Physiologically-Based Pharmacokinetic-Pharmacodynamic Modeling of 1α,25-Dihydroxyvitamin D₃ in Mice

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ABSTRACT

1α,25-Dihydroxyvitamin D₃ [1,25(OH)₂D₃] concentrations are regulated by renal CYP27B1 for synthesis and CYP24A1 for degradation. Published plasma and tissue 1,25(OH)₂D₃ concentrations and mRNA fold change expression of Cyp24a1 and Cyp27b1 following repetitive i.p. injections to C57BL/6 mice (2.5 μg × kg⁻¹ every 2 days for 4 doses) were fitted with a minimal and full physiologically-based pharmacokinetic-pharmacodynamic models (PBPK-PD). The minimal physiologically-based pharmacokinetic-pharmacodynamic linked model (mPBPK-PD) related Cyp24a1 mRNA fold changes to linear changes in tissue/tissue baseline 1,25(OH)₂D₃ concentration ratios, whereas the full physiologically-based pharmacokinetic-pharmacodynamic model (PBPK-PD) related measured tissue Cyp24a1 and Cyp27b1 fold changes to tissue 1,25(OH)₂D₃ concentrations with indirect response, sigmoidal maximal stimulatory effect/maximal inhibitory effect functions. Moreover, the intestinal segregated flow model (SFM) that describes a low and partial intestinal (blood/plasma) flow to enterocytes was nested within both models for comparison with the traditional model for intestine (TM) where the entire flow perfuses the intestine. Both the mPBPK(SFM)-PD and full PBPK(SFM)-PD models described the i.p. plasma and tissue 1,25(OH)₂D₃ concentrations and fold changes in mRNA expression significantly better than the TM counterparts with F test comparisons. The full PBPK(SFM)-PD model performed the best among the tested models for describing the complex pharmacokinetic-pharmacodynamic interplay among Cyp27b1, Cyp24a1, and 1,25(OH)₂D₃.

Introduction

Vitamin D is a collection of fat-soluble prohormone steroids with a diverse range of biologic effects that are primarily endocrine in nature (Norman et al., 1992). The two major lipophilic forms are vitamin D₃/cholecalciferol and vitamin D₂/ergocalciferol, which are extracted from food sources and produced upon sun exposure to the skin and activation of 7-dehydrocholesterol. The two forms exist bound to the vitamin D binding protein in plasma and are activated sequentially via hydroxylation, first in liver to form the circulating metabolite 25-hydroxyvitamin D₃ [25(OH)D₃] by CYP2R1 and CYP27A1, and then by 1α-hydroxylase or CYP27B1, the rate-limiting enzyme in kidney to form the active ligand, 1α,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] (Jones et al., 1998). In circulation, 1,25(OH)₂D₃ is present at very low concentrations that are not readily monitored by conventional means; the 25(OH)D₃ concentration (30 ng/mL) is 1000-fold higher compared with 1,25(OH)₂D₃ and is used to determine the vitamin D status (Holick, 2009). The active ligand, 1,25(OH)₂D₃, binds to the vitamin D receptor (VDR) in multiple tissues throughout the body to regulate the expression of genes relating to various biologic processes (Jones et al., 1998) and plays a vital role in regulating the calcium-phosphate mineral balance by enhancing calcium and phosphate absorption by the intestine. Active 1,25(OH)₂D₃ is known to exhibit antiproliferative, immunosuppressive, and anti-inflammatory effects (Clemens et al., 1983; Lemire, 2000; Topilski et al., 2004). New, potential therapeutic targets of the VDR-bound 1,25(OH)₂D₃ relating to cholesterol (Chow et al., 2014) and cerebral beta-amyloid (Durk et al., 2014)–lowering properties, have also been reported.

Processes controlling the disposition of 1,25(OH)₂D₃ are complex. Endogenous concentrations of 1,25(OH)₂D₃ in circulation and tissues are tightly regulated, especially its synthesis by CYP27B1 and
degradation by CYP24A1. CYP27B1, the rate-limiting synthetic enzyme that tightly regulates 1α-hydroxylation of 25(OH)D3 to form 1,25(OH)2D3, is inhibited by the 1,25(OH)2D3-bound VDR via the calcium-sensing receptor and parathyroid hormone (Shinki et al., 1992; Lemay et al., 1995). CYP24A1 or 25-hydroxyvitamin D-24-hydroxylase (24-hydroxylase) is a mitochondrial P450 enzyme that catalyzes the hydroxylation of both 25(OH)D3 and 1,25(OH)2D3 at carbon-24 to form 24,25(OH)2D3 and 1,24,25(OH)3D3 (Jones et al., 1998; Henry, 2001). This inactivation pathway eventually leads to the production of the more polar metabolite, calcitroic acid, and induces elimination of 1,25(OH)2D3 (St-Arnaud, 1999). Upregulation of CYP24A1 by 1,25(OH)2D3 exerts feedback control to reduce 1,25(OH)2D3 concentrations and is a hallmark of 1,25(OH)2D3 upregulation.

Few mathematical models exist to relate these complex interactions on the absorption, distribution, metabolism, and elimination of 1,25(OH)2D3. A recent compartmental model showed that changes in the pharmacodynamics of Cyp27b1 and Cyp24a1, when incorporated into the pharmacokinetic model, greatly improved description of the kinetic profiles of 1,25(OH)2D3 in mice after increasing i.v. doses of 1,25(OH)2D3 (Quach et al., 2015), suggesting the need to simultaneously incorporate pharmacodynamics into modeling. In this study, we revisited the rich data obtained from repeated i.p. injections of 1,25(OH)2D3 to mice, which included 1,25(OH)2D3 tissue concentrations and fold changes (FC; ratio of changed/basal mRNA level) of renal Cyp27b1 and Cyp24a1 mRNA expressions in kidney, ileum, liver, and brain (Chow et al., 2013). We examined the utility of a minimal physiologically based pharmacokinetic model (mPBPK-PD) to parsimoniously describe tissue concentrations versus time, a model that was originally designed to use plasma concentrations and tissue to plasma and blood partition coefficients (Kp), flow terms expressed in terms of the fraction of cardiac output with Fick’s law of perfusion (fd), and intrinsic clearances (Cao and Jusko, 2012); the pharmacodynamic component was simplified by relating Cyp24a1 FCs to 1,25(OH)2D3 tissue/baseline tissue concentration ratios. We also employed a full physiologically-based pharmacokinetic-pharmacodynamic model (PBPK-PD), consisting of the same number of “lumped” tissue compartments as the mPBPK-PD model, to fit to the full set of plasma and tissue 1,25(OH)2D3 concentration-time data, together with FC of Cyp24a1 and Cyp27b1 concentrations.

![Fig. 1. Schematic presentation of the mPBPK-PD models for 1,25(OH)2D3 kinetics in mice.](image-url)
mRNA expressions, using indirect response models consisting of maximal stimulatory effect ($E_{\text{max}}$) or maximal inhibitory effect ($I_{\text{max}}$) and EC$_{50}$ (or IC$_{50}$) terms. Moreover, the subtleties of intestinal tissue perfusion patterns that describe route-dependent intestinal metabolism and distribution were compared, as follows: the traditional intestine model (TM) in which the entire blood flow perfuses the intestine tissue as a whole; the segregated flow model (SFM) that describes the intestine as the enterocyte region, perfused by a low and partial intestinal blood flow (5–30%); and a serosal region, perfused by the remaining flow (Cong et al., 2000). The SFM describes a greater extent of intestinal elimination with oral compared with i.v. dosing and delimits access of drug after i.v. dosing to enterocytes due to the low flow to that region (Cong et al., 2000).

Materials and Methods

Published 1,25(OH)$_2$D$_3$ Data and FC in mRNA Expressions

Data used for the modeling of plasma and tissue 1,25(OH)$_2$D$_3$ (or calcitriol, chemical structure obtainable from DrugBank: http://www.drugbank.ca/drugs/DB00136) concentrations and FC in mRNA expressions of Cyp24a1 in various tissues and for Cyp27b1 in kidney were obtained from previously published in vivo pharmacokinetic studies. In these studies, 0 (control) or 2.5 $\mu$g $\times$ kg$^{-1}$ 1,25(OH)$_2$D$_3$ (120 pmol or 0.05 $\mu$g $\times$ mouse$^{-1}$) dissolved in sterile corn oil, was administered i.p. to male C57BL/6 mice (8 weeks old) every other day over 8 days, or q2d $\times$ 4 (Chow et al., 2013).

The PBPK-PD Models

The mPBPK-PD Model. Cao and Jusko (2012) described a minimal physiologically-based pharmacokinetic model (PBPK) with a minimum number of compartments with extensive lumping: the plasma, the liver, and two lumped compartments, together with KT as the fitted constant. The plasma flows (Q$_T$) are expressed as f$_Q$Q$_{CO}$ for the lumped tissue compartments, with f$_Q$ as the fraction of plasma cardiac output (Q$_{CO}$) to the lumped compartment. Our mPBPK-PD consists of 11 compartments, with 6 representing various tissues [plasma, brain, liver, kidney, ileum, and peripheral (or other) compartments] that are interconnected in a physiologically relevant manner. Four subcompartments corresponding to Cyp24a1 enzyme in liver, kidney, ileum, and brain were used to account for the synthesis and degradation of the enzyme. For simplicity,
Cyp2ba1 as a subcompartment of the kidney was not considered, because the synthesis rate was low (50 fmol h⁻¹) (Hsu et al., 1987) and Cyp2ba1 synthesis was immediately and completely inhibited upon administration of 1,25(OH)D₃ (Quach et al., 2013). The turnover of the Cyp2ba1 enzyme was defined experimentally to be 0.14, 0.34, 0.40, and 0.05 for the liver, kidney, ileum, and brain, respectively (Chow et al., 2013). The turnover of the Cyp2ba1 enzyme was defined with a zero-order synthesis rate (kₚCp₀baseline) and first-order degradation rate (kₚCp₀baseline). Numerical values of kₚ and kₚ are identical in each Cyp2ba1baseline values are the pharmacokinetic changes [FC of Cyp2ba1, or Cyp2ba1mRNA (mRNA expression/control expression value)] were assumed to change proportionally with the ratio of the relevant tissue 1,25(OH)D₃ concentration (Cₜ) to its baseline value (Cₜbaseline) (see eq. A7 in Appendix).

The Full PBPK-PD Model. The full PBPK-PD model incorporated in the mRNA expression of both Cyp2ba1 and Cyp2ba2 (Fig. 2). The model is similar to the minimal model in most respects except for the definition of the pharmacodynamics. Induction of Cyp2ba1 and inhibition of Cyp2ba2 were described using indirect response equations (Daynka et al., 1993; Sharma and Jusko, 1996; Mager et al., 2003), comprising the full sigmoidal Eₘₐₓ (Iₘₐₓ, EC₅₀ (IC₅₀), and Hill coefficients.

Nested TM versus SFM in PBPK-PD Modeling. To account for differences in intestinal blood/plasma flow to the enterocyte region of the intestine, we highlighted the SFM to contrast with the TM for both minimal PBPK and full PBPK models (right panels of Figs. 1 and 2). Pang and colleagues have viewed the intestine as two tissue subcompartments for the SFM, with an enterocyte compartment consisting of absorptive/secretory transporters at the apical membrane facing the lumen, metabolic enzymes within and a basolateral side facing the blood, and a serosal compartment acting only as a storage or distribution compartment (Cong et al., 2000; Doherty and Pang, 2000; Pang, 2003; Fan et al., 2010). For SFM, the fraction of intestinal blood flow perfusing the enterocyte region (fₑ) is 5–30% of the total intestinal blood flow (Cong et al., 2000; Pang and Chow, 2012). The model suggests that drug in systemic circulation (e.g., from i.v. dosing) would be partially shunted away from the enterocyte region, whereas for by-mouth (PO) dosing, the entire dose would first reach the enterocytes prior to entering the circulation. In contrast, the TM suggests that the entire intestinal blood flow perfuses the enterocyte region that is indistinguishable from the serosal region. Hence, the SFM suggests the occurrence of route-dependent intestinal metabolism (see eqs. A14 and A15 in Appendix), and that a greater extent of intestinal metabolism occurs for PO or i.p. over i.v. dosing (Cong et al., 2000).

Data Fitting and Simulations

ADAPTS (version 5.1, Biomedical Simulations Resource, University of Southern California, Los Angeles, CA) was used for model fitting and simulations. The mPBPK-PD (Fig. 1) and full PBPK-PD (Fig. 2) were used, with the intestinal compartment being described the traditional way (TM) or as SFM (for intestinal compartment), with two subcompartments representing the enterocyte and serosal regions (Cong et al., 2000). The initial condition for the amount of 1,25(OH)D₃ in gut lumen was the i.p. dose administered (eq. A11 in Appendix). In contrast, the i.v. dose was administered to plasma compartment directly (eq. A6 in Appendix).

Fitting. Fitting with equations shown in the Appendix for the minimal and full PBPK-PD models to repeated i.p. data (120 pmol × 4) was performed. Rate equations for plasma flows (Qₜ) and tissue volumes (Vₜ), common physiologic parameters (Davies and Morris, 1993; Brown et al., 1997) (Table 1), were assigned for fitting of the PBPK-PD models with nested SFM and TM (see Appendix). The initial condition or the baseline plasma concentration of 1,25(OH)D₃ (C₀baseline) was assigned the measured value (217 pM) (Chow et al., 2013). The baseline concentration of 1,25(OH)D₃ in tissue volumes (V) and plasma flow (Q) were obtained from Davies and Morris, 1993, and Brown et al., 1997; KT and plasma baseline concentrations, C₀baseline, were obtained experimentally (Chow et al., 2013).
(C_T,baseline) was expressed as a function of C_P,baseline, as described in eq. A4 in the Appendix.

We assumed that 1,25(OH)_2D_3 is confined to the plasma space and inter-relate plasma concentrations and plasma flows in the rate equations that also relate blood concentrations and blood flows; we further defined K_T as the tissue to plasma concentration ratio (C_T/C_P). The assumption that 1,25(OH)_2D_3 is confined to the plasma space is consistent with compartmental estimates of 61.5 mL x kg^{-1} or 1.23 mL for a 20 g mouse for V_1, the central volume of distribution of 1,25(OH)_2D_3 for mice given the 120 pmol i.v. dose (Quach et al., 2015). By assuming a hematocrit (Hct) of 0.45, then the estimated blood volume for the central compartment is 1.23 (1-0.45) or 2.24 mL. The value is similar to the published value for blood volume for the mouse (1.5-2.5 mL; web.jhu.edu/animalcare/procedures/mouse.html), suggesting that 1,25(OH)_2D_3 is indeed confined to the plasma.

For a drug that is confined to the plasma and does not distribute into red blood cells, the measured plasma concentration may be converted to blood concentration, from the equality: V_B (1-Hct)C_P = C_BV_B.

\[ C_P = \frac{C_B}{1-Hct} \]  (1)

Plasma volume (V_P) and plasma flow (Q_P) could be expressed in terms of blood volume (V_B) and blood flow (Q_B).

\[ V_P = (1-Hct) \times V_B \]  (2)
\[ Q_P = (1-Hct) \times Q_B \]  (3)

A Hct value of 0.45 was used for mice (average value obtained from Charles River Laboratories, St. Constance, QC, Canada). Equations in the Appendix were

Fig. 3. FC in renal Cyp27b1 as well as renal, intestinal, hepatic, and brain Cyp24a1 mRNA expression versus the corresponding tissue 1,25(OH)_2D_3 concentrations following the first, second, third, fourth, and all four doses of 120 pmol [data of (Chow et al., 2013)]. Equation 5 for induction Cyp24a1_{FC,T} = (1 + \frac{E_{max} C_{T}}{EC_{50} + C_{T}}) and eq. 6 for inhibition Cyp27b1_{FC,K} = (1 - \frac{E_{max} C_{K}}{EC_{50} + C_{K}}) were used here, where \( \gamma_1 \) and \( \gamma_2 \) denote the Hill coefficient (\( \gamma = 1 \)) of Cyp27b1 and Cyp24a1, respectively, and baseline values of Cyp24a1_{FC,T} and Cyp27b1_{FC,K} = 1.
used for model fitting. The initial 1,25(OH)2D3 baseline concentration (CT,\textsubscript{baseline}) in tissue was estimated from KT and CP\textsubscript{baseline} for both models (eq. A4, Appendix). Because a rich data set existed, tissue 1,25(OH)2D3 concentrations were used for fitting, not only for the full PBPK-PD models, but also for the mPBPK-PD models. For data fitting of both models, a naive-pooled data analysis approach was used, in which all data were modeled simultaneously in ADAPT5 using the maximum likelihood estimator. The variance model was defined as:

\[
VAR_i = (\sigma_1 + \sigma_g \cdot Y(\theta_i))^2
\]  

with \(\sigma_1\) and \(\sigma_g\) as the variance model parameters, and \(Y(\theta_i)\) as the \(i\)th predicted value from the pharmacokinetic model. Different variance parameters were used for 1,25(OH)2D3 concentrations in plasma/tissues and the mRNA expression of VDR genes (Cyp27b1 and Cyp24a1). The final model was selected based on goodness-of-fit criteria, which included model convergence, parameter precision, and visual inspection of predicted versus observed values and residual plots. The F test was used to compare goodness of fit of the nested TM and SFM models (Boxenbaum et al., 1974).

The mPBPK-PD Model. For fitting of the mPBPK-PD model, Cyp27b1\textsubscript{FC,K} was omitted in the kidney compartment due to the low synthesis rate of the enzyme (see eqs. A6 - A16, Appendix). The FC of mRNA expression of Cyp24a1, or Cyp24a1\textsubscript{FC,T}, was expressed as a linear function of C/CT,\textsubscript{baseline} (see eq. A7, Appendix).

The Full PBPK-PD Model. For full PBPK-PD model fitting, the indirect response models with E\textsubscript{max} (or I\textsubscript{max}) and E\textsubscript{CO} (or I\textsubscript{CO}) values were used for description of induction of Cyp24a1 and inhibition of Cyp27b1, respectively (see eqs. A18 and A19, Appendix). These constants were estimated by regression of the FC of the enzymes against the relevant tissue 1,25(OH)2D3 concentration (Fig. 3). The Cyp24a1\textsubscript{FC,T} in tissue was expressed as:

\[
\text{Cyp24a1}_{\text{FC,T}} = \left(1 + \frac{E_{\text{max}}C_T}{E_{\text{CO}} + C_T}\right)
\]  

with E\textsubscript{max} as the maximum stimulatory effect, E\textsubscript{CO} as the tissue 1,25(OH)2D3 concentration producing 50% of E\textsubscript{max}, and \(\gamma_1\) as the Hill coefficient of Cyp24a1 in tissue (Mager et al., 2003; Quach et al., 2015).

The Cyp27b1\textsubscript{FC,K} in the kidney was expressed as:

\[
\text{Cyp27b1}_{\text{FC,K}} = \left(1 + \frac{I_{\text{max}}C_K}{I_{\text{CO}} + C_K}\right)
\]  

with I\textsubscript{max} as the maximum inhibitory effect, I\textsubscript{CO} as the renal 1,25(OH)2D3 concentration producing 50% of I\textsubscript{max}, and \(\gamma_2\) as the Hill coefficient of Cyp27b1 in kidney (Mager et al., 2003; Quach et al., 2015). Regression of data from each dose and for the entire data set was performed according to eqs. 5 and 6. Because there were no trends among these estimates, final estimates were obtained from the fit to all data. Values of E\textsubscript{max} and I\textsubscript{max} were assigned based on our final estimates, whereas the E\textsubscript{CO} and I\textsubscript{CO} estimates were used as initial estimates, and final values were obtained from model fitting.

**Simulations**

Simulations were performed to examine whether the mPBPK-PD and full PBPK-PD models, with TM- or SFM-nested as the intestine compartment, were able to predict the rebound phenomenon from previous repeated and single i.p. administration (120 pmol) (Chow et al., 2013) as well as assayed data for repeated i.v. administration with different (2, 60, and 120 pmol) 1,25(OH)2D3 doses (Quach et al., 2015). Final parameter estimates and assigned constants were used for simulations. The prediction errors (PE), defined as PE\textsubscript{i} = C\textsubscript{pred,i} - C\textsubscript{obs}, were calculated to compare the precision of mPBPK-PD and full PBPK-PD model when predicting single i.p. and repeated i.v. data. The median prediction error (MPE) and median absolute prediction error (MAPE) were used to estimate accuracy and precision, respectively:

\[
\text{MPE} = \text{Median}\left\{\frac{C_{\text{pred,i}} - C_{\text{obs,i}}}{|C_{\text{obs,i}}|}\right\}_0^{8\text{ days}}
\]  

\[
\text{MAPE} = \text{Median}\left\{\frac{|C_{\text{pred,i}} - C_{\text{obs,i}}|}{|C_{\text{obs,i}}|}\right\}_0^{8\text{ days}}
\]  

where MPE is the median value of the prediction error from time 0 to 8 days.

**Quantitative Real-Time Polymerase Chain Reaction**

To evaluate the relative expressions of Cyp24a1 in tissue, total RNA, obtained from kidney, ileum, liver, and brain samples from control mice, was extracted using the TRizol extraction method (Sigma-Aldrich, Mississauga, ON, Canada), according to manufacturer’s protocol with modifications (Chow et al., 2011). cDNA (total of 1.5 μg) was synthesized from RNA using the high capacity cDNA reverse transcription kit (Applied Biosystems by Life Technologies, Burlington, ON, Canada), followed by quantitative real-time polymerase chain reaction using the SYBR Green detection system. mRNA data were normalized to cyclophilin for calculation of the relative change in gene expression in terms of FC (Chow et al., 2011).

**Results**

**Estimation of E\textsubscript{max}, E\textsubscript{CO}, I\textsubscript{max}, and I\textsubscript{CO}**

Plots of FCs of Cyp24a1 and Cyp27b1 mRNA expression levels in kidney, liver, ileum, and brain versus tissue 1,25(OH)2D3 concentration are shown in Fig. 3. The I\textsubscript{max} (and I\textsubscript{CO}) and E\textsubscript{max} (and E\textsubscript{CO}) values were obtained after regression of Cyp27b1\textsubscript{FC,K} and Cyp24a1\textsubscript{FC,T} expression against the corresponding tissue 1,25(OH)2D3 concentrations with eqs. 5 and 6. Parameters for inhibition of Cyp27b1 and induction of Cyp24a1 mRNA expression for each dose and for the combined doses are summarized in Table 2. Similar values of E\textsubscript{CO} and E\textsubscript{max} for the induction and I\textsubscript{CO} and I\textsubscript{max} for the inhibition were obtained among the first, second, third, and fourth doses. Finally, the E\textsubscript{CO} and I\textsubscript{CO} values for the composite fit (combined doses) were used.
as initial estimates in model fitting, and the $E_{\text{max}}$ and $I_{\text{max}}$ values were assigned. The $E_{\text{max}}$ values estimated in this study are about 61–70% of those estimated by Quach et al. (2015). The estimated $I_{\text{max}}$ was slightly lower than that by Quach et al. (2015), but the $IC_{50}$ estimate was similar. Overall, there was good correspondence between the fits to both datasets. Fitted values for $EC_{50}$ were much lower than those obtained by Quach et al. (2015), who used plasma instead of tissue $1,25(OH)_2D_3$ concentrations for fitting. Parameters estimated from fitting with tissue $1,25(OH)_2D_3$ concentrations are more appropriate.

Fig. 4. Observed (closed circles) and fitted (spline lines) concentration-time profiles of $1,25(OH)_2D_3$ and time course of FC of tissue Cyp24a1 and Cyp27b1 mRNA after multiple i.p. doses [data of (Chow et al., 2013)] using mPBPK-PD model with nested TM (dashed lines) and SFM (solid lines).
The observed and model-fitted concentration-time profiles using the minimal and full PBPK-PD models with nested TM and SFM are shown in Figs. 4 and 5, respectively. All of the models characterized the naive-pooled 1,25(OH)₂D₃ concentrations and Cyp24a₁₉₉₉₉₉₉₉ mRNA expression for the multiple i.p. doses well. These models predicted similar trends for the plasma and tissue 1,25(OH)₂D₃ profiles and showed that the peak concentration (C_max) was reached within

Fig. 5. Observed (closed circles) and fitted (spline lines) concentration-time profiles of 1,25(OH)₂D₃ and time course of FC of tissue Cyp24a₁ and Cyp27b1 mRNA after multiple i.p. doses [data of (Chow et al., 2013)] using the full PBPK-PD model with nested TM (dashed lines) and SFM (solid lines).

Fitting to Minimal and Full PBPK-PD Models
The observed and model-fitted concentration-time profiles using the minimal and full PBPK-PD models with nested TM and SFM are shown in Figs. 4 and 5, respectively. All of the models characterized the
<table>
<thead>
<tr>
<th>Fitted Parameters</th>
<th>Definition</th>
<th>mPBPK(TM)-PD</th>
<th>mPBPK(SFM)-PD</th>
<th>Full PBPK(TM)-PD</th>
<th>Full PBPK(SFM)-PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_d$</td>
<td>Fraction of intestinal flow to enterocyte region</td>
<td>1</td>
<td>0.114 (2.26)</td>
<td>1</td>
<td>0.105 (0.533)</td>
</tr>
<tr>
<td>$k_{in}$ (h⁻¹)</td>
<td>Fraction of cardiac output to peripheral compartment</td>
<td>0.0021 (0.021)</td>
<td>0.0032 (0.025)</td>
<td>0.0035 (0.002)</td>
<td>0.0048 (0.001)</td>
</tr>
<tr>
<td>$k_{deg}$ (h⁻¹)</td>
<td>Degradation rate constant of 1,25(OH)₂D₃</td>
<td>1.43 (0.011)</td>
<td>1.26 (0.013)</td>
<td>1.50 (0.004)</td>
<td>1.61 (0.0021)</td>
</tr>
<tr>
<td>$K_{peri}$ or $K_{other}$</td>
<td>Partition coefficient of peripheral/other compartment</td>
<td>0.0042 (6.06)</td>
<td>0.0013 (11.6)</td>
<td>0.0017 (2.54)</td>
<td>0.0012 (6.65)</td>
</tr>
<tr>
<td>$R_{syn}$ (fmol × h⁻¹)</td>
<td>Endogenous synthesis rate of 1,25(OH)₂D₃</td>
<td>50.9 (0.26)</td>
<td>23.0 (0.238)</td>
<td>31.0 (0.247)</td>
<td>21.5 (0.116)</td>
</tr>
<tr>
<td>$k_{in,Cyp27b1,K}$ or $k_{out,Cyp27b1,K}$ (h⁻¹)</td>
<td>Turnover rates constant of renal $C_{yp27b1}$</td>
<td>—</td>
<td>—</td>
<td>0.220 (11.5)</td>
<td>0.245 (9.72)</td>
</tr>
<tr>
<td>$\gamma_2$</td>
<td>Hill coefficient for indirect response of renal $C_{yp27b1}$ function</td>
<td>—</td>
<td>—</td>
<td>3.57 (14.0)</td>
<td>2.71 (12.1)</td>
</tr>
<tr>
<td>$f_{CL,inmet,1}$ (mL × h⁻¹)</td>
<td>Hepatic metabolic intrinsic clearance of 1,25(OH)₂D₃ via hepatic $C_{yp24a1}$</td>
<td>0.031 (0.841)</td>
<td>0.0782(2.82)</td>
<td>0.0043 (1.78)</td>
<td>0.0010 (3.03)</td>
</tr>
<tr>
<td>$f_{CL,inmet,1}$ (mL × h⁻¹)</td>
<td>Intestinal metabolic intrinsic clearance of 1,25(OH)₂D₃ via intestinal $C_{yp24a1}$</td>
<td>0.229 (0.348)</td>
<td>0.220 (0.196)</td>
<td>0.0011 (0.038)</td>
<td>0.0014 (0.412)</td>
</tr>
<tr>
<td>$f_{CL,inmet,K}$ (mL × h⁻¹)</td>
<td>Renal metabolic intrinsic clearance of 1,25(OH)₂D₃ via renal $C_{yp24a1}$</td>
<td>0.053 (0.435)</td>
<td>0.0641 (0.782)</td>
<td>0.0242 (0.008)</td>
<td>0.0280 (0.0066)</td>
</tr>
<tr>
<td>$f_{CL,inmet,Br}$ (mL × h⁻¹)</td>
<td>Brain metabolic intrinsic clearance of 1,25(OH)₂D₃ via brain $C_{yp24a1}$</td>
<td>0.030 (15.0)</td>
<td>0.0345 (3.37)</td>
<td>0.0006 (8.30)</td>
<td>0.0003 (0.412)</td>
</tr>
<tr>
<td>$\gamma_2$</td>
<td>Hill coefficient for indirect response of hepatic $C_{yp24a1}$</td>
<td>—</td>
<td>—</td>
<td>1.24 (2.45)</td>
<td>1.75 (0.020)</td>
</tr>
<tr>
<td>$\gamma_2$</td>
<td>Hill coefficient for indirect response of renal $C_{yp24a1}$ function</td>
<td>—</td>
<td>—</td>
<td>2.64 (0.665)</td>
<td>3.59 (0.081)</td>
</tr>
<tr>
<td>$\gamma_1$</td>
<td>Hill coefficient for indirect response of intestinal $C_{yp24a1}$</td>
<td>—</td>
<td>—</td>
<td>0.985 (1.22)</td>
<td>2.09 (0.002)</td>
</tr>
<tr>
<td>$\gamma_1$</td>
<td>Hill coefficient for indirect response of function of hepatic $C_{yp24a1}$</td>
<td>—</td>
<td>—</td>
<td>0.878 (0.148)</td>
<td>0.576 (0.076)</td>
</tr>
<tr>
<td>$k_{in,Cyp24a1,L}$ or $k_{out,Cyp24a1,L}$ (h⁻¹)</td>
<td>Turnover rate constant of hepatic $C_{yp24a1}$</td>
<td>0.0413 (0.052)</td>
<td>0.044 (0.708)</td>
<td>0.045 (0.006)</td>
<td>0.047 (0.008)</td>
</tr>
<tr>
<td>$k_{in,Cyp24a1,I}$ or $k_{out,Cyp24a1,I}$ (h⁻¹)</td>
<td>Turnover rate constant of intestinal $C_{yp24a1}$</td>
<td>0.218 (0.278)</td>
<td>0.174 (0.485)</td>
<td>0.489 (0.005)</td>
<td>0.287 (0.067)</td>
</tr>
<tr>
<td>$k_{in,Cyp24a1,K}$ or $k_{out,Cyp24a1,K}$ (h⁻¹)</td>
<td>Turnover rate constant of renal $C_{yp24a1}$</td>
<td>0.0256 (0.038)</td>
<td>0.0238 (0.026)</td>
<td>0.012 (0.065)</td>
<td>0.047 (0.008)</td>
</tr>
<tr>
<td>$k_{in,Cyp24a1,Br}$ or $k_{out,Cyp24a1,Br}$ (h⁻¹)</td>
<td>Turnover rate constant of brain $C_{yp24a1}$</td>
<td>1.20 (2.73)</td>
<td>2.10 (4.52)</td>
<td>1.28 (0.051)</td>
<td>1.87 (5.10)</td>
</tr>
<tr>
<td>$SM_{Cyp24a1,L}$</td>
<td>Power coefficient on renal $C_{yp24a1}$ enzyme turnover</td>
<td>0.931 (0.112)</td>
<td>0.766 (0.933)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$SM_{Cyp24a1,K}$</td>
<td>Power coefficient on hepatic $C_{yp24a1}$ enzyme turnover</td>
<td>1.27 (0.120)</td>
<td>1.33 (0.056)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$SM_{Cyp24a1,Br}$</td>
<td>Power coefficient on brain $C_{yp24a1}$ enzyme turnover</td>
<td>0.804 (0.389)</td>
<td>0.880 (0.498)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$SM_{Cyp24a1,I}$</td>
<td>Power coefficient on intestinal $C_{yp24a1}$ enzyme turnover</td>
<td>1.40 (0.116)</td>
<td>1.42 (0.160)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$HK$</td>
<td>Power coefficient on renal intrinsic clearance</td>
<td>0.828 (0.0622)</td>
<td>0.784 (0.0372)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$HL$</td>
<td>Power coefficient on hepatic intrinsic clearance</td>
<td>1.86 (0.0769)</td>
<td>0.470 (2.23)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$HBr$</td>
<td>Power coefficient on brain intrinsic clearance</td>
<td>0.142 (17.8)</td>
<td>0.374 (5.68)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$HI$</td>
<td>Power coefficient on intestinal intrinsic clearance</td>
<td>0.230 (0.246)</td>
<td>0.054 (7.82)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike information criteria</td>
<td>9367</td>
<td>9270</td>
<td>10,006</td>
<td>9993</td>
</tr>
<tr>
<td>WSSR</td>
<td>Weighted sum of squared residuals</td>
<td>1684</td>
<td>1644</td>
<td>1664</td>
<td>1478</td>
</tr>
<tr>
<td>$df^*$</td>
<td>Degrees of freedom (critical $F = 3.84$)</td>
<td>998</td>
<td>988</td>
<td>986</td>
<td>985</td>
</tr>
<tr>
<td>$F$ value$^*$</td>
<td>Calculated $F$ score</td>
<td>24.04$^*$ versus mPBPK(TM)-PD</td>
<td>5.93$^*$ versus mPBPK(TM)-PD</td>
<td>55.3$^*$ versus mPBPK(SFM)-PD</td>
<td>124$^*$ versus PBPK(TM)-PD</td>
</tr>
</tbody>
</table>

$^*$Fitted $CL_{inmet,2}$ values were adjusted by corresponding unbound fraction of 1,25(OH)₂D₃ in tissue ($f_d$).

$^*$Degrees of freedom are the number of data points ($n = 1010$) used in the model minus the number of parameters being fitted.

$^*_F$ score was calculated using $\frac{n - k - 1}{k}$, where $df = n - k$.

$^*_F$ score suggests a significant improvement in the goodness of fit (critical $F = 3.84$) (Boxenbaum et al., 1974); the rank order of goodness of fit (from poorest to best for optimal fit: mPBPK(TM)-PD < mPBPK(SFM)-PD < full PBPK(TM)-PD < full PBPK(SFM)-PD model.
for fitted f TCLint,met,T values for Cyp24a1-mediated metabolism in kidney to 1.0 for brain (Fig. 6). The rank order was similar to the pattern of fmol1.2
value of fQ was

The results showed that the fit with the SFM was superior compared with the TM. Predictions from the full PBPK(SFM)-PD model were also better for describing the route-dependent concentration-time profiles compared with those from the full PBPK(TM)-PD model (Fig. 5). Values from the F test attests that the full PBPK(SFM)-PD is the better model (Table 3).

Validation of the Models. To validate the robustness of the models, simulations were performed for the repetitive i.p. dosing of 120 pmol (Chow et al., 2013) and escalating i.v. doses (2, 60, and 120 pmol) (Quach et al., 2015). Simulations described well the plasma and tissue concentrations rising and then falling below baseline after 24–48 hours and gradually returning back to basal level following repetitive (Figs. 7 and 8) or single (Figs. 9 and 10) i.p. administration (120 pmol). Both PBPK-PD models also predicted the Cyp24a1 mRNA levels in tissue returning to basal level (=1), suggesting that the up- and downregulation of VDR target genes disappeared within 10–14 days after discontinuation of treatment (Figs. 7–10).

The robustness of the model for prediction of escalating i.v. doses (2, 60, and 120 pmol every other day for 6 days; Figs. 11 and 12) was also examined. All models were able to predict the pharmacokinetics of 1,25(OH)2D3 following repeated i.v. administration, although data for the low doses were not as well predicted by the mPBPK-PD models. After comparison of prediction errors (P < .05; Table 4), the full PBPK-PD model was found to be more consistent with data and more robust than the mPBPK-PD model, as defined by the MPE and MAPE values in Table 4. Moreover, both observed and predicted Cmax in ileum following i.v. administration were unexpectedly lower than the Cmax after i.p. administration (Fig. 12 versus Fig. 5) despite the bioavailability of 0.84 ± 0.16 [from the dose-corrected area under the curve ratios of i.p./ i.v., (AUC0–t /Dose i.p. )/(AUC0–t /Dose i.v.) for all 4 models], suggesting a less distribution of 1,25(OH)2D3 into the enterocyte after i.dosing, a flow pattern lending support to the SFM model. The full PBPK(SFM)-PD model was better for describing the route-dependent kinetics of 1,25(OH)2D3 for i.p. dosing than other types of PBPK-PD models as it captured the lower Cmax in ileum following i.v. administration (Fig. 11 versus Fig. 12).

Discussion

Vitamin D synthesis and disposition is a complex, multistage process occurring in different tissues and is tightly regulated. Hence, we revisited the rich i.p. and i.v. data of 1,25(OH)2D3 in mice to gain a more physiologically relevant perspective. The modeling design was based on measurements of 1,25(OH)2D3 concentrations and regulatory enzymes that are under feedback control by 1,25(OH)2D3-bound VDR over a sufficiently long duration following multiple i.p. and i.v. doses (Chow et al., 2013; Quach et al., 2015). Moreover, a dense sampling frequency had been adopted previously to provide rich temporal profiles that best capture the disposition of 1,25(OH)2D3 in plasma and tissues. In addition, the dynamics of critical enzymes involved in the metabolism of 1,25(OH)2D3 were measured in parallel.

Quach et al. (2015) employed compartmental pharmacokinetic/pharmacodynamic (PK/PD) modeling for i.v. 1,25(OH)2D3 data obtained from escalating doses to relate 1,25(OH)2D3 kinetics and the feedback inhibition of Cyp27b1 and induction of Cyp24a1. Finer mechanistic details can be obtained by applying PBPK models that are parameterized based on physiologic system components, functions, and tissue compartments that are connected by plasma or blood flow rates. The minimal PBPK approach is proven to be superior over compartmental models and provides a greater mechanistic insight and more interpretable and physiologically meaningful pharmacokinetic parameters, especially when only plasma or blood data are available (Cao and Jusko, 2012). It adopts Fick’s law of perfusion by incorporating fractional distribution (fD) to account for organ/tissue lumping and

![Fig. 6. Baseline levels of relative mRNA expression for Cyp24a1 in kidney, ileum, liver, and brain in control mice.](https://example.com/image)
variability in cardiac output ($Q_{cc}$), and has been successfully applied to describe the kinetics of small molecules as well as disposition pathways and sites of elimination of monoclonal antibodies (Cao et al., 2013; Li et al., 2014), although additional parameters, including vascular reflection coefficients, may be needed. The dimensionality and complexity of these types of developed PBPK models are conveniently reduced using lumping approaches, in which tissues with similar kinetics are grouped together to provide a simpler approach with fewer compartments than a whole-body PBPK model incorporating all tissues and organs (Nestorov et al., 1998; Pilari and Huisinga, 2010).

Fig. 7. Simulated (lines) concentration-time profiles of 1,25(OH)$_2$D$_3$ and time course of FC of tissue Cyp24a1 and Cyp27b1 mRNA expression following repeated i.p. doses [data of (Chow et al., 2013)] over 30 days to show the rebound phenomenon. Data were simulated using mPBPK-PD models with nested TM (dashed lines) and SFM (solid lines) described in the Appendix and parameters from Tables 2 and 3.
We first adopted a minimal PBPK-PD model to describe the pharmacokinetics and pharmacodynamics. The mPBPK-PD models provide a more simplistic framework and relate Cyp24a1 relative expression with tissue 1,25(OH)₂D₃ concentrations (eq. A7, Appendix). The FC of enzymes are related to CT/CT baseline linearly without any knowledge of Emax/EC₅₀ and Imax/IC₅₀ values. The model was further simplified by

Fig. 8. Simulated (lines) concentration-time profiles of 1,25(OH)₂D₃ and time course of FC of tissue Cyp24a1 and Cyp27b1 mRNA expression following repeated i.p. doses [data of (Chow et al., 2013)] over 30 days to show the rebound phenomenon. Data were simulated using the full PBPK-PD models with nested TM (dashed lines) and SFM (solid lines) described in the Appendix and parameters from Tables 2 and 3.
assuming the Cyp27b1 effects on Rsyn as minimal or negligible. The mPBPK-PD model adequately described the 1,25(OH)_{2}D_{3} and Cyp24a1FC,T (Figs. 4, 7, and 9). The final PK/PD parameters were estimated with good precision (low coefficient of variation) (Table 3). This simplified approach is particularly useful when pharmacodynamic data are scarce and E_{max}/I_{max} or EC_{50}/IC_{50} values are unavailable. However, the mPBPK-PD model that correlated enzyme expressions with baseline 1,25(OH)_{2}D_{3} concentrations via simplified, linear relationships is less able to adequately describe the concentration-dependent pharmacodynamic behavior of 1,25(OH)_{2}D_{3} (Figs. 11 and 12; Table 4).

We also employed the full PBPK-PD models that use indirect response equations (eqs. A18 and 19, Appendix) for describing the

Fig. 9. Observed (closed circles) versus simulated (lines) concentration-time profiles of 1,25(OH)_{2}D_{3} and the rebound to baseline levels after a single i.p. dose for the TM (dashed lines) and SFM (solid lines) nested within mPBPK-PD models [data of (Chow et al., 2013)] to show the rebound phenomenon.
pharmacokinetics and pharmacodynamics of 1,25(OH)₂D₃. The full PBPK-PD model requires dense sampling in multiple tissues and a diverse array of assigned parameters, including Cyp27b1 for the synthesis of 1,25(OH)₂D₃ and Cyp24a1 for catabolism, thereby providing a more complete description of the complex kinetics of 1,25(OH)₂D₃. Expectedly, inclusion of the inhibitory effect of 1,25(OH)₂D₃ on Cyp27b1-mediated endogenous synthesis (R_syn) of 1,25(OH)₂D₃ in kidney, which normally accounts for the bulk

Fig. 10. Observed (closed circles) versus simulated (lines) concentration-time profiles of 1,25(OH)₂D₃ and the rebound to baseline levels after a single i.p. dose for the TM (dashed lines) and SFM (solid lines) nested within the full PBPK-PD models [data of (Chow et al., 2013)] to show the rebound phenomenon.
of circulating 1,25(OH)$_2$D$_3$ (Bell, 1998), aptly described the 1,25(OH)$_2$D$_3$ profiles, the Cyp24a1 and Cyp27b1 expression, as well as the rebound phenomenon well (Figs. 5, 8, and 10). The full PBPK-PD model was found to be superior compared with the mPBPK-PD model in terms of precision according to prediction errors and prediction accuracy (Table 4), especially when characterizing repeated i.v. dose data ranging from low to higher doses (2 versus 60 and 120 pmol). This is expected because Cyp24a1$_{FC,T}$ and Cyp27b1$_{FC,K}$ are related nonlinearly by saturable sigmoidal $E_{max}/I_{max}$ equations, providing a more accurate account of concentration-dependent pharmacodynamics (shown in eqs. A18 and A19 in the Appendix).

Fig. 11. Observed (closed circles) versus simulated (spline lines) concentration-time profiles of 1,25(OH)$_2$D$_3$ and time course of FC of tissue Cyp24a1 and Cyp27b1 mRNA after multiple i.v. doses (given every 2 days for 6 days) in plasma, kidney, liver, ileum, and brain using mPBPK-PD models with nested TM (dashed lines) and SFM (solid lines) for describing the intestine compartment [data of (Quach et al., 2015)]. Data were simulated with PBPK-PD using inhibition and induction functions described in the Appendix and parameters in Tables 2 and 3.
The SFM (versus the TM) of the intestinal compartment defines the nonvascular (oral or i.p.) route of administration in more physiologically meaningful terms and distinguishes the differences in plasma/blood flow, transporter, channel, and metabolic enzyme density in the enterocyte and serosal regions of the intestine (Cong et al., 2000). With the peritoneal cavity serving as a reservoir for i.p. dosing, passive, nonsaturable, and continuous absorption of $1,25(\text{OH})_2\text{D}_3$ into the enterocyte compartment ensues (Hollander et al., 1978). The SFM was shown to be superior over the TM when these intestinal models are nested in the mPBPK-PD and full PBPK-PD models. With

**Fig. 12.** Observed (closed circles) versus simulated (spline lines) concentration-time profiles of $1,25(\text{OH})_2\text{D}_3$ and time course of FC of tissue Cyp24a1 and Cyp27b1 mRNA after multiple i.v. doses (given every 2 days for 6 days) in plasma, kidney, liver, ileum, and brain using the full PBPK-PD models with nested TM (dashed lines) and SFM (solid lines) for describing the intestine compartment [data of (Quach et al., 2015)]. Data were simulated with PBPK-PD using inhibition and induction functions described in the Appendix, and parameters in Tables 2 and 3. In particular, $C_{\text{max}}$ $1,25(\text{OH})_2\text{D}_3$ values in ileum were much overestimated for the TM. The extents of overestimation were less for the full PBPK(SFM)-PD and mPBPK(SFM)-PD models (Fig. 11), with the full PBPK(SFM)-PD model being the best.
1,25(OH)2D3 given i.p., part of the dose must traverse the enterocyte layer before reaching systemic circulation, whereas with i.v. administration, the entire dose directly enters the circulation. Consequently, more 1,25(OH)2D3 is available for intestinal metabolism following i.p. or PO than i.v. administration (Cong et al., 2000), allowing a greater extent of intestinal metabolism (see Figs. 1 and 2). For 1,25(OH)2D3, however, the extent of first-pass intestinal removal is small because the total clearance is low (Quach et al., 2015). The distinct presence of a serosal storage compartment better defines the distribution space in the intestine (Cong et al., 2000). The data support the SFM as the preferred model over the TM, inferring that there is intestinal route-dependent metabolism, namely, a drug given systemically will be less extracted by the intestine due to the low blood flow rate perfusing the enterocyte region (Cong et al., 2000; Pang, 2003; Pang and Chow, 2012).

In conclusion, a biologically plausible model has been developed for the quantitative characterization of the roles of Cyp24a1 and Cyp27b1 in regulating the complex pharmacokinetics of 1,25(OH)2D3 in mice. Based on the study design of the present study, PBPK-PD modeling provides a mechanism-based framework for discerning the tissue-specific disposition characteristics of 1,25(OH)2D3. The pharmacodynamic effects of 1,25(OH)2D3 are tightly regulated by the endogenous tissue concentrations, and the current models provide various platforms to integrate the absorption, distribution, and metabolism/excretion of 1,25(OH)2D3 to biologic effects observed in preclinical studies. We demonstrated good utility of the mPBPK-PD as a rational and simplistic approach for describing PK/PD interplay when limited pharmacodynamics data are available. The full PBPK-PD model, however, is superior to mPBPK-PD for describing dose-dependent kinetics and utilizes dynamic constants such as Emax, EC50, Imax, and IC50, and lastly, the nested SFM better characterizes the route-dependent intestinal removal/distribution as compared with the TM. The pharmacokinetic models developed in this study could be extended to understand the pharmacodynamic regulation of 1,25(OH)2D3 as well as other endogenous compounds in a quantitative manner. The models may also find utility in predicting 1,25(OH)2D3 disposition for interspecies scaling and for exploration of alternative dosing schemes and routes of administration to describe the dynamics of 1,25(OH)2D3 in its new therapeutic roles.

**Appendix**

**Definition of Q, V, KT, and CT,baseline**

For a noneliminating tissue, the rate of change in the tissue under basal conditions is given by:

\[ V_T \frac{dC_T}{dt} = Q_T \left( \frac{C_{P,baseline}}{C_T,baseline} - \frac{C_{T,baseline}}{K_T} \right) \quad (A1) \]

where \( C_{P,baseline} \), \( C_{T,baseline} \), \( V_T \), and \( Q_T \) are the baseline plasma and tissue concentrations, tissue volume, and plasma flow rate through the tissue compartment, respectively. At steady state, \( K_T \), the tissue to plasma partition coefficient, is given by the ratio of the tissue to plasma concentration:

\[ \frac{C_{T,baseline}}{C_{P,baseline}} = K_T \quad (A2) \]

For an eliminating tissue, the rate of change in the tissue at the basal level, in absence of induction of the degradating enzyme, Cyp24a1, is given by:

\[ V_T \frac{dC_T}{dt} = Q_T \left( \frac{C_{P,baseline} - C_{T,baseline}}{K_T} \right) - f_T C_{T,baseline} C_{int.met,T} \]

\[ \quad (A3) \]

where \( f_T \) is the unbound fraction and \( C_{int.met,T} \) is the intrinsic metabolic clearance in the tissue compartment. At steady state,

\[ C_{T,baseline} = \frac{Q_T C_{P,baseline}}{K_T + f_T C_{int.met,T}} \quad (A4) \]

and

\[ K_{T,app} = \frac{C_{T,baseline}}{C_{P,baseline}} = \frac{Q_T K_T}{K_T + f_T C_{int.met,T}} = \frac{1}{1 + \frac{Q_T}{K_T} C_{int.met,T}} \quad (A5) \]

As shown in eq. A5, \( C_{T,baseline}/C_{P,baseline} \) for eliminating organs yields only the apparent \( K_T \) (\( K_{T,app} \)) and underestimates the true \( K_T \) (Chen and Gross, 1979), in view of the fact that there is elimination within tissues with Cyp24a1. These \( K_T \) values, however, will not deviate much from the true values because the clearance of 1,25(OH)2D3 is low (Quach et al., 2015), and the error in underestimation of \( K_T \), based on \( C_{T,baseline}/C_{P,baseline} \), will be small. We further assumed that the \( K_T \) would stay constant under conditions when Cyp27b1 and Cyp24a1 are altered, and equal \( K_T \) under basal conditions.

In the equations to follow, \( Q_{peri}, Q_{Br}, Q_{K}, Q_{I}, \) and \( Q_L \) are the plasma flow rates to the peripheral, brain, kidney, intestine, and liver.
compartments, respectively; $V_{\text{peri}}$, $V_{\text{Br}}$, $V_K$, $V_P$, and $V_{\text{L}}$ are the corresponding tissue volumes, respectively. $Q_{\text{HA}}$ and $V_P$ are the plasma arterial flow rate and plasma volume, respectively.

### The mPBPK(TM)-PD Model (Fig. 1)

**For the Rate of Change of mRNA Expression**

$$\frac{dC_{\text{p}}}{dt} = \left( Q_L + Q_K + Q_{\text{Br}} + Q_{\text{peri}} \right) C_{\text{v}} + \left( Q_{\text{L}} + Q_{\text{K}} \right) C_{\text{KL}} + \frac{Q_K}{K_K} + \frac{Q_{\text{Br}}}{K_{\text{Br}}} + Q_{\text{peri}} \frac{C_{\text{peri}}}{K_{\text{peri}}} - C_{\text{p}}(0) = C_{\text{p baseline}} = 217 \text{ pM} \quad (A6)$$

$Q_{\text{peri}}$ is the plasma flow rate for the peripheral compartment (where $Q_{\text{peri}} = f_i Q_{\text{CVO}}$) and is expressed as a function of plasma cardiac output ($Q_{\text{CVO}}$), and $Q_L = (Q_i + Q_{\text{IA}})$ is the total hepatic plasma flow rate, where $Q_{\text{HA}}$ and $Q_i$ are the plasma flow of the hepatic artery and portal vein, respectively.

Changes in 1,25(OH)$_2$D$_3$ concentrations in tissues must first consider the rate of change of the degradation enzyme, Cyp24a1. In absence of enzyme induction, $\frac{dC_{\text{y24}}}{dt}$ (adapted from the corresponding baseline concentration $\left( \frac{C_{\text{T baseline}}}{T_{baseline}} \right)$, where $k_{\text{yp}}$, $k_{\text{yp base}}$, is the zero-order production rate constant and $k_{\text{yp base}}$ is the first-order degradation rate constant of the enzyme. Values of $k_{\text{in Cyp24a1 T}}$ and $k_{\text{out Cyp24a1 T}}$ are identical when Cyp24a1T baseline = 1.

For the mPBPK-PD model, Cyp24a1T FC was assumed to change directly with the relevant tissue 1,25(OH)$_2$D$_3$ concentration ($C_{\text{T}}$), which in turn is expressed relative to the corresponding baseline concentrations ($\left( \frac{C_{\text{T baseline}}}{T_{baseline}} \right)$). With induction of Cyp24a1, $k_{\text{in Cyp24a1 T}}$ is increased by the factor, $\left( \frac{C_{\text{T baseline}}}{T_{baseline}} \right)^{\text{power coefficient}}$ where SM$_T$ is the tissue-specific power coefficient.

$$\frac{dC_{\text{y24}}}{dt} = k_{\text{in Cyp24a1 T}} \left( \frac{C_{\text{T baseline}}}{T_{baseline}} \right)^{\text{power coefficient}} - k_{\text{out Cyp24a1 T}} C_{\text{y24}} \quad (A6)'$$

Upon normalization to Cyp24a1T baseline, we obtain FC of mRNA expression of Cyp24a1.

$$\frac{dC_{\text{y24}}}{dt} = k_{\text{in Cyp24a1 T}} \left( \frac{C_{\text{T baseline}}}{T_{baseline}} \right)^{\text{power coefficient}} - k_{\text{out Cyp24a1 T}} C_{\text{y24}}$$

or

$$\frac{dC_{\text{y24}}}{dt} = k_{\text{in Cyp24a1 T}} \left( \frac{C_{\text{T baseline}}}{T_{baseline}} \right)^{\text{power coefficient}} - k_{\text{out Cyp24a1 T}} C_{\text{y24}}$$

since

$$C_{\text{y24}} \text{ baseline} = 1$$

For the mPBPK-PD model, the FC of Cyp24a1 (Cyp24a1 FC) was described with the use of $(k_{\text{in Cyp24a1 T}}, k_{\text{out Cyp24a1 T}})$.

**For the Rate of Change in Brain.** $V_{\text{Br}} \frac{dC_{\text{Br}}}{dt} = Q_{\text{Br}} C_{\text{Br baseline}} = f_{\text{Br}} C_{\text{Br}} Cl_{\text{int,met,Br}}$ (Cyp24a1 FC Br) HBr, where HBr is the power coefficient for the brain.

After the discontinuation of 1,25(OH)$_2$D$_3$, levels of 1,25(OH)$_2$D$_3$ will rebound to basal conditions. After return to steady state, $V_{\text{Br}} \frac{dC_{\text{Br}}}{dt} = 0$ and $C_{\text{Br}}(0) = C_{\text{Br baseline}}$.

$$C_{\text{Br}}(0) = C_{\text{Br baseline}} = \frac{Q_{\text{Br}} C_{\text{Br baseline}}}{K_{\text{Br}} + f_{\text{Br}} Cl_{\text{int,met,Br}}}$$

### For the Rate of Change in Kidney.** $V_{\text{K}} \frac{dC_{\text{K}}}{dt} = Q_{\text{K}} C_{\text{K baseline}} + R_{\text{syn}}$ since Cyp24a1FC = 1

$$C_{\text{K}}(0) = C_{\text{K baseline}} = \frac{Q_{\text{K}} C_{\text{K baseline}} + R_{\text{syn}}}{K_{\text{K}} + f_{\text{K}} Cl_{\text{int,met,K}}}$$

### For the Rate of Change in Intestine.** $V_{\text{I}} \frac{dC_{\text{I}}}{dt} = k_i A_{\text{lumen}} + Q_{\text{I}} C_{\text{I baseline}} = f_{\text{I}} Cl_{\text{int,met,I}}$ (Cyp24a1 FC I) Hl, where Hl is the power coefficient for the intestine.

$$C_{\text{I}}(0) = C_{\text{I baseline}} = \frac{Q_{\text{I}} C_{\text{I baseline}}}{K_I + f_{\text{I}} Cl_{\text{int,met,I}}}$$

### For the Rate of Change in Gut Lumen.

$$\frac{dA_{\text{lumen}}}{dt} = -(k_a + k_{\text{deg}}) A_{\text{lumen}}; A_{\text{lumen}}(0) = \text{Dose}_\text{p}$$

Here, absorption of the i.p. dose is assumed to occur solely from the gut lumen, and is parameterized by the first-order absorption ($k_a$) and degradation ($k_{\text{deg}}$) rate constants in the gut lumen; $A_{\text{lumen}}$ is the amount of 1,25(OH)$_2$D$_3$ in the gut lumen.

**For the Rate of Change in Liver.** $V_{\text{L}} \frac{dC_{\text{L}}}{dt} = Q_{\text{L}} C_{\text{L baseline}} = \left[ 1 + \left( \frac{Q_{\text{I}} C_{\text{I baseline}}}{K_I} + Q_{\text{K}} C_{\text{K baseline}} \right) \right] C_{\text{P baseline}}$$

since Cyp24a1 FC = 1

and $C_{\text{L}}(0) = C_{\text{L baseline}} = \frac{Q_{\text{L}} C_{\text{L baseline}}}{K_L + f_{\text{L}} Cl_{\text{int,met,L}}}.$

When $f_{\text{L}} Cl_{\text{int,sec,L}} \leq Q_{\text{L}}$ (assumption is justified since the clearance of 1,25(OH)$_2$D$_3$ is low),

$$C_{\text{L baseline}} \text{ simplifies to } C_{\text{L}}(0) = C_{\text{L baseline}} = \frac{Q_{\text{L}} C_{\text{L baseline}}}{K_L + f_{\text{L}} Cl_{\text{int,met,L}}}$$

**For the Rate of Change in the Peripheral Tissues:**

$$\frac{dA_{\text{peri}}}{dt} = Q_{\text{peri}} \left( \frac{C_{\text{peri baseline}}}{K_{\text{peri}}} \right) C_{\text{peri}}(0) = \text{Cperi baseline} = K_{\text{peri}} C_{\text{P}}$$

where $A_{\text{peri}}$ is the amount of 1,25(OH)$_2$D$_3$ in the peripheral compartment.

### The mPBPK(SFM) Model

Additional equations are defined for the enterocyte and serosa regions for the SFM:
where \(C_T\) is the 1,25(OH)\(_2\)D\(_3\) concentration in tissue (kidney, liver, ileum, or brain), and \(\gamma_1\) and \(\gamma_2\) are the corresponding Hill coefficient for Cyp24a1 and Cyp27b1, respectively. \(E_{\text{max}}\) and \(I_{\text{max}}\) are the maximum inductive and inhibitory FC, respectively. \(EC_{50}\) and \(IC_{50}\), the tissue concentration that results in 50% of \(E_{\text{max}}\) and \(I_{\text{max}}\), respectively, were fitted with the initial estimates obtained from plotting FC against tissue concentrations (Table 2).

Additional equations for full PBPK(SFM) model are shown previously (see eq. A14–A16).

**Authorship Contributions**

 Participated in research design: Ramakrishnan, Young, Cao, Mager, Pang.
 Conducted experiments: Quach, Chow.
 Performed data analysis: Ramakrishnan, Young, Quach, Cao, Mager, Pang.
 Suggested and used of mPBPK-PD model for fitting: Cao
 Wrote or contributed to the writing of the manuscript: Yang, Ramakrishnan, Quach, Mager, Pang.

**References**


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