Pharmacokinetic-Pharmacodynamic Modeling of Rifampicin-Mediated Cyp3a11 Induction in Steroid and Xenobiotic X Receptor Humanized Mice

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ABSTRACT

The purpose of this study was to develop a mechanistic pharmacokinetic-pharmacodynamic (PK-PD) model to describe the effects of rifampicin on hepatic Cyp3a11 RNA, enzymatic activity, and triazolam pharmacokinetics. Rifampicin was administered to steroid and xenobiotic X receptor (SXR) humanized mice at 10 mg/kg p.o. (every day for 3 days) followed by triazolam (4 mg/kg p.o.) 24 h after the last dose of rifampicin. Rifampicin and triazolam concentrations and Cyp3a11 RNA expression and activity in the liver were measured over the 4-day period. Elevations in Cyp3a11 RNA expression were observed 24 h after the first dose of rifampicin, reaching a maximum (10 times baseline) after the third dose and were sustained until day 4 and began declining 48 h after the last rifampicin dose. Similar changes in enzymatic activity were also observed. The triazolam serum area under the curve (AUC) was 5-fold lower in mice pretreated with rifampicin, consistent with enzyme induction. The final PK-PD model incorporated rifampicin liver concentration as the driving force for the time-delayed Cyp3a11 induction governed by in vitro potency estimates, which in turn regulated the turnover of enzyme activity. The PK-PD model was able to recapitulate the delayed induction of Cyp3a11 mRNA and enzymatic activity by rifampicin. Furthermore, the model was able to accurately anticipate the reduction in the triazolam plasma AUC by integrating a ratio of the predicted induced enzyme activity and basal activity into the equations describing triazolam pharmacokinetics. In conjunction with the SXR humanized mouse model, this mathematical approach may serve as a tool for predicting clinically relevant drug-drug interactions via pregnane X receptor-mediated enzyme induction and possibly extended to other induction pathways (e.g., constitutive androstanse receptor).

Introduction

The induction of drug-metabolizing enzymes often leads to a reduced level of therapeutically active parent drug or increased production of reactive metabolites. The clinical manifestations of such events range from loss of therapeutic efficacy to increased toxicity of drugs that are metabolized by these enzymes (Lin, 2006). Thus, screening for potential inducers has been at the core of lead optimization during drug development (Tucker et al., 2001; Hewitt et al., 2007a). To aid in screening, many in vitro tools have been developed, which can be grouped into two approaches: utilization of the receptor-ligand interaction and employment of hepatocyte or hepatocyte-like cells. Pregnan X receptor (PXR) ligand binding and transactivation assays have been widely used to rapidly screen potential inducers of CYP3A4, CYP2B6, CYP2C, and transporters (e.g., multiresistance protein-2 and P-glycoprotein) (Zhu et al., 2004; Sinz et al., 2006), whereas the primary human hepatocyte model is recognized as a gold standard assay throughout the pharmaceutical industry and regulatory agencies because it readily responds to a wide variety of inductive stimuli, even beyond those mediated by PXR (Hewitt et al., 2007b). There are also several known immortalized cell lines (such as Fa2N-4 and HepaRG), which mimic primary hepatocytes in their induction responses (Ripp et al., 2006; Hariparsad et al., 2008; McGinnity et al., 2009).

At issue with these current practices is the tendency to focus on providing a qualitative measure of the magnitude of induction. In addition, the potency of the inducer is studied...
under static conditions and ignores the relationship between exposure of the inducing drug and time, the resultant alterations in mRNA levels and enzymatic activity, and the downstream impact on the pharmacokinetics of a specific probe substrate. To study this dynamic/temporal relationship between inducers and the downstream induction response, one has to investigate such events in vivo. However, animal models that can provide a quantitative measure of the magnitude of a drug-drug interaction (DDI) are limited because of species differences in induction responses. Humanization of mice by replacing mouse PXR with the human homolog [steroid and xenobiotic X receptor (SXR)] overcomes this obstacle and opens the possibility of using mice as a quantitative model to predict human CYP3A4 induction (Xie et al., 2000). The availability of such models is particularly valuable during the early stages of compound selection and lead optimization, because chemical modifications could still be performed to mitigate the potential for serious induction liabilities.

We previously described the use of an SXR humanized mouse model that accurately quantified the impact of rifampicin-mediated Cyp3a11 induction on RNA expression, enzyme activity, and triazolam pharmacokinetics in vivo (Kim et al., 2008). Although the study demonstrated good correlation of triazolam AUC reduction between SXR humanized mice and humans under similar in vivo exposures, it required resource-intensive repeat-dose studies to identify the rifampicin dose that produced therapeutically relevant exposures and a corresponding Cyp3a11 RNA induction response.

The objective of the current investigation was to find a more efficient way to conduct DDI studies in SXR humanized mice without significantly comprising predictability. To this end, we attempted to create a mathematical framework that would eventually provide a prediction of the magnitude of change in the pharmacokinetics of a probe substrate in SXR humanized mice (and ultimately in patients) using only knowledge of the in vitro potency in mouse hepatocytes and single-dose in vivo pharmacokinetics of the inducer. Using rifampicin as a prototypical inducer, we characterized the time course of Cyp3a11 RNA induction and downstream elevations in CYP3A11 enzyme activity using a turnover model, wherein the in vitro induction potency [capacity (Smax, RIF) and sensitivity (SC50, RIF)] of rifampicin was fixed as a constant and the impact on the pharmacokinetics of a CYP3A11 probe substrate was simulated. This approach allowed estimation of the rates governing the system-dependent synthesis and degradation of this pathway, which could be used for future predictions/simulations of the induction potential of unknown compounds, given a measure of the compound-dependent in vitro potency.

Materials and Methods

Chemicals and Reagents. Rifampicin, triazolam, and PEG400 were obtained from Sigma-Aldrich (St. Louis, MO). 1-Hydroxytriazolam was purchased from QIAGEN (Valencia, CA). TaqMan primers and sequence detection probes were obtained from Applied Biosystems (Foster City, CA). The generic names for all commonly used chemical reagents have been used.

Experimental Animals. SXR humanized mice were bred at Bristol-Myers Squibb (Wallingford, CT). Mice were provided food and water ad libitum and housed in a temperature-controlled room under a 12-h light/dark cycle. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH Publication 85-23, revised in 1985).

SXR Humanized Mouse Hepatocyte Induction Study. Mouse hepatocytes were freshly isolated from SXR humanized mice and plated onto 24-well collagen-coated plates at CellzDirect (Durham, NC). After a 24-hr acclimation, hepatocytes were incubated in serum-free William’s E medium containing either dimethyl sulfoxide (0.1% v/v) or rifampicin (0.4–50 μM in 0.1% dimethyl sulfoxide) for 3 consecutive days. The hepatocytes were incubated with lysis buffer from an SV-96 RNA purification kit (Promega, Madison, WI). The purified RNA was used in a real-time reverse-transcriptase polymerase chain reaction to measure the expression of Cyp3a11 RNA as described previously (Kim et al., 2008). Nonlinear regression analysis for determination of the Smax, RIF and SC50, RIF parameters was performed with GraphPad Prism (version 4.0 for Windows; GraphPad Software, Inc., San Diego, CA).

In Vivo Induction Studies in SXR Humanized Mice. Male SXR humanized mice were dosed orally with 10 mg/kg rifampicin or vehicle (80:20 PEG400-water) once daily for 3 days. On day 4, all animals were dosed orally with 4 mg/kg triazolam (50:50 PEG400-saline). After each daily dose of rifampicin (days 1–3), mice were euthanized at 0.5, 1, 2, 4, 8, and 24 h postdose (n = 3 mice/time point). Blood was collected by cardiac puncture and centrifuged to obtain serum. Liver tissue was also harvested, and a portion was preserved in RNAlater and stored at 4°C for subsequent RNA isolation. Both serum samples and an additional piece of liver were frozen at −20°C for subsequent rifampicin and triazolam quantitation. The remaining liver tissues were frozen at −80°C for microsomal preparation. On day 4, 24 h after the final dose of rifampicin, all rifampicin- and vehicle-treated mice were euthanized at various time points (0.08, 0.17, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, and 24 h) after triazolam administration (3 mice/time point). Likewise, a group of animals receiving vehicle on day 1 were euthanized, and samples were collected as described above to establish basal Cyp3a11 mRNA expression and CY3A11 enzyme activity levels. TaqMan reverse-transcriptase polymerase chain reaction, microsomal isolation/incubation, and liquid chromatography with tandem mass spectrometry analysis of rifampicin and triazolam was performed as described previously (Kim et al., 2008).

Pharmacokinetic/Pharmacodynamic Model. Using a naive-pooled approach, noncompartmental pharmacokinetic analysis was performed on the mean rifampicin plasma and liver concentration versus time profiles on days 1 to 3 and on the mean triazolam plasma concentration versus time profiles on the 4th day of the study using WinNonlin 5.0 (Pharsight, Mountain View, CA). A semiphysiologically based PK-PD model describing the time course of rifampicin concentration in the blood and liver, its induction of the Cyp3a11 pathway, and the downstream effects on the pharmacokinetics of triazolam is shown in Fig. 1. Compartmental analysis was performed by first fitting the pharmacokinetic components of the model. The drug is introduced into the gut followed by first-order absorption into the liver where it is distributed to a secondary compartment, which constitutes the plasma and the rest of the body. The hepatic elimination of rifampicin was described by a well stirred model (Wilkinson, 1987). Equations 1 and 2 describe the gut and hepatic disposition of rifampicin after oral administration:

\[
\frac{dA_{\text{gut,RIF}}}{dt} = -k_{a,RIF} \cdot A_{\text{gut,RIF}}
\]

\[
\begin{align*}
\frac{dC_{\text{plasma,RIF}}}{dt} &= k_{a,RIF} \cdot A_{\text{gut,RIF}} - C_{\text{plasma,RIF}} \cdot V_H^{-1} \cdot A_{\text{liver,RIF}} \\
&\quad + k_{l,RIF} \cdot A_{\text{liver,RIF}} - CL_{\text{RIF}} \cdot V_H^{-1} \cdot A_{\text{liver,RIF}}
\end{align*}
\]

where \( k_{a,RIF} \), \( A_{\text{gut,RIF}} \), \( A_{\text{liver,RIF}} \), \( A_{\text{plasma,RIF}} \), and \( CL_{\text{RIF}} \) represent the first-order absorption rate constant, the amount of rifampicin in...
the gut, liver, and plasma compartment, and the hepatic rifampicin concentration, respectively. The hepatic blood flow ($Q_H$) and liver volume ($V_H$) were fixed physiological parameters (Davies and Morris, 1993), and $k_{a, RIF}$ represents the first-order distribution rate from the plasma compartment to the liver. The hepatic extraction ($E_{H, RIF}$), clearance ($CL_{H, RIF}$), and bioavailability ($F_{H, RIF}$) were described by a well stirred model according to eqs. 3 to 5:

$$E_{H, RIF} = CL_{mol, RIF} \cdot f_u, RIF \cdot Q_H + CL_{mol, RIF} \cdot f_u, RIF$$

$$CL_{H, RIF} = Q_H \cdot E_{H, RIF}$$

$$F_{H, RIF} = 1 - E_{H, RIF}$$

where $E_{H, RIF}$ is the hepatic extraction, $CL_{mol, RIF}$ is the intrinsic clearance, and $f_u, RIF$ is the experimentally determined plasma unbound fraction (Bauer et al., 2006). The differential equation governing the rate of change in the plasma compartment is described in eq. 6:

$$V_{plasma} \frac{dC_{plasma, RIF}}{dt} = Q_H \cdot F_{H, RIF} \cdot A_{liver, RIF} - k_{a, RIF} \cdot A_{plasma, RIF}$$

where $A_{plasma, RIF}$ and $C_{plasma, RIF}$ represent the amount and concentration of rifampicin in the plasma, respectively, and $V_{plasma, RIF}$ is the plasma volume of distribution. The first-order distribution of rifampicin from the liver to the plasma is driven by hepatic blood flow ($Q_H$) and the hepatic bioavailability ($F_{H, RIF}$).

The final parameters obtained from the pharmacokinetic fitting were then fixed and used to fit the pharmacodynamic data. The pharmacodynamic component of this model is also shown in Fig. 1. The differential equations used to describe the rate of change of Cyp3a11 mRNA expression (Cyp3a11m) are given in eqs. 7 to 10:

$$\frac{dTC_1}{dt} = \frac{1}{\tau} (Cyp3a11m - TC_1)$$

$$\frac{dTC_2}{dt} = \frac{1}{\tau} (TC_1 - TC_3)$$

$$\frac{dTC_3}{dt} = \frac{1}{\tau} (TC_2 - TC_3)$$

$$Cyp3a11m = S_{max, RIF} \cdot C_{liver, RIF} \cdot SC_{50, RIF} + C_{liver, RIF}$$

where the stimulatory effects of rifampicin on Cyp3a11 mRNA levels (Cyp3a11m) were described using a series of three transit compartments (TCi) to account for the onset delay, with a transit time of $\tau$. $C_{liver, RIF}$ was responsible for stimulation of Cyp3a11m, involving both $S_{max, RIF}$ and $SC_{50, RIF}$ parameters, which were fixed as constants on the basis of in vitro Cyp3a11 mRNA induction response in SXR mouse hepatocytes.

The expression of CYP3A11 enzyme activity and CYP3A11 protein levels were assumed to be proportional to one another and translated from Cyp3a11m. The regulation of CYP3A11 enzyme activity was described by two first-order rate constants governing the impact of enzyme induction on triazolam disposition ($k_{deg, CYP3A11}$) and loss of activity ($k_{deg, CYP3A11}$). The amplification factor ($\gamma$) indicates that multiple copies of protein are translated from a single copy of Cyp3a11 mRNA. Equation 11 describes the regulation of CYP3A11 activity:

$$\frac{dC_{CYP3A11}}{dt} = k_{syn, CYP3A11} \cdot TC_3 \cdot TC_3 - k_{deg, CYP3A11} \cdot C_{CYP3A11}$$

$$k_{deg, CYP3A11} = k_{syn, CYP3A11} \cdot C_{CYP3A11(0)} / C_{CYP3A11}$$

(12)

For the CYP3A11 probe substrate, triazolam, disposition in the plasma was described using a one-compartment pharmacokinetic model with first-order oral absorption ($k_{a, TRZ}$) and linear elimination ($k_{el, TRZ}$) as described in eqs. 13 and 14:

$$\frac{dA_{TRZ}}{dt} = -k_{el, TRZ} \cdot A_{TRZ} - k_{a, TRZ} \cdot A_{plasma, TRZ}$$

$$V_{plasma, TRZ} / F_{TRZ} \frac{dC_{plasma, TRZ}}{dt} = k_{a, TRZ} \cdot A_{TRZ} - k_{el, TRZ} \cdot A_{plasma, TRZ} \cdot M$$

where $A_{TRZ}$ is the amount of triazolam in the absorption compartment, and $A_{plasma, TRZ}$ and $C_{plasma, TRZ}$ are the amount and concentration of triazolam in the plasma, respectively. The term $V_{plasma, TRZ} / F_{TRZ}$ represents a composite term combining both the triazolam volume of distribution and its oral bioavailability, which were not resolved independently. The induction ratio, $M$, was used to account for the impact of enzyme induction on triazolam disposition after rifampicin pretreatment and is defined as follows:

$$M = \frac{CYP3A11}{CYP3A11(0)}$$

(15)

where CYP3A11 represents the time-variable enzyme activity (as previously described in eq. 11) and CYP3A11(0) is the basal enzyme activity. In naive or vehicle-treated mice, in which induction has not
occurred, CYP3A11 enzyme activity would be equivalent to the basal activity and the ratio would collapse to unity.

Data from multiple animals were pooled, and all PK-PD model fittings and parameter estimations were performed by nonlinear regression analysis using ADAPT II with the maximum likelihood method (Biomedical Simulations Resource, Los Angeles, CA). The variance models for rifampicin and triazolam pharmacokinetics (eq. 16) and for the pharmacodynamics (eq. 17) are given by

\[
\text{Var}(\sigma_1, \theta, t_i) = (\sigma_1 + Y(\theta, t_i))^2 \quad (16)
\]

\[
\text{Var}(\sigma_2, \theta, t_i) = \sigma_2^2 \cdot Y(\theta, t_i)^{\mu_2} \quad (17)
\]

where \(Y\) represents the predicted value, \(\sigma_1\) and \(\sigma_2\) are the variance parameters that were fitted, and \(\mu_2\) represents the structural parameters. The goodness of fit was determined by visual inspection, Akaike information criterion, Schwartz criterion, examination of the residuals, and the coefficient of variation of the parameter estimates.

### Results

**Cyp3a11 Induction in SXR Humanized Mouse Hepatocytes.** After 3 days of treatment with rifampicin, RNA expression of Cyp3a11 in the hepatocytes was increased in a concentration-dependent manner (Fig. 2). Nonlinear regression analysis of the concentration-response curve yielded \(S_{50, \text{RIF}}\) and \(S_{\text{max, RIF}}\) estimates of 6.2 \(\mu\text{M}\) and 7.86-fold, respectively (Table 3).

**Rifampicin Pharmacokinetics.** The 3-day time course of serum and liver rifampicin concentrations after a 10 mg/kg oral administration (given once a day for 3 days) is shown in Fig. 3. The serum maximum concentration (\(C_{\text{max}}\)) for rifampicin after the first, second, and third dose (days 1, 2, and 3) were 10.2, 16.7, and 10.0 \(\mu\text{M}\), respectively and the corresponding liver \(C_{\text{max}}\) were relatively higher at 38.7, 44.1, and 26.0 \(\mu\text{M}\), respectively. Likewise, total liver concentrations of rifampicin were greater than those observed in serum on all days of the study (average liver-to-serum AUC ratio = 4.3).

Two-fold increase in CYP3A11 enzyme activity was observed. The model appears to provide a reasonable fit of the observed serum and liver rifampicin concentrations with reasonable variability in parameter estimation (Fig. 3; Table 2).

**Pharmacodynamics of Cyp3a11 Induction In Vivo.** The Cyp3a11 mRNA levels in vehicle-treated animals remained relatively constant on days 1 and 4 (data not shown), indicating that baseline levels were stationary. After rifampicin administration, the Cyp3a11 message began increasing between 8 and 24 h after the first dose (Fig. 4). Cyp3a11 message levels reached a maximum by the end of day 2, which was sustained throughout days 3 and 4 and trending back toward baseline 48 h after the last dose of rifampicin.

The CYP3A11 activity profile, as shown in Fig. 5, followed an increase similar to that for Cyp3a11 mRNA with a comparable lag time in the observed elevations relative to baseline. As expected, the baseline CYP3A11 enzyme activity was also stationary (data not shown). The observed stimulation of Cyp3a11 mRNA production and subsequent elevations in CYP3A11 activity were adequately described using the in vitro derived estimates of \(S_{\text{max, RIF}}\) and \(S_{50, \text{RIF}}\), which were fixed as constants in the model. The temporal profiles of Cyp3a11 mRNA and CYP3A11 enzyme activity were cap-

### Table 1

Noncompartmental analysis of rifampicin concentration versus time profiles in mice

<table>
<thead>
<tr>
<th>Day</th>
<th>Matrix</th>
<th>(T_{\text{max}}) h</th>
<th>(C_{\text{max}}) (\mu\text{M})</th>
<th>AUC(_{0-24\ h}) (\mu\text{M} \cdot \text{h})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serum</td>
<td>4</td>
<td>10.2</td>
<td>120</td>
</tr>
<tr>
<td>2</td>
<td>Liver</td>
<td>4</td>
<td>38.7</td>
<td>571</td>
</tr>
<tr>
<td>3</td>
<td>Serum</td>
<td>4</td>
<td>16.7</td>
<td>254</td>
</tr>
<tr>
<td>4</td>
<td>Liver</td>
<td>8</td>
<td>44.1</td>
<td>1036</td>
</tr>
</tbody>
</table>

Data are expressed as mean values from three mice per time point per day.
tured well by this model (Figs. 4 and 5). The values of the estimated Cyp3a11 dynamics are given in Table 3.

Triazolam Pharmacokinetics. As shown in Fig. 6, the serum pharmacokinetics of triazolam (4 mg/kg p.o.) was assessed in mice 72 h after pretreatment with either 10 mg/kg rifampicin or vehicle (orally; once a day for 3 days). In vehicle-treated mice, the serum concentrations were reasonably well described using a simple one-compartment model with first-order rates of absorption and elimination (Table 2; Fig. 6). As shown in Table 4, the predicted serum triazolam AUC in vehicle-treated mice was close to the observed value; however, the predicted $C_{\text{max}}$ was nearly two times lower than the observed value. To account for the impact of Cyp3a11 induction on the disposition of triazolam in the serum, its elimination rate was multiplied by a ratio of the time-variable enzyme activity over the basal enzyme activity (eq. 15). The previously determined triazolam pharmacokinetic parameters determined from fitting of the vehicle-treated data (Table 2) were fixed and along with all the other final model parameters the concentration versus time profile of triazolam after 10 mg/kg rifampicin pretreatment was simulated. The $C_{\text{max}}$ and AUC predictions from the final model are given in Table 4. The simulated triazolam pharmacokinetic profile agreed well with the observed data obtained from rifampicin-pretreated mice.

### Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rifampicin pharmacokinetics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_a, \text{RIF} (h^{-1})$</td>
<td>0.69</td>
<td>18.3</td>
</tr>
<tr>
<td>$k_{\text{el}}, \text{RIF} (h^{-1})$</td>
<td>26.4</td>
<td>14.6</td>
</tr>
<tr>
<td>$V_{\text{plasma}}, \text{RIF} (l/kg)$</td>
<td>0.90</td>
<td>14.8</td>
</tr>
<tr>
<td>$CL_{\text{int}}, \text{RIF} (l/kg)$</td>
<td>0.087</td>
<td>7.9</td>
</tr>
<tr>
<td>$Q_H (l/kg)$</td>
<td>5.40</td>
<td>N.E.</td>
</tr>
<tr>
<td>$V_H (l/kg)$</td>
<td>0.065</td>
<td>N.E.</td>
</tr>
<tr>
<td>$f_a, \text{RIF}$</td>
<td>0.12</td>
<td>N.E.</td>
</tr>
<tr>
<td>$E_H, \text{RIF}$</td>
<td>0.0019</td>
<td>N.E.</td>
</tr>
<tr>
<td>$F_{\text{RIF}}$</td>
<td>1.00</td>
<td>N.E.</td>
</tr>
<tr>
<td>$CL_{\text{H}, \text{RIF}} (l/kg)$</td>
<td>0.010</td>
<td>N.E.</td>
</tr>
<tr>
<td><strong>Triazolam pharmacokinetics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{\text{a, TRZ}} (h^{-1})$</td>
<td>0.43</td>
<td>11.2</td>
</tr>
<tr>
<td>$k_{\text{el, TRZ}} (h^{-1})$</td>
<td>27.9</td>
<td>11.4</td>
</tr>
<tr>
<td>$V_{\text{plasma, TRZ/FTRZ}} (l/kg)$</td>
<td>0.38</td>
<td>11.4</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; N.E., not estimated.

- **Parameter fixed to literature/experimental value.**
- **Secondary parameter.**
TABLE 4
Observed versus predicted pharmacokinetics of triazolam in vehicle and rifampicin-treated mice
Data are expressed as mean values from three mice per time point.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( C_{\text{max}} )</th>
<th>( \text{AUC}_{0-8 \text{h}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Predicted</td>
</tr>
<tr>
<td>Vehicle</td>
<td>853</td>
<td>442</td>
</tr>
<tr>
<td>Treated</td>
<td>106</td>
<td>146</td>
</tr>
<tr>
<td>Fold change</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

Discussion

The pharmacokinetics of rifampicin in SXR humanized mice was comparable to that in previously published studies conducted in wild-type mice, indicating that genetic modifications do not alter rifampicin pharmacokinetics (Bruzzese et al., 2000; Kim et al., 2008). In addition, these data were comparable to previously reported data from our group in SXR humanized mice (Kim et al., 2008). The semiphysiological pharmacokinetic model was used to describe rifampicin absorption from the gut into the liver and subsequent distribution into the central serum compartment. This approach provides a better representation of the physiological factors governing absorption, distribution, and elimination than traditional mammillary models. Although the present model does not take nonhepatic tissue distribution/elimination into consideration, it adequately described the observed liver and serum disposition of rifampicin. Moreover, with the hepatic elimination of rifampicin being described by a well stirred model, known physiological parameters (\( V_{H} \) and \( Q_{H} \)) and rifampicin mouse protein plasma protein binding (\( f_{\text{RIF}} \)) were able to be used as model constants. The final approximations of \( C_{\text{H,RIF}} \), \( F_{\text{H,RIF}} \), and \( V_{\text{plasma,RIF}} \) were consistent with what is known regarding rifampicin pharmacokinetics in mice being a low extraction compound with nearly complete gastric absorption (Bruzzese et al., 2000; Kim et al., 2008). A similar model was previously reported (Gordi et al., 2005) to describe the pharmacokinetics of an antimalarial drug, artemisinin, in humans and allows hepatic rifampicin concentrations to be tied directly to its effects on hepatic enzymes (CYP3A11).

The data from this study illustrate that chronic oral administration of rifampicin results in elevations of Cyp3a11 mRNA, leading to a downstream increase in the CYP3A11 enzyme activity and is consistent with prior observations in the literature (Xie et al., 2000; Gonzalez and Yu, 2006). To the best of our knowledge, this is the first study examining the entire time course of PXR-mediated enzyme induction by rifampicin in vivo. As noted with other inducers, we observed a significant time delay between rifampicin administration and the onset of induction, with the peak response occurring approximately 48 h after the first dose of rifampicin (Gordi et al., 2005; Magnusson et al., 2006). This delayed onset is related to the complex series of events that must occur to trigger an increase in transcription (Moore et al., 2002; Milnes et al., 2008). We accounted for this temporal delay by using a series of transduction compartments, which introduces time lags between the rifampicin liver concentration and the increase in mRNA levels. The final estimated 21-h delay for the initiation of induction is similar to literature estimates for other P450 inducers in both human (Gordi et al., 2005) and rat (Magnusson et al., 2006). As mentioned previously, rifampicin liver concentrations served as the driving function for stimulation of mRNA production. Subsequently, the enhanced transcription of Cyp3a11 mRNA regulates the increases in CYP3A11 enzymatic activity. The elevations in both the mRNA and enzyme activity were adequately captured by the model.

On day 4, animals dosed for 3 days with vehicle or rifampicin received triazolam orally to assess the impact of induction on the pharmacokinetics of a CYP3A11 probe substrate. A 5-fold reduction in the triazolam serum AUC in rifampicin-treated mice was observed relative to the vehicle group (Table 4). These data are comparable to results of a previous study, although in that study the time course of induction was not fully characterized (Kim et al., 2008). The pharmacokinetic model for triazolam was constructed on the basis of the concentration versus time relationship in the vehicle treatment group, and it appears to adequately capture the observed data. To account for the impact of enhanced CYP3A11 activity on triazolam pharmacokinetics, we integrated a ratio of the time-variable CYP3A11 enzyme activity and basal enzyme activity. In vehicle-treated mice, in which no induction occurred, CYP3A11 activity equaled the basal activity and the ratio collapsed to unity. After rifampicin pretreatment, the time-variable CYP3A11 enzyme activity increased, yielding reductions in triazolam exposure. Using this approach, the simulated triazolam pharmacokinetics in rifampicin-treated mice was consistent with the observed data. Our model provided a better prediction of the observed triazolam AUC, whereas the \( C_{\text{max}} \) was underestimated, which may be due to the limited amount of data collected during the triazolam absorption phase. The current SXR humanized mouse lacks the expression of gut PXR (mouse and human) because of the knock out of mouse PXR and targeted expression of hPXR in the liver (via an albumin promoter) (Xie et al., 2000). Therefore, rifampicin cannot induce gut Cyp3a11 RNA, which might have reduced the absorption of triazolam by induction of gut first-pass metabolism. However, the ability to predict fold changes on the basis of AUC should be sufficient for assessment of potential DDI occurrences.

The attractive feature of this model is that it uses the in vitro Cyp3a11 RNA induction potency estimates determined from humanized mouse hepatocytes as fixed-model constants. It is conceivable that by integrating in vivo pharmacokinetic data and in vitro induction potency, one could predict the induction profile in mice for unknown compounds during the stages of drug discovery and approximate a potential fold induction in human. The main assumption of this approach is that the synthesis and degradation rates of mRNA and enzyme are compound-independent, and the induction potential would be driven by the inducer potency and its hepatic concentrations. On the basis of our fitted parameter estimates, the half-life for synthesis and degradation of CYP3A11 enzyme in mouse was 0.02 and 0.6 h, respectively. Values for human CYP3A4 degradation half-lives are reported to be within the range of 26 to 140 h (Yang et al., 2008) and are at least 40 times longer than our estimates for mouse CYP3A11. There are several potential explanations for this difference. There could be differences in the turnover rate of actual enzyme (i.e., human CYP3A4 versus mouse CYP3A11). Although the reported turnover half-lives of a variety of
human hepatic P450s appear to be longer than 20 h (Ghanbari et al., 2006), another explanation is that there may be species differences in the hepatic P450 turnover rates wherein mice have a quicker turnover than humans. Although plausible, Magnusson et al. (2006) have reported enzyme turnover half-lives in vivo for a variety of rat P450s in the range of ~40 h, which are consistent with values reported for humans. A more probable explanation for this discrepancy is related to the model predicted estimate of the CYP3A11 degradation rate. In the present study, the pharmacodynamic effect of rifampicin was only monitored for 4 days after the initial dose of rifampicin. The levels of Cyp3a11 mRNA and enzymatic activity had not returned to predose baseline levels, and, therefore, the reliability of the estimate of the enzyme degradation rate could be suspect. Whereas an extended sampling paradigm would be required to adequately capture the entire pharmacodynamic profile, the present study was able to anticipate the effect of induction on the CYP3A11 probe-substrate pharmacokinetics.

In a drug discovery setting, the proposed PK-PD model, combined with in vitro potency estimates from humanized mice and in vivo plasma and liver exposure data, could aid in the assessment of the induction potential of unknown compounds in the stages of lead optimization/characterization. Given the possibility that the rate constants governing the time course of induction of the CYP3A pathway are compound-independent, one could use the proposed model to simulate the effect of induction on the pharmacokinetics of a probe substrate for unknown compounds. The only required model inputs would be the in vivo Cyp3a11 mRNA induction potency in hepatocytes and an understanding of the pharmacokinetics of the inducer in mice. If we assume that the unknown compound has linear pharmacokinetics and is not solely metabolized by CYP3A enzyme (i.e., not a victim of auto-induction), only a single-dose pharmacokinetic study in mice with sampling of plasma and liver concentrations would be required. This approach would alleviate the need for multiple-dose in vivo induction studies and dramatically minimize the compound requirements and in-life resources that are typically constrained in the discovery setting. Knowledge of target in vivo exposures required for efficacy would aid in dose selection for the pharmacokinetic screen so that therapeutically concentrations could be achieved. Moreover, information obtained from these exercises could be used to aid in subsequent induction studies in larger nonclinical species (e.g., nonhuman primates) as well as placing the potential for induction into context with anticipated efficacious human exposure to enable design of clinical DDI studies.

Overall, the results from this study suggest that the SXR humanized mouse and proposed PK-PD model could be valuable in predicting the outcome of CYP3A4 induction in patients while reducing the amount of data required for such predictions. However, there are caveats in using this approach that have to be taken into consideration. First, the PK-PD model assumes that metabolism, pharmacokinetics, and liver exposure are similar between mouse and human. If there is significant deviation from this underlying assumption, the predictive power of the model would be diminished. Second, the lack of gut PXR expression can lead to underprediction of human induction if the inducer or probe-substrate (victim drug) undergoes extensive first-pass metabolism. Third, the current mouse model expresses mouse CAR, not human CAR. Because of the cross-talk between PXR and CAR in inducing CYP3A4 (and CYP2B) enzymes, it would be ideal if both nuclear hormone receptors were humanized for prediction of human drug-drug interactions.

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Authorship Contributions

Participated in research design: Raybong, Zheng, Sinz, and Kim. Conducted experiments: Pray and Zoeckler. Contributed new reagents or analytic tools: Morgan. Performed data analysis: Raybong and Kim. Wrote or contributed to the writing of the manuscript: Raybong, Sinz, and Kim.

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