Physiologically-Based Pharmacokinetic Model of Mechanism-Based Inhibition of CYP3A by Clarithromycin

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Abbreviations: CYP3A, cytochrome P450 3A; DDI, drug-drug interaction; PK, pharmacokinetic; PBPK, physiologically based pharmacokinetic; MIC, metabolic intermediate complex; $K_i$, concentration of inhibitor required for half maximal inactivation; $k_{\text{inact}}$, maximum inactivation rate constant; $CL_{\text{int}}$, intrinsic clearance; $K_i$, reversible equilibrium inhibition constant; $k_{\text{deg}}$, degradation rate constant; $f_u$, fraction unbound; $Q_H$, hepatic blood flow; AUC, area under the plasma concentration-time curve.
Abstract
The prediction of clinical drug-drug interactions (DDI’s) due to mechanism-based inhibitors of CYP3A is complicated when the inhibitor itself is metabolized by CYP3A, as in the case of clarithromycin. Previous attempts to predict the effects of clarithromycin on CYP3A substrates, e.g. midazolam, fail to account for nonlinear metabolism of clarithromycin. A semi-physiologically based pharmacokinetic model was developed for clarithromycin and midazolam metabolism, incorporating hepatic and intestinal metabolism by CYP3A and nonCYP3A mechanisms. CYP3A inactivation by clarithromycin occurred at both sites. $K_i$ and $k_{inact}$ values for clarithromycin obtained from in vitro sources were unable to accurately predict the clinical effect of clarithromycin on CYP3A activity. An iterative approach determined the optimum values to predict in vivo effects of clarithromycin on midazolam to be 5.3 µM for $K_i$ and 0.4 and 4 h⁻¹ for $k_{inact}$ in the liver and intestines, respectively. The incorporation of CYP3A-dependent metabolism of clarithromycin enabled prediction of its nonlinear pharmacokinetics. The predicted 2.6 fold-change in i.v. midazolam AUC following 500 mg clarithromycin p.o. twice daily was consistent with clinical observations. While the mean predicted 5.3 fold-change in the AUC of p.o. midazolam was lower than mean observed values, it was within the range of observations. Intestinal CYP3A activity was less sensitive to changes in $K_i$, $k_{inact}$, and CYP3A half-life than hepatic CYP3A. This semi-PBPK model incorporating CYP3A inactivation in the intestine and liver accurately predicts the nonlinear pharmacokinetics of clarithromycin and the DDI observed between clarithromycin and midazolam. Further, this model framework can be applied to other mechanism-based inhibitors.
Introduction

The macrolide antibiotic clarithromycin is extensively metabolized by CYP3A to 14-hydroxycellarithromycin and N-desmethylclarithromycin (Suzuki et al., 2003), and exhibits non-linear pharmacokinetics, demonstrated by reduced clearance with increasing doses. Accordingly, the ratio of 14-hydroxycellarithromycin to clarithromycin decreases with increasing doses, indicating saturation or auto-inhibition of CYP3A-mediated metabolism (Chu et al., 1992). Clarithromycin forms a non-covalent metabolic-intermediate complex (MIC) with CYP3A(Mayhew et al., 2000), leading to a time-dependent decrease in the clearance of midazolam by CYP3A. Covalent binding of a clarithromycin intermediate complex to CYP3A leads to an increased rate of enzyme degradation, defined by the maximum inactivation rate constant ($k_{\text{inact}}$) and the concentration of inhibitor needed to achieve half of the maximum inactivation ($K_i$). CYP3A inactivation leads to a reduction in the in vivo intrinsic clearance ($CL_{\text{int}}$), reflected by an increase in the AUC of a CYP3A probe substrate, e.g. midazolam, and the nonlinear kinetics of clarithromycin. Clinically, clarithromycin increases the AUC of oral midazolam by 7-fold, resulting in prolonged sedation (Gorski et al., 1998).

Several studies have evaluated the effect of clarithromycin on oral or intravenous midazolam disposition (Yeates et al., 1996; Gorski et al., 1998; Gorski et al., 2002b; Wang et al., 2004; Pinto et al., 2005). Midazolam is a commonly used in vivo probe for CYP3A activity that undergoes CYP3A-mediated hydroxylation to form 1’-hydroxymidazolam and 4-hydroxymidazolam (Kronbach et al., 1989; Gorski et al., 1994). A small proportion, 1-2% of the dose, is glucuronidated (Hyland et al., 2009) and less than 1% of the administered dose is excreted unchanged in the urine. In contrast to other CYP3A substrates, midazolam does not appear to undergo p-glycoprotein mediated transport (Schmiedlin-Ren et al., 1997; Kim et al., 1999). The clearance of intravenously administered midazolam correlates with hepatic CYP3A protein expression and activity (Thummel et al., 1994a; Thummel et al., 1994b). Administration of both oral and intravenous midazolam enables one to determine intestinal CYP3A activity (Gorski et al., 1998).

Clinical effects of mechanism based inhibitors have been predicted from in vitro data using non-physiological and semi-physiological models (Mayhew et al., 2000; Takanaga et al., 2000; Ito et al., 2003; Venkatakrishnan and Obach, 2005; Polasek and Miners, 2006; Einolf, 2007; Fahmi et al., 2008). Previous models of drug-drug interactions with clarithromycin (Ito et al., 2003; Polasek and Miners, 2006) fail to
account for changes in CYP3A-mediated metabolism of clarithromycin, thus, not accounting for nonlinearity in the clearance of clarithromycin. Furthermore, these models do not incorporate the time-course of intestinal CYP3A inactivation by clarithromycin.

A semi-physiologically based pharmacokinetic (PBPK) model including significant covariates, i.e. age, weight, sex, was developed and validated to illustrate the effect clarithromycin on CYP3A activity \textit{in vivo} as demonstrated by changes in the AUC of midazolam. The time-course for inactivation of CYP3A is predicted in both gut wall and liver, and both hepatic and intestinal enzymes influence the clearance of midazolam and clarithromycin. Additionally, this model accounts for the nonlinear clearance of clarithromycin. Sensitivity of the model to changes in various parameters was evaluated to estimate the importance of accurate determination of inactivation parameters.
Methods

Overview. The semi-PBPK models for clarithromycin and midazolam were separately developed using data from clinical studies and physiological parameters from literature. The interaction models between clarithromycin and midazolam for the gut wall and liver were established from in vitro data, and were subsequently validated by clinical studies (Gorski et al., 2002b; Wang et al., 2004; Pinto et al., 2005). Further simulations and sensitivity analyses were performed from the validated model.

Simulated Study Population. The simulated study population was drawn at random from an equal distribution of males and females. Weight was included as a covariate for calculation of hepatic blood flow ($Q_H$), and described by a normal distribution with males having a mean of 72 kg and females a mean of 65 kg, with a 20% coefficient of variation, similar to our study population.

Semi-PBPK Model. The semi-PBPK models derived for clarithromycin and midazolam were identical except that clarithromycin is fit to a one-compartment model, while midazolam fits a two compartment model. Both models incorporated compartments representing the gut lumen, gut wall, portal vein, and liver. The model describing midazolam PK (Figure 1, Table 1) was modified from Chien, et al. (Chien et al., 2006) and has been described previously by Zhang et al. (Zhang et al., 2009). The differential equations used to define this model are provided in equations 1-6. Pharmacokinetic parameters of clarithromycin were incorporated from literature sources (Table 1) to construct a similar one-compartment model for clarithromycin, incorporating first-pass intestinal metabolism and the well-stirred model for hepatic clearance (Figure 1). The mass-balance equations for clarithromycin are provided by equations 1-4 and equation 5b.

\[
\frac{dA_{GL}}{dt} = -C_{GL} \times V_{GL} \times k_{GL} \quad (1)
\]

\[
\frac{dA_{GW}}{dt} = C_{GL} \times V_{GL} \times k_{GL,MDZ} - C_{GW} \times V_{GW} \times k_{PV} - C_{GW} \times CL_{tot,GW} \times \frac{C_{E(0),GW}}{C_{E(0),GW}} \quad (2)
\]

\[
\frac{dA_{PV}}{dt} = C_{GW} \times V_{GW} \times k_{PV} + C_1 \times Q_{PV} - C_{PV} \times Q_{PV} \quad (3)
\]
\[
\frac{dA_H}{dt} = C_{PV} \times Q_{PV} + C_1 \times Q_{HA} - C_H \times Q_H - f_u \times C_H \times \left( CL_{int,3A} \times \frac{C_{E(t),H}}{C_{E0,H}} + CL_{int,non3A} \right) \tag{4}
\]

\[
\frac{dA_1}{dt} = C_H \times Q_H - C_1 \times Q_{HA} - C_1 \times Q_{PV} + C_2 \times CL_{per} - C_1 \times CL_{per} - C_1 \times CL_R \tag{5}
\]

\[
\frac{dA_2}{dt} = C_1 \times CL_{per} - C_2 \times CL_{per} \tag{5b}
\]

\[
\frac{dA_3}{dt} = C_1 \times CL_{per} - C_2 \times CL_{per} \tag{6}
\]

where \( A_{GL, GW, APV, AH, A1, } \) and \( A_2 \) are the amounts of drug in the gut lumen, gut wall, portal vein, liver, central and peripheral compartments, respectively. \( V_{GL} \) and \( V_{GW} \) are the volumes of the gut lumen and gut wall. \( V_{GL} \) is equivalent to the amount of fluid administered with drug (250 mL). \( C_{GL, GW, CPV, CH, C1, } \) and \( C_2 \) are the volumes and concentrations of drug in gut lumen, gut wall, portal vein, liver, central and peripheral compartments, respectively. \( CL_{per,} \) is the clearance between the peripheral and central compartments. \( f_u \) is the fraction of drug unbound in the plasma. \( k_{GL} \) and \( k_{PV} \) are the first order rate constants for absorption from gut lumen to gut wall and gut wall to portal vein, respectively. \( Q_H \) is hepatic blood flow calculated as an allometric expression of total body weight (Eq. 7) (Brown et al., 1997)

\[
Q_H = 3.75 \times (\text{body weight})^{0.75} \tag{7}
\]

\( Q_{HA} \) and \( Q_{PV} \) are hepatic artery and portal venous blood flow and represent 25% and 75% of \( Q_H \), respectively. \( CL_R \) is the renal clearance. \( CL_{int, GW} \) and \( CL_{int,3A} \) and \( CL_{int,non3A} \) indicate intrinsic clearances of drug in the gut wall, by CYP3A-mediated pathways in the liver, and by other non-CYP3A pathways in the liver, respectively.

\( CL_{int,3A} \) and \( CL_{int,non3A} \) are the CYP3A and non-CYP3A mediated intrinsic clearances of midazolam in the liver and are calculated by

\[
CL_{int,3A} = \frac{V_{\text{max,3A}}}{K_m + f_u \times C_H} \tag{8}
\]

and

\[
CL_{int,non3A} = \frac{V_{\text{max,non3A}}}{K_m + f_u \times C_H} \tag{9}
\]
where $V_{\text{max},3A}$ and $V_{\text{max,non}3A}$ are the maximum velocity of metabolism by CYP3A and nonCYP3A mechanisms, and $K_m$ is the Michaelis-Menten constant. Intrinsic clearance of drug in the gut wall ($\text{CL}_{\text{int,GW}}$) was assumed to be entirely by CYP3A, thus

$$\text{CL}_{\text{int,GW}} = \frac{V_{\text{max,GW}}}{K_m + C_{GW}}$$

(10)

Where $V_{\text{max,GW}}$ indicates the maximum velocity of metabolism by CYP3A in the gut wall. Gut wall $K_m$ was assumed to be equivalent to hepatic $K_m$.

Prior to initial dosing, no drug was present in the system. Orally dosed midazolam was assumed to be immediately present in the gut lumen and completely absorbed into the gut wall (Gorski et al., 1998). Intravenously dosed midazolam was infused into the central compartment. Midazolam PK parameters were obtained from our previous published studies and the literature (Table 1) (Chien et al., 2006). Clarithromycin was administered orally and assumed to be immediately present in the gut lumen and completely absorbed into the gut wall.

Since clarithromycin is a substrate for CYP3A, mechanistic inactivation of CYP3A by clarithromycin results in auto-inhibition of its metabolism. Therefore, it was necessary to incorporate the inhibition of CYP3A into the model (see below). However, at lower (e.g. 100 mg) doses of clarithromycin, it was assumed that minimal CYP3A inactivation occurs, and that this amount of inactivation is inconsequential to clarithromycin’s pharmacokinetics. Thus, intrinsic clearance was adjusted such that the model, assuming 100% CYP3A activity, would reproduce the pharmacokinetic parameters for a single 100 mg dose (Chu et al., 1992).

**CYP3A enzyme model.** At steady-state, the amount of active CYP3A enzyme ($E_0$) available in the liver or intestinal wall is determined by a zero-order synthesis rate ($R_0$) and first-order degradation rate ($k_{\text{deg}}$) of the enzyme. The rate of change of active enzyme ($E_t$) in the absence of a modulating drug is given by (11)

$$\frac{dE_t}{dt} = R_0 - k_{\text{deg}} \times E_0$$

(11)

At steady state,

$$R_0 = k_{\text{deg}} \times E_0$$

(12)
Where $E_0$ is the amount of active CYP3A at time 0.

In the presence of an inactivator, the degradation rate of enzyme is increased by an inactivation rate constant, $k_{obs}$ (13)

$$Rate\ of\ inactivation = k_{obs} \times E_t = \frac{k_{inact} \times I_t \times E_t}{K_I + I_t}$$

(13)

where $k_{inact}$ is the maximum rate of enzyme inactivation, $K_I$ is the dissociation rate constant of the inhibitor, and $I_t$ is the unbound concentration of inhibitor at the enzyme site at time $t$ (Zhou et al., 2004). The rate of change of CYP3A in response to inactivation may be described by

$$\frac{dE_t}{dt} = R_0 - k_{deg} \times E_t - \frac{k_{inact} \times I_t \times E_t}{K_I + I_t}$$

(14)

where $E_t$ is the amount of active CYP3A enzyme present at time $t$. Enzyme turnover was assumed to be 28 hours based on in vivo data obtained by our group (Gorski et al., 2002a; Wang et al., 2004). This resulted in $R_0$ and $k_{deg}$ of 0.025 h$^{-1}$ in both the liver and gut wall. Initial estimates of $K_I$ and $k_{inact}$ were obtained from in vitro studies as reported in the literature (Table 2). Microsomal binding of clarithromycin and midazolam was assumed to be negligible (Hallifax and Houston, 2006; Gertz et al., 2008).

**Clarithromycin and Midazolam Interaction Models.** Changes in the CYP3A enzyme pool due to clarithromycin inactivation were integrated into the pharmacokinetic models of clarithromycin and midazolam, resulting in the alteration of intestinal and hepatic metabolism. Thus gut wall intrinsic clearance of clarithromycin (CLAR) can be defined by:

$$CL_{int, GW, CLAR} = \frac{V_{max, GW, CLAR} \times E_{t, GW}}{K_m, CLAR + C_{GW, CLAR}}$$

(15)

Where $E_{t, GW}$ and $E_{0, GW}$ are the enzyme concentration at time $t$ and at baseline (steady state) in the gut wall. The intrinsic clearance of clarithromycin by CYP3A in the liver is calculated as:

$$CL_{int, 3A, CLAR} = \frac{V_{max, 3A, CLAR} \times E_{t, H}}{K_m, CLAR + (C_{H, CLAR} \times f_u, CLAR)}$$

(16)

Where $E_{t, H}$ and $E_{0, H}$ are the enzyme concentration at time $t$ and at baseline in the liver.
In addition to irreversibly inactivating CYP3A, Clarithromycin is also a weak competitive inhibitor of CYP3A, so the clearance term for midazolam (MDZ) incorporated both mechanism-based and competitive inhibition:

\[
CL_{int, GW, MDZ} = \frac{V_{max, GW, MDZ}}{K_m, MDZ (1 + \frac{C_{GW, CLAR}}{K_i}) + C_{GW, MDZ}} \times \frac{E_{i, GW}}{E_{0, GW}}
\]  \( (17) \)

\[
CL_{int, 3A, MDZ} = \frac{V_{max, 3A, MDZ}}{K_m, MDZ (1 + \frac{C_{H, CLAR} \times f_{u, CLAR}}{K_i}) + (C_{H, MDZ} \times f_{u, MDZ})} \times \frac{E_{i, H}}{E_{0, H}}
\]  \( (18) \)

where \( K_i \) is the reversible equilibrium inhibition constant of clarithromycin determined in vitro (Obach et al., 2006).

**Validation Data.** The clarithromycin and midazolam interaction model was validated using data from two clinical trials performed at the Indiana University School of Medicine (Gorski et al., 2002b; Wang et al., 2004; Pinto et al., 2005). In both studies, healthy adult volunteers were administered clarithromycin 500 mg orally every 12 hours for 7 days. In one study (n=10), upper intestinal biopsies were obtained prior to and 12 hours following the last dose of clarithromycin (Pinto et al., 2005). Subjects received intravenous midazolam (2-13 mg) to achieve conscious sedation for endoscopy, and a single point 1'-hydroxymidazolam to midazolam serum ratio, normalized to dose of midazolam, was used to determine hepatic CYP3A activity. Intestinal CYP3A activity was determined by formation of 1'-hydroxymidazolam in intestinal homogenates. In a separate study (Gorski et al., 2002b; Wang et al., 2004), healthy adult volunteers (n=15) were simultaneously administered 4-mg of \(^{15}\)N\(_3\)-midazolam oral solution and 0.05 mg/kg of midazolam (Versed®) intravenously over 30-minutes period prior to initiation of clarithromycin therapy, 1 hour after the 13\(^{th}\) dose of clarithromycin, and 36, 72, and 144 hours after the 14\(^{th}\) dose of clarithromycin. Plasma samples were collected up to 12 hours after midazolam administration for analysis of midazolam, 1'-hydroxymidazolam, \(^{15}\)N\(_3\)-midazolam, 1'-hydroxy-\(^{15}\)N\(_3\)-midazolam, and clarithromycin.

**Model Validation.** Based on previously published data, the clarithromycin and midazolam interaction was simulated using Trial Simulator v.2.1.2 (Pharsight Corp, Mountain View, CA). WinNonlin (Pharsight Corp, Mountain View, CA) was used to calculate AUC\(_{\infty}\) from the simulated data and the AUC ratio (AUCR) of midazolam in presence and absence of clarithromycin was estimated as:
\[ AUCR = \frac{AUC_{\infty, \text{inhibitor}}}{AUC_{\infty, \text{control}}} \]  

(19)

Observed and simulated midazolam \( AUCRs \) were compared. In order to improve the model prediction of the observed midazolam \( AUCR \), model validation was performed by tuning PK parameters such as \( K_i \) and \( k_{\text{inact}} \). A grid search algorithm was performed to minimize \( \sum_{i=1,...,I} [AUCR_{\text{obs},i} - AUCR_{\text{pred},i}(V_{\text{max,MDZ}}, K_i, k_{\text{inact}})]^2 \), where \( i \) indicates study \( i \). This optimization approach was implemented through an interactive procedure within Trial Simulator.

**Sensitivity analysis.** Sensitivity analyses were performed on the final model to determine the effect of altering key parameters in the inactivation equation. Each parameter (\( k_{\text{deg}}, K_i \), and \( k_{\text{inact}} \)) was altered for intestinal and hepatic CYP3A while holding all other parameters constant. The effect of each parameter on extent of hepatic or intestinal enzyme inactivation and recovery was evaluated.
Results

Clarithromycin AUC$_{\infty}$'s following single doses (100-1200 mg) were simulated. Predicted AUC$_{\infty}$'s were within 25% of observed values (Chu et al., 1992) for all doses (Figure 2A). Importantly, predicted AUC$_{\infty}$'s were within 5% of observed values for the 400 and 600 mg doses. Nonlinearity in clarithromycin bioavailability is clearly seen in figure 2B. Model predicted bioavailability was within 25% of observed bioavailability at all doses. Clinical studies of the clarithromycin-midazolam interaction (Gorski et al., 2002b; Pinto et al., 2005; Wang et al., 2005) employed the typical clinical dose of 500 mg clarithromycin every 12 hours for 7 days. Predicted and observed steady-state plasma concentrations of clarithromycin on day 6, after the 12th dose of clarithromycin are shown in Figure 3. Although time to maximum concentration is earlier for the observed data, the predicted AUC$_{12}$ (11±0.73 µg/ml·hr, mean±SD) closely matched the observed AUC$_{12}$ (11±2.1 µg/ml·hr) (Gorski et al., 2002b; Wang et al., 2004).

A previously described PBPK model for midazolam (Chien et al., 2006) was modified to incorporate the inactivation of CYP3A enzyme on hepatic and intestinal clearance. Initial $K_I$ and $k_{inact}$ values were obtained from in vitro experiments (Table 2). However, these values were unable to correctly estimate the effects of clarithromycin on CYP3A activity and midazolam clearance. A grid search algorithm was therefore used to identify the optimal $K_I$ (5.3 µM) and $k_{inact}$ (0.4 h$^{-1}$ in liver and 4 h$^{-1}$ in intestine). This model accurately predicted mean change in the AUC$_{\infty}$ of intravenous midazolam from baseline following a 7-day course of oral clarithromycin (Table 3, Figure 4). The mean rate of enzyme recovery in a simulated population of 100 individuals, indicated by fold-changes in AUC of midazolam administered 36, 72, and 144 hours after the final dose of clarithromycin compared to baseline, was similar to that observed (Figure 3). Although the mean predicted change in AUC of oral midazolam from baseline to day 7 of clarithromycin treatment was lower than the mean observed change (5.3±0.85 vs. 8.6±4.5-fold), the predicted fold-changes in AUC fell within the ranges of those observed. The predicted rate of return of oral midazolam AUC to baseline after discontinuation of clarithromycin was similar to the observed. To further validate the model, the fold-change in oral midazolam AUC following 250 mg clarithromycin twice daily for 5 days was simulated. The predicted increase in midazolam AUC for this regimen (3.0-fold) was similar to that observed (3.6-fold, Table 3) (Yeates et al., 1996).
Figure 5A depicts the predicted plasma concentration-time course of clarithromycin following a standard regimen of 500 mg orally every 12 hours. Steady-state plasma concentrations of clarithromycin were not achieved until the seventh dose, corresponding to the decline in hepatic CYP3A activity (Panel B), for which the maximum 70% loss of activity was reached on day 7. Intestinal CYP3A activity decreased after the initial dose of clarithromycin by 88%. With the second and subsequent doses of clarithromycin, intestinal CYP3A activity was reduced to 4% of baseline activity before recovering to approximately 24% of baseline within the 12 hour dosing interval. The model-predicted recovery of intestinal and hepatic CYP3A activity to 99% of baseline required 7.5 days following the final dose of clarithromycin. The predicted reduction in intestinal CYP3A activity (24%) was in agreement with the observed change (25.7%) in 1'-hydroxymidazolam formation rate in homogenates from intestinal pinch biopsies obtained prior to and after the 13th dose of clarithromycin (500 mg orally every 12 hours, Figure 5) (Pinto et al., 2005).

Effects of a mechanism-based inactivator on enzyme activity are dependent on \( k_{\text{inac}}, K_I \), and \( k_{\text{deg}} \). Sensitivity analyses were conducted for each of these parameters to determine their importance in the final model. The extent of reduction in intestinal CYP3A activity was insensitive to changes in \( k_{\text{inac}}, K_I \), and \( k_{\text{deg}} \) (Figure 6). The rate of return to baseline CYP3A activity in the intestine was dependent on the \( k_{\text{deg}} \) with a longer enzyme half-life leading to a slower return to baseline conditions. Hepatic CYP3A activity was more sensitive to changes in all parameters. \( k_{\text{inac}} \) and \( K_I \) primarily alter the extent of inactivation, while reductions in \( k_{\text{deg}} \) increased the time required for enzyme to return to baseline following discontinuation of clarithromycin (Figure 6).
Discussion

Modeling and simulation is becoming more prevalent for evaluation of potential drug-drug interactions. Physiologic and non-physiologic models have been developed describing the effect of mechanism-based inhibitors on enzyme activity (Mayhew et al., 2000; Takanaga et al., 2000; Gorski et al., 2002b; Ito et al., 2003; Wang et al., 2004; Venkatakrishnan and Obach, 2005; Polasek and Miners, 2006; Einolf, 2007). A recent comparison of prediction methods found the mechanistic static model with a single inhibitor concentration more accurately predicted mechanism based interactions than the Simcyp® dynamic model (Einolf, 2007). However, a dynamic model enables incorporation of inter-individual variability and extrapolation to various dosing regimens. We developed a semi-PBPK model for the interaction between clarithromycin and midazolam incorporating CYP3A inactivation in both the gut wall and liver. Furthermore, this model incorporates effects of this inactivation on the disposition of clarithromycin.

Oral and i.v. midazolam exposure increases 7-fold and 2.7-fold following 500 mg oral clarithromycin twice daily for 7 days (Gorski et al., 1998). Several approaches to predict this effect of clarithromycin on midazolam disposition have been reported. Using a non-physiological model, our group predicted that a steady-state unbound plasma concentration of 0.1 µM of clarithromycin reduces hepatic CYP3A4 by 61%, leading to a 2.6-fold increase in the AUC of oral midazolam (Mayhew et al., 2000). Polasek and Miners used a similar non-PBPK model with $K_I$'s of 2.25 and 29.5 µM and $k_{inact}$'s of 0.04 and 0.05 min$^{-1}$, based on inactivation of testosterone $\beta$-hydroxylation in recombinant CYP3A4 and human liver microsomes, respectively, to predict a change in the oral AUC of midazolam of 12.7 and 4.6-fold (Polasek and Miners, 2006). A physiologically-based model by Ito et al, found the AUC of oral midazolam increased 2.5-fold following 7 days of treatment of clarithromycin 500 mg twice daily (Ito et al., 2003). Galetin et al. found that incorporating increased bioavailability in presence of inhibitors improved the accuracy of drug-drug interaction predictions, including clarithromycin (Galetin et al., 2006). The approach described herein, incorporating time-dependent changes in intestinal and hepatic metabolism and nonlinear pharmacokinetics of clarithromycin, predicts a 5.3-fold increase in the oral AUC of midazolam and a 2.6-fold increase for i.v. midazolam. This increase in the AUC of i.v. midazolam is consistent with in
vivo data. While the predicted AUC increase for oral data is less than the mean observed change in AUC following oral dosing, it is within the range of variability observed in vivo (Figure 4).

CYP3A is highly expressed in the gastrointestinal tract (Kolars et al., 1994; Paine et al., 2005; Paine et al., 2006). However, most predictive models of CYP3A inactivation fail to account for time-dependent changes in gut wall CYP3A activity. Some models incorporate intestinal CYP3A by increasing gut wall availability of midazolam (Ito et al., 2003; Galetin et al., 2006; Polasek and Miners, 2006). This results in over-prediction of availability as intestinal CYP3A activity recovers due to synthesis of new enzyme. The model described in this paper defines the time-course of intestinal CYP3A inactivation and recovery. A limitation to this approach is the accurate determination of gut wall concentration. Gut lumen volume was defined as the volume of fluid administered with drug, resulting in a relatively high concentration of drug in the gut. At inhibitor concentrations greater than $K_i$, enzyme inactivation is determined by $k_{\text{inact}}$ (Eq. 20). Maximal inactivation will occur in the gut wall following each dose of clarithromycin. Drug exposure is transient as gastrointestinal motility and absorption rapidly reduce drug concentration, allowing the rate of enzyme recovery to be primarily determined by $k_{\text{deg}}$ (Figure 6). Thus, the overriding factor of CYP3A inhibition in gut wall is duration of exposure to inhibitor and not inhibitor concentration.

CYP3A metabolizes clarithromycin to 14-hydroxylarithromycin and N-desmethyl-clarithromycin (Suzuki et al., 2003). While these metabolites may also inactivate CYP3A, they occur at low systemic concentrations in vivo such that circulating metabolites will likely have an insignificant effect on CYP3A (Chu et al., 1992; Chu et al., 1993). It is possible that metabolites may inactivate CYP3A without exiting the liver. In this case, inactivation constants for clarithromycin account for inhibition by metabolites.

Following a 250 mg oral dose of clarithromycin, 60% of the drug is excreted as 14-hydroxylarithromycin and N-desmethyl-clarithromycin metabolites (Ferrero et al., 1990). However, these metabolites account for only 35% of a single oral 1200 mg dose (Ferrero et al., 1990). Thus, the nonlinear pharmacokinetics of clarithromycin may be explained by saturation of CYP3A. In this model, inactivation of CYP3A directly affects the metabolism of clarithromycin. As predicted by the model, the first dose of clarithromycin reduces hepatic CYP3A by approximately 25%. However, as clarithromycin accumulates after multiple doses, hepatic CYP3A is inactivated to a greater extent, resulting in a 75% decrease in
hepatic CYP3A activity at steady state. Previous models of mechanism-based inhibition (Ito et al., 2003; Polasek and Miners, 2006) have not included effects of the inactivation of CYP3A on the inactivating drug. While these prior models may be adequate to describe the pharmacokinetic profile of the inactivator for the dosage regimen initially modeled, extrapolation to other doses will be limited. In contrast, incorporation of the nonlinear clearance of clarithromycin enables accurate predictions at different doses of inactivator.

In vitro $K_i$ and $k_{inact}$ values have been determined by a number of investigators (Table 2) (Mayhew et al., 2000; Ito et al., 2003; Polasek and Miners, 2006). A high degree of variability was observed among the studies. This inconsistency may be due to variability of enzyme sources for the in vitro experiments; $K_i$ determined in recombinant CYP3A4 systems was generally 10-fold lower than that determined in human liver microsomes (Table 2). Such differences between recombinant systems and human liver microsomes may be due to differential non-specific binding or increased CYP3A: NADPH oxidoreductase molar ratio (Polasek and Miners, 2007).

In contrast to our diltiazem model (Zhang et al., 2009), no combination of in vitro $K_i$ and $k_{inact}$ values estimated the in vivo effect of clarithromycin on midazolam clearance. Additionally, the in vivo estimate of $k_{inact}$ in the gastrointestinal tract was 10-fold higher than in the liver. The finding that in vivo inactivation efficiency was lower than in vitro estimates is consistent with rate limiting, slow access of drug to the inactivation site. This slowness may be tissue selective. At present, no mechanistic model of drug distribution is available to accurately predict rate of drug movement into hepatocytes or intestinal epithelial cells. Thus, it remains difficult to determine the effective concentration of inhibitor at the enzyme site.

Sensitivity analyses indicate intestinal CYP3A is less responsive to changes in $K_i$, $k_{inact}$, and $k_{deg}$ than hepatic CYP3A. Altering $k_{inact}$ or $K_i$ affects the extent of hepatic CYP3A inactivation, with $k_{inact}$ being more sensitive to smaller changes than $K_i$ (figure 6). The time-line of the reduction and recovery of CYP3A activity following inactivation is influenced by the half-life of the CYP3A enzyme, a value that is debatable. In vivo approaches estimate the half-life for CYP3A to be between 1 and 6 days (Lai et al., 1978; Fromm et al., 1996; Hsu et al., 1997; Greenblatt et al., 2003; Obach et al., 2007; Yang et al., 2008). We have examined the recovery of intestinal and hepatic CYP3A activity following a week-long course of clarithromycin (Gorski et al., 2002b; Wang et al., 2004). By fitting the inactivation rate equation to the data, a half-life of CYP3A was determined to be 28 hours ($k_{deg} = 0.025 \text{ h}^{-1}$). The population-based ADME
simulator Simcyp®, using a default hepatic CYP3A half-life of 90 hours, was found to over-predict the AUC change for mechanism-based inhibitors (Einolf, 2007). Reducing the half-life of CYP3A to 36 hours improved the predictions such that the change in AUC for 7 out of 9 trials were predicted within 2-fold of the observed values. In our model, with CYP3A half-life estimated as 28 hours, the predicted AUC ratio for i.v. midazolam was nearly identical to that observed.

Although our model predicted mean changes in the AUC of midazolam in the presence of clarithromycin, inter-individual variability was typically under-predicted (Figure 4). Variability was incorporated into the model for $k_{GL}$, $V_1$, $V_2$ and $V_{max}$ of the drugs and body weight of individuals. Further exploration into inter-individual differences in CYP3A half-life, pharmacogenetic variants in CYP3A enzymes, or impact of transport on clarithromycin’s PK may enhance the predictive capability of the model with regards to inter-individual variability.

Mechanistic models such as this are important tools in collecting and explaining data regarding a drug and in designing future trials. This model incorporating the CYP3A metabolism of clarithromycin and midazolam in the liver and intestine predicts the nonlinear pharmacokinetics of clarithromycin and the effects of clarithromycin on midazolam clearance. Additionally, this model allows the prediction of drug interactions with varying dosage regimens of clarithromycin. This general framework will be useful in the prediction of other mechanism-based drug-drug interactions. Application of this model to clarithromycin illustrates, however, that not all drug interactions can be accurately estimated from in vitro data.

Advancements in mechanistic models of transport are needed to further improve in vitro–in vivo predictions.

Acknowledgement

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References


Footnotes

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**Figure Legends**

**Figure 1.** *Midazolam and clarithromycin PK models.* \( k_{GL} \) and \( k_{PV} \) are the 1st-order absorption rate constants from the gut lumen to the gut wall and gut wall to portal vein, respectively. \( V_{GL}, V_{GW}, V_{PV}, V_H, V_1, \) and \( V_2, \) volumes of the gut lumen, gut wall, portal vein, liver, central and peripheral compartments (midazolam only), respectively. \( CL_{per} \) is the clearance rate between the peripheral and central compartments for midazolam. \( Q_H \) is the hepatic blood flow. \( Q_{HA} \) and \( Q_{PV} \) are the hepatic artery and portal venous blood flow, respectively. Drug is cleared renally and through CYP3A in the gut wall (\( CL_{int, GW} \)) and CYP3A and nonCYP3A (midazolam only) mechanisms in the liver (\( CL_{int, H} \)).

**Figure 2.** *Clarithromycin AUC\( \infty \) and relative bioavailability following single doses of clarithromycin.*

A.) Predicted (grey bars) and observed (black bars) clarithromycin AUC\( \infty \) following single doses of clarithromycin. Error bars represent standard error in 100 simulated subjects (predicted) or in observed subjects (n=6 for 100 mg clarithromycin, n=8 for all other doses) (Chu et al., 1992). B.) Relative bioavailability of clarithromycin compared to 100 mg oral dosage. Filled circles represent observed data, open triangles indicate predicted values. Error bars indicate standard error.

**Figure 3.** *Simulated and observed clarithromycin plasma concentrations.* A simulated dose of 500 mg clarithromycin was administered every 12 hours for a total of 12 doses to 100 virtual subjects. The mean (solid line) and 95% CI (dashed lines) of the concentration-time curve of the 12th dose of clarithromycin is shown. The observed mean ± SD of 15 subjects is indicated by black dots and error bars (Gorski et al., 2002b; Wang et al., 2004).

**Figure 4.** *Predicted (filled circles) and observed (empty circles) fold-change in intravenous and oral midazolam AUC from baseline.* Clarithromycin (500 mg) was administered orally every 12 hours for 7 days. Intravenous midazolam (0.05 mg/kg) and oral \( ^{15} \text{N}_3 \)-midazolam (4 mg) were administered simultaneously 1 hour after the 13th dose of clarithromycin (day 7), and 36 (day 9), 72 (day 11), and 144 (day 13) hours after the 14th dose of clarithromycin.
Figure 5. Predicted plasma concentrations of clarithromycin during week-long course of 500 mg orally every 12 hours (upper panel) and effect on intestinal (dashed line) and hepatic (solid line) CYP3A activity. Circles and bars indicate observed rate of 1'-hydroxymidazolam formation in intestinal biopsies obtained prior to and after dose 13 of clarithromycin (Pinto et al., 2005).

Figure 6. Effect of altering $k_{inact}$, $K_I$, and $k_{deg}$ on inactivation of CYP3A during 7 day course of clarithromycin 500 mg orally every 12 hours. Upper panel: Intestinal/hepatic $k_{inact}$ were 2/0.2 (dotted line), 4/0.4 (solid line), and 8/0.8 (dashed line). Middle panel: $K_I$ values were 10 (dotted line), 4 (solid line) and 1 (dashed line). Lower panel: CYP3A half-life was 20 hours (dotted line); 28 hours (solid line) and 40 hours (dashed line).
Table 1. Model parameters for clarithromycin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Midazolam PK Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{GL}$ (h$^{-1}$)</td>
<td>1.2±0.52</td>
<td>(Chien et al, 2006)</td>
</tr>
<tr>
<td>$V_1$ (L)</td>
<td>43±8.6</td>
<td>(Chien et al, 2006)</td>
</tr>
<tr>
<td>$V_2$ (L)</td>
<td>88.4±17.7</td>
<td>(Chien et al, 2006)</td>
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<tr>
<td>$f_u$</td>
<td>0.04</td>
<td>(Chien et al, 2006)</td>
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<tr>
<td>$CL_{per}$ (L/h)</td>
<td>59.5</td>
<td>(Chien et al, 2006)</td>
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<tr>
<td>$CL_R$ (L/h)</td>
<td>0.06</td>
<td>(Chien et al, 2006)</td>
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<tr>
<td>$CL_{int,3A}$ (L/h)</td>
<td>439</td>
<td>(Chien et al, 2006)</td>
</tr>
<tr>
<td>$CL_{int,non3A}$ (L/h)</td>
<td>36</td>
<td>(Chien et al, 2006)</td>
</tr>
<tr>
<td>$V_{max,3A}$ (mg/h)</td>
<td>2600$^a$</td>
<td></td>
</tr>
<tr>
<td>$V_{max,non3A}$ (mg/h)</td>
<td>250$^a$</td>
<td></td>
</tr>
<tr>
<td>$K_m$ (µM)</td>
<td>5.8</td>
<td>(Chien et al, 2006)</td>
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<tr>
<td><strong>Clarithromycin PK Parameters</strong></td>
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<td></td>
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<tr>
<td>$k_{GL}$ (h$^{-1}$)</td>
<td>1.7±0.73</td>
<td>(Chu et al., 1992)</td>
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<tr>
<td>$V_1$ (L)</td>
<td>123±24.6</td>
<td>(Chu et al., 1992b)</td>
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<td>$CL_R$ (L/h)</td>
<td>7.5</td>
<td>(Chu et al., 1992)</td>
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<tr>
<td>$CL_{int}$ (L/h)</td>
<td>67</td>
<td>(Chu et al., 1992)</td>
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<tr>
<td>$V_{max,3A}$ (mg/h)</td>
<td>4002±800.4$^b$</td>
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<tr>
<td>$K_m$ (µM)</td>
<td>60</td>
<td>(Rodrigues et al, 1997)</td>
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<tr>
<td>$f_u$</td>
<td>0.28</td>
<td>(Ito et al, 2003)</td>
</tr>
<tr>
<td><strong>Physiological Parameters</strong></td>
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<tr>
<td>$V_{GW}$ (L)</td>
<td>0.25</td>
<td>(Chien et al, 2006)</td>
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<tr>
<td>$V_{PV}$ (L)</td>
<td>0.07</td>
<td>(Ito et al, 2003)</td>
</tr>
<tr>
<td>$V_{H}$ (L)</td>
<td>2.8</td>
<td>(Ito et al, 2003)</td>
</tr>
<tr>
<td><strong>CYP3A Enzyme Parameters</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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*a* Based on mean of 3 subjects.

*b* Based on mean of 2 subjects.
$k_{deg} (h^{-1})$ & 0.025$^c$

$K_{i,clar} (\mu M)$ & 300 (Obach et al, 2006)

$^a K_m$ was estimated in house using human liver microsomes. $V_{max}$ was estimated by $CL_{int,3A} \times K_m$. $V_{max,non3A}$ was estimated as 10% of $V_{max,3A}$. $V_{max,3A}$ was assumed to be equivalent between the gut wall and liver.

$^b$ Calculated from $CL_{int}$ (Chu et al., 1992) and in vitro $K_m$ (Rodrigues et al., 1997).

$^c k_{deg}$ was estimated from in vivo data on CYP3A recovery following clarithromycin (Gorski et al., 2002a; Wang et al., 2004).
Table 2. Comparison of $K_I$ and $k_{inact}$ for clarithromycin.

<table>
<thead>
<tr>
<th>Source</th>
<th>$K_I$ (µM)</th>
<th>$k_{inact}$ ($h^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human liver microsomes</td>
<td>5.49</td>
<td>4.32</td>
<td>(Mayhew et al., 2000)</td>
</tr>
<tr>
<td>CYP3A4+b5 supersomes</td>
<td>4.12</td>
<td>13.8</td>
<td>(Jones et al., 2007)</td>
</tr>
<tr>
<td>Human liver microsomes</td>
<td>37.0-41.4</td>
<td>2.5-2.8</td>
<td>(Ito et al., 2003)</td>
</tr>
<tr>
<td>Recombinant CYP3A4*</td>
<td>2.25</td>
<td>2.4</td>
<td>(Polasek and Miners, 2006)</td>
</tr>
<tr>
<td>Human liver microsomes*</td>
<td>29.5</td>
<td>3.0</td>
<td>(Polasek and Miners, 2006)</td>
</tr>
<tr>
<td><strong>In Vivo estimate</strong></td>
<td><strong>5.3</strong></td>
<td><strong>0.4</strong> (liver)</td>
<td><strong>4</strong> (intestine)</td>
</tr>
</tbody>
</table>

* Determined by 6β-hydroxylation of testosterone
Table 3. Observed and predicted fold-change in oral or intravenous midazolam AUC following indicated course of clarithromycin

<table>
<thead>
<tr>
<th>Clarithromycin Dose</th>
<th>Midazolam Dose</th>
<th>Observed Fold-change in AUC</th>
<th>Predicted Fold-change in AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 mg p.o. BID for 5 days</td>
<td>15 mg p.o.</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0</td>
</tr>
<tr>
<td>500 mg p.o. BID for 7 days</td>
<td>4 mg p.o.</td>
<td>7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.3</td>
</tr>
<tr>
<td>500 mg p.o. BID for 7 days</td>
<td>0.05 mg/kg i.v. over 30 min.</td>
<td>2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> (Yeates et al., 1996)

<sup>b</sup> (Gorski et al., 1998)

<sup>c</sup> (Wang et al., 2004)
Figure 2

AUC (mg hr/L)

Dose (mg)

Relative Bioavailability

Dose (mg)
Figure 4

Intravenous Midazolam

Day

Oral Midazolam

Day