 14 days) and PB (200 mg QD 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.

Table 1. Input parameters of three CYP3A4 inducers in Simcyp for DDI simulations.

Parameters	Rifampin	Carbamazepine	Phenobarbital
MW (g/mol)	823 ^b	236 ^e	232 ^e
Log P _{octanol:water}	3.28 ^b	2.3 ^e	1.47 ^e
pK _{a1}	7.9 ^b	-	7.3 ^e
pK _{a2}	1.7 ^b	-	-
Blood to plasma ratio	0.9 ^b	1.0	1.0
f _{u, plasma}	0.175 ^c	0.26 ^f	0.7 ^c
Polar surface area (PSA, Å ²)	-	46.33 ^g	78.27 ^g
Count of hydrogen bond donors (HBD)	-	1 ^g	2 ^g
First-order absorption rate constant - K _a (1/h)	0.51 ^b	1.91 ^h	0.48 ^h
Volume of distribution - V _{d,ss} (L/Kg)	0.33 ^b	1.4 ^f	0.54 ^f
HLM ^a CL _{int} for CYP3A4 (μL/min/mg)	-	0.69 ⁱ	-
HLM ^a CL _{int} for other enzymes (μL/min/mg)	-	0.56 ⁱ	-
Renal clearance - CL _R (L/h)	1.2 ^b	0 ^f	0.07 ^f
Oral plasma clearance - CL _{po} (L/h)	-	-	0.3 ^f
Systemic plasma clearance - CL _{iv} (L/h)	7 ^b	-	-
CYP3A4 Ki (μM)	18.5 ^d	-	-
f _{u, hepatocytes}	0.419 ^c	0.52 ^c	1.0 ^c

^a Human liver microsomes.

^b Default parameters implemented in Simcyp.

^c Shou et al., 2008.

^d Kajosaari et al., 2005

^e The DrugBank database (<http://www.drugbank.ca/>)

^f Thummel et al., 2006

^g Calculated from the software ACD labs log D suite.

^h Predicted by Simcyp using PSA and HBD.

ⁱ Pelkonen et al., 2001 and assuming that f_{m, CYP3A4} for CBZ is 55% based on the clinical DDI data between fluconazole (as a CYP3A4 inhibitor) and CBZ (Nair and Morris, 1999).

Table 2. EC_{50} , E_{max} and Hill coefficient (n) values of the inducers for CYP3A4 activity in human hepatocytes.^a

Inducer	Donor	EC_{50} (μ M)	E_{max}	n
Rifampin	1	0.25	10.6	1.2
	2	0.51	12.51	1.2
Carbamazepine	1	15.3	15.73	2.1
	2	27.7	4.57	2.6
	3	26.4	10.1	0.37
Phenobarbital	1	250	22.65	0.9
	2	120.3	8.65	1.4

^aFrom *Shou et al.* (2008) except for donor 3

Table 3. $f_{m, CYP3A4}$ of various CYP3A4 substrates (n = 100 individuals) used in Simcyp for simulation.

	$f_{m, CYP3A4}$ (%)	
	Mean (S.D.)	Median (5 th – 95 th %)
Nifedipine	98.1 (4.1)	100 (88.6 – 100)
Simvastatin	83.1 (12.0)	87.1 (56.4 – 96.6)
Midazolam	83.7 (29.8)	100 (20.0 – 100)
Zolpidem	40.8 (17.8)	39.3 (15.3 -72.8)

Table 4. Predicted and observed pharmacokinetic parameters of CYP3A4 inducers after multiple oral doses.

Inducer	Dose Regimen	C _{max, ss} (µg/mL)		AUC _{ss} (µg*h/mL)		Reference
		Predicted	Observed	Predicted	Observed	
Rifampin	300 mg BID for 14 days	4.04	4.5 ± 1.44	33.2	13.5 ± 4.2	(Drusano et al., 1986)
Carbamazepine	200 mg BID for 32 days	6.78 – 12.2 ^a	6.97 ± 0.64	98.7 - 182 ^a	70.3 ± 8.92	(Rapeport et al., 1996)
Phenobarbital	100 mg QD for 38 days	13.4	16.8	354	342	(Reidenberg et al., 1995)

^a The predicted C_{max, ss} and AUC_{ss} values for CBZ are the range of Simcyp predictions using the in vitro CYP3A4 induction data from the respective three hepatocyte donors (donor 1, 2 or 3 in Table 2).

Table 5. Predicted and observed pharmacokinetic parameters of CYP3A4 substrates after a single oral dose.

Substrate	Dose	C _{max} (ng/mL)		AUC (ng*h/mL)		Reference
		Predicted	Observed	Predicted	Observed	
Midazoalm	8 mg	28.1	28.3 ± 14.1	175	167 ± 104	(Uchida et al., 2006)
Nifedipine	10 mg	75.2	64.8 ± 34.1	341	216 ± 93.2	(Lang et al., 2003)
Simvastatin	40 mg	5.54	6.87 ± 3.30	43.4	25.2 ± 16.6	(Backman et al., 2000)
Zolpidem	10 mg	107	112 ± 50	751	528 ± 337	(Saari et al., 2007)

Table 6. Dose regimens of three inducers in drug interaction studies from literature

Inducer	Dosing Regimen	No. of Studies	% of Studies
Rifampin	600 mg once daily (600 mg QD)	254	76
	450 mg once daily (450 mg QD)	25	7
	300 mg twice daily (300 mg BID)	18	5
	Other	39	12
	Total	336	-
Carbamazepine	200 mg once daily (200 mg QD)	8	12
	200 mg twice daily (200 mg BID)	12	18
	600 mg once daily (600 mg QD)	5	7
	300 mg twice daily (300 mg BID)	2	3
	Other	41	60
	Total	68	-
Phenobarbital	60 mg once daily (60 mg QD)	1	2
	100 mg once daily (100 mg QD)	24	46
	200 mg once daily (200 mg QD)	1	2
	Other	26	50
	Total	52	-

Data extracted from the Metabolism and Transporter Drug Interaction Database® (searched in July 2010).

Table 7. Recommended CYP3A4 inducer and dose regimens for clinical DDI studies.

Inducer	Dose Regimen	Duration of Induction
Rifampin	450 - 600 mg QD or 200 - 300 mg BID	~7 days

Fig. 1

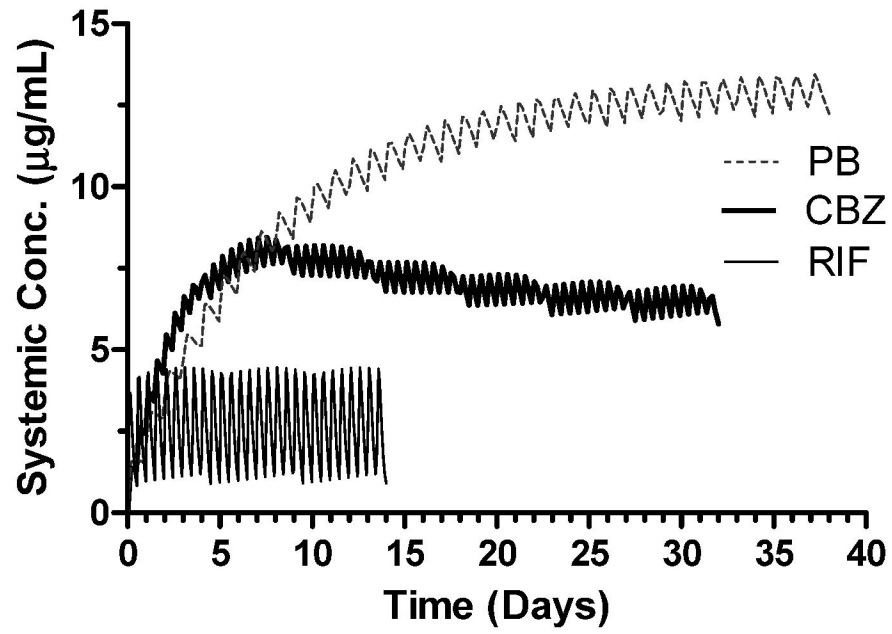
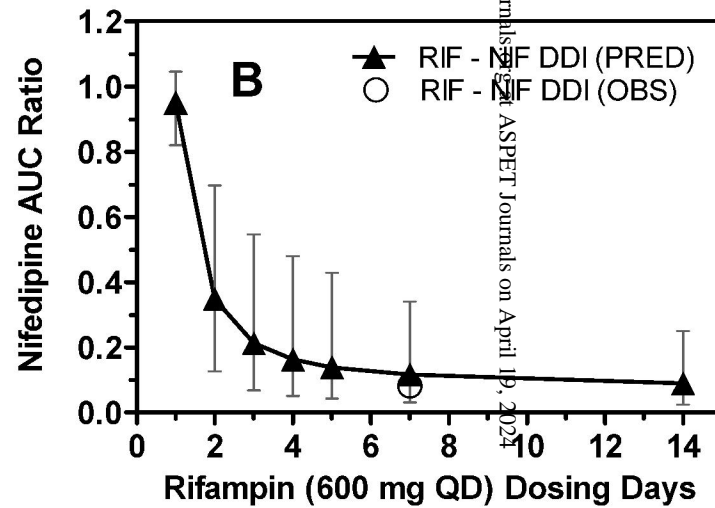
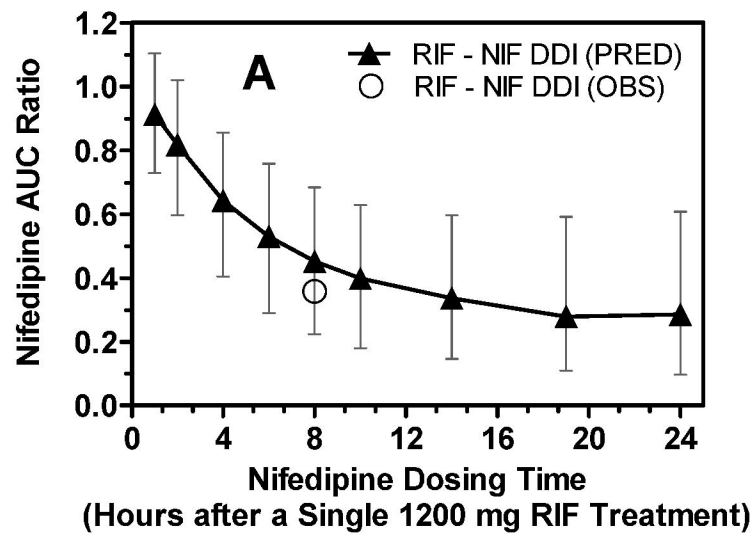
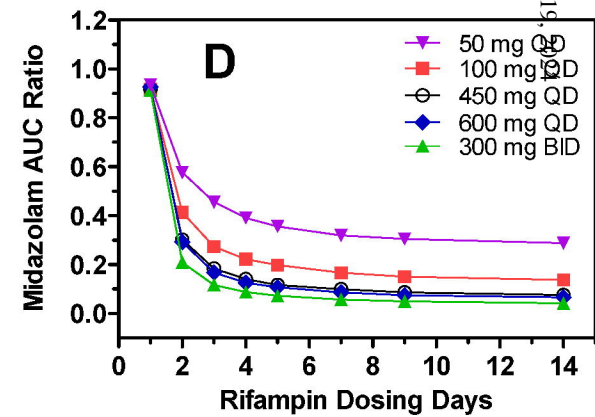
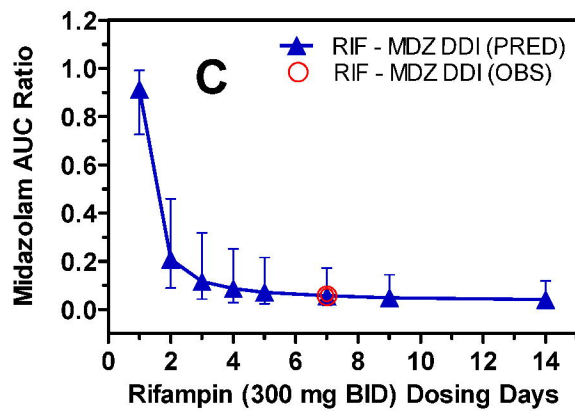
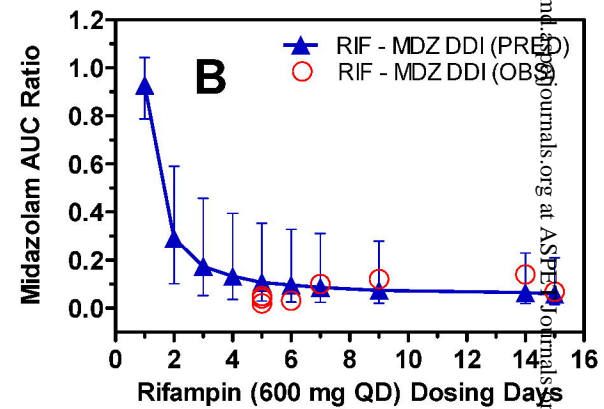
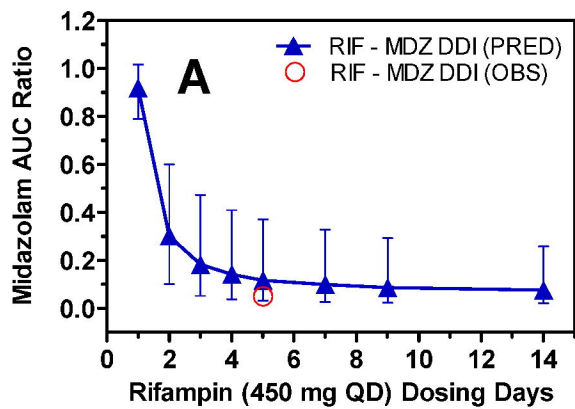


Fig. 2



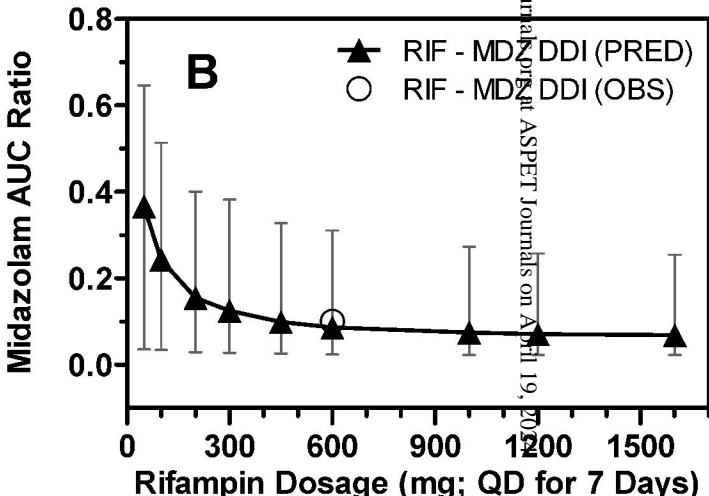
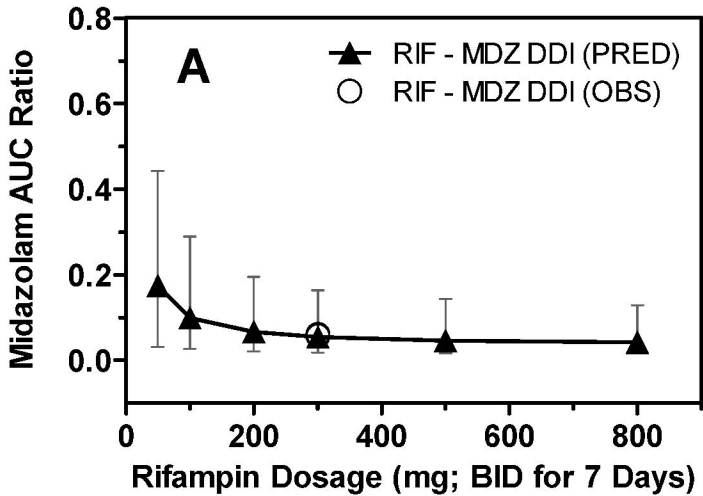
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Fig. 3



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Fig. 4



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Fig. 5

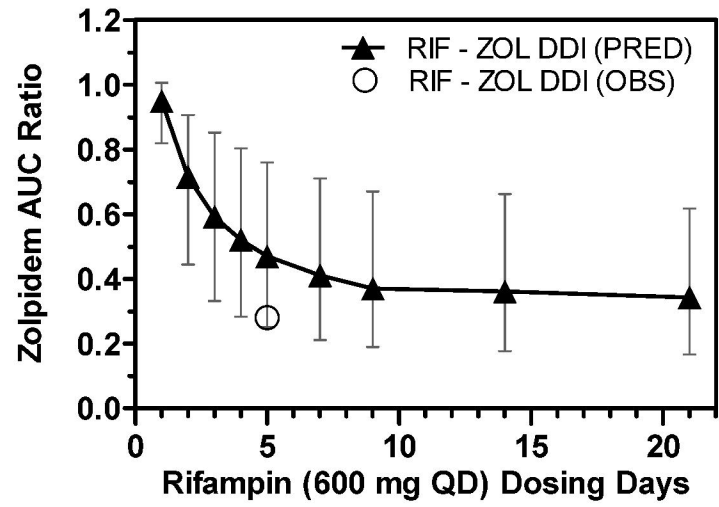


Fig. 6

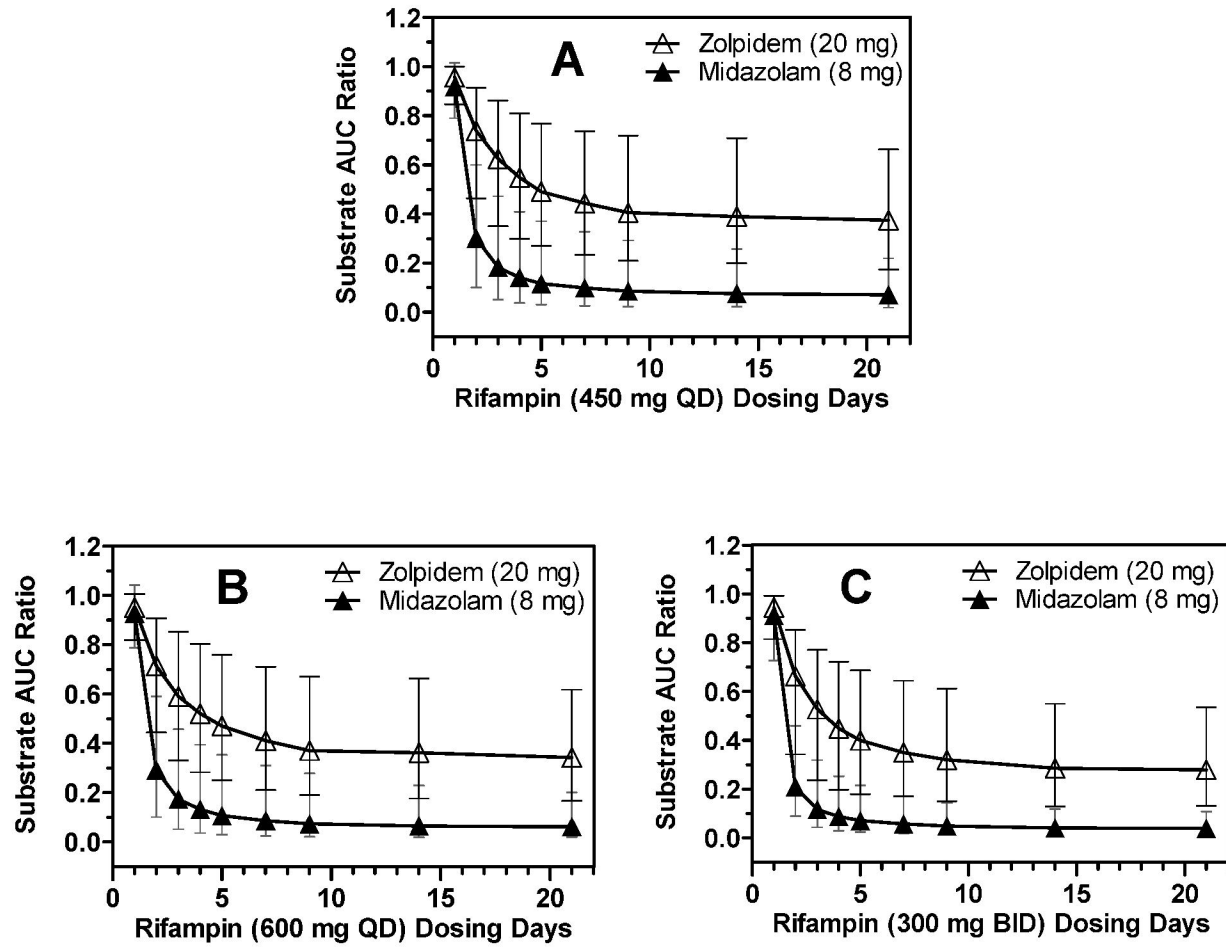


Fig. 7

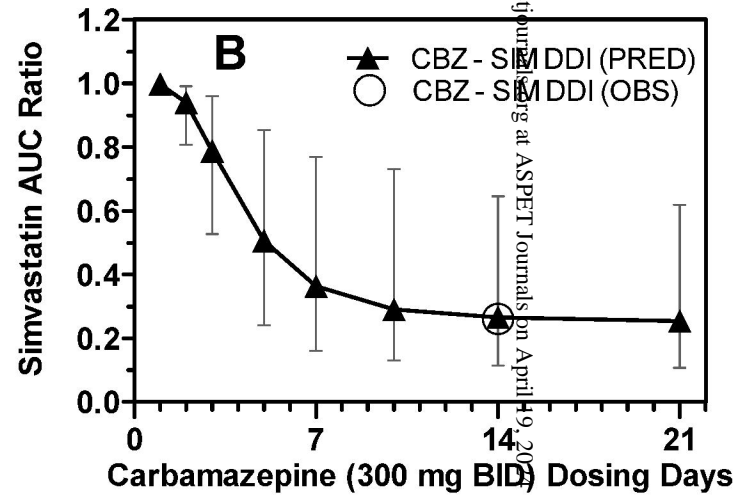
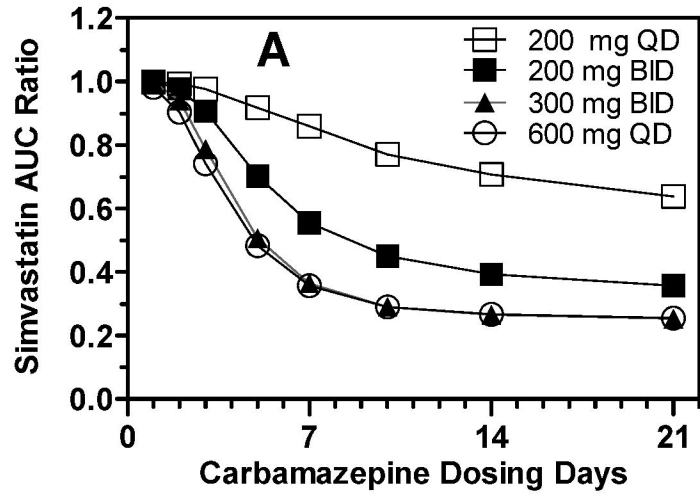
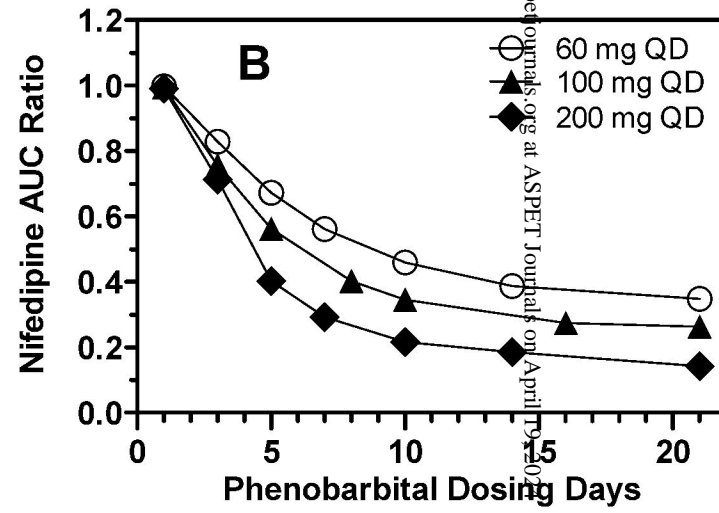
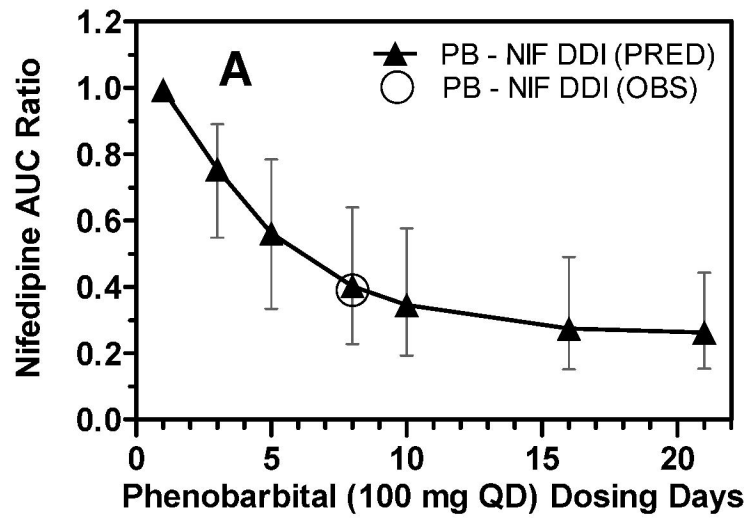


Fig. 8



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Fig. 9

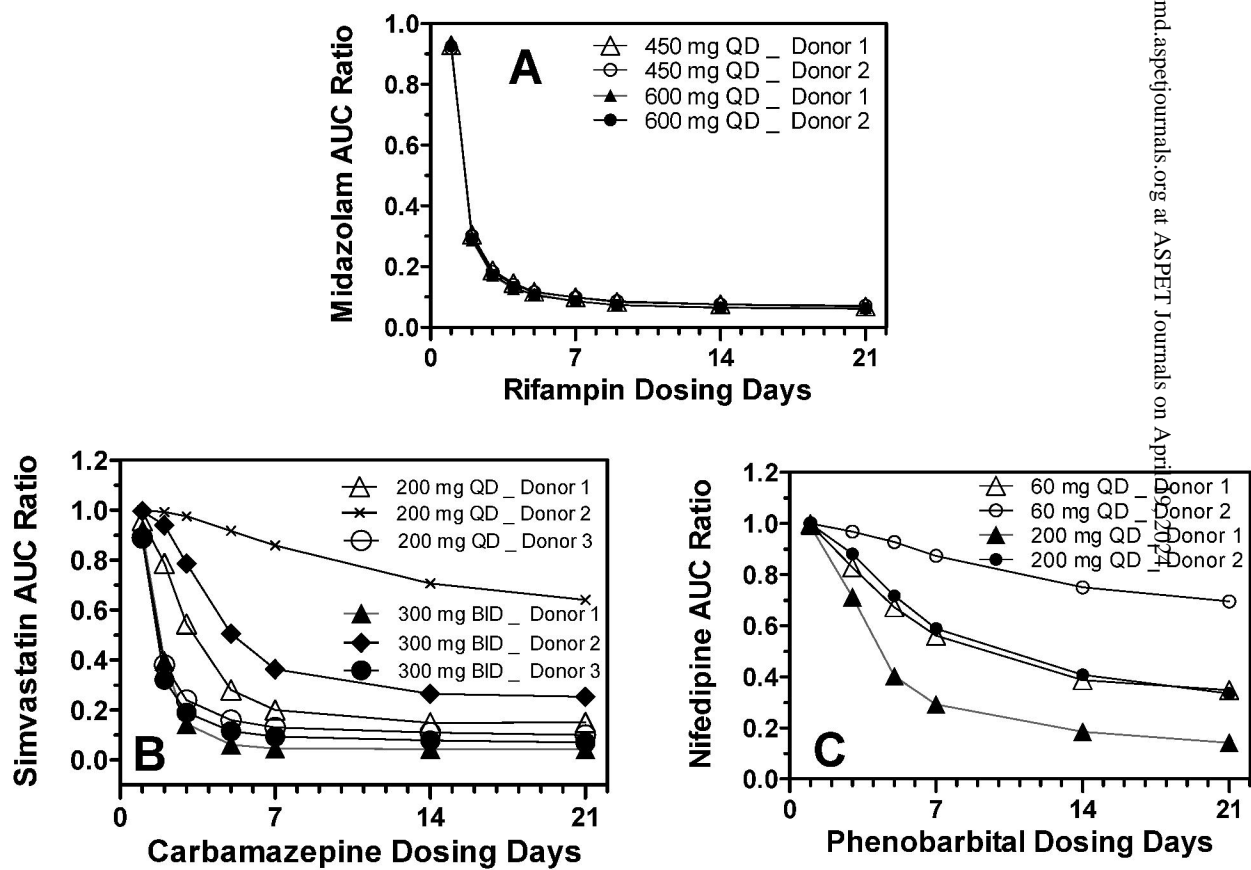


Fig. 10

