# Physiologically Based Pharmacokinetic Modeling Involving Nonlinear Plasma and Tissue Binding: Application to Prednisolone and Prednisone in Rats ${ }^{\text {® }}$ 

Xiaonan Li, Debra C. DuBois, Richard R. Almon, and William J. Jusko<br>Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences (X.L., D.C.D., R.R.A., W.J.J.) and Department of Biological Sciences (D.C.D., R.R.A.), State University of New York at Buffalo, Buffalo, New York

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#### Abstract

The pharmacokinetics (PK) of prednisolone (PNL) exhibit nonlinearity related to plasma protein binding, tissue binding, metabolic interconversion with prednisone (PN), and renal elimination. Blood and 11 tissues were collected from male Wistar rats after steady-state (SS) infusion and after subcutaneous boluses of $50 \mathrm{mg} / \mathrm{kg}$ of PNL. Concentrations of PNL and PN were measured by liquid chromatography-tandem mass spectrometry. Plasma and tissue profiles were described using a complex physiologically based pharmacokinetics (PBPK) model. Concentrations of PN and PNL were in rapid equilibrium in plasma and tissues. The tissue partition coefficients $\left(K_{p}\right)$ of PNL calculated from most subcutaneously dosed tissue and plasma areas were similar to SS infusion and in silico values. The blood-to-plasma ratio of PNL was 0.71 with similar red blood cell and unbound-plasma concentrations. Plasma protein binding (60\%-90\%) was related to corticosteroid-binding globulin (CBG) saturation. Tissue distribution was nonlinear. The equilibrium dissociation constant $\left(K_{d}\right)$ of PNL shared by all tissues was $3.01 \mathrm{ng} / \mathrm{ml}$, with the highest binding in muscle, followed by liver, heart, intestine, and bone and the lowest binding in skin, spleen,


fat, kidney, lung, and brain. Fat and bone distribution assumed access only to interstitial space. Brain PNL concentrations ( $K_{p}=$ 0.05) were low owing to presumed P-glycoprotein-mediated efflux. Clearances of CBG-free PNL were 1789 from liver and $191.2 \mathrm{ml} / \mathrm{h}$ from kidney. The PN/PNL ratio was nonlinear for plasma, spleen, heart, intestine, bone, fat, and linear for the remaining tissues. Our PBPK model with multiple complexities well described the PK profiles of PNL and PN in blood, plasma, and diverse tissues.

## SIGNIFICANCE STATEMENT

Because steroids, such as prednisolone and prednisone, have similar and complex pharmacokinetics properties in various species, receptors in most tissues, and multiple therapeutic and adverse actions, this physiologically based pharmacokinetics (PBPK) model may provide greater insights into the pharmacodynamic complexities of corticosteroids. The complex properties of these compounds require innovative PBPK modeling approaches that may be instructive for other therapeutic agents.

## Introduction

Corticosteroids (CSs) have long been frequently and intensively used in clinical practice for various indications, such as inflammatory (e.g., rheumatoid arthritis and asthma) and autoimmune diseases (e.g., solid organ transplant rejection and systemic lupus erythematosus) owing to their potent antiinflammatory and immunosuppressive properties (Czock et al., 2005; Rhen and Cidlowski, 2005; Bergmann et al., 2012; Kadmiel and Cidlowski, 2013). As synthetic glucocorticoids (GCs), CSs act primarily through genomic mechanisms

[^0]by binding to cytosolic glucocorticoid receptors (GRs) in tissues and causing inhibition of transcriptional factors, such as nuclear factor- $\kappa$ B and activator protein-1, thus suppressing the activation of genes encoding various proinflammatory mediators that promote inflammation and tissue damage (Coutinho and Chapman, 2011; Oakley and Cidlowski, 2011). As a result of the ubiquitous expression of GRs across the body, the CSs show multiple organ sites of activity (both desired and undesired), of which the commonly occurring dose- and time-dependent side effects, including growth retardation, metabolic disturbances, and osteoporosis (Saag et al., 1994), often compromise the overall therapeutic effects and limit the sustained use of CSs.

Prednisolone (PNL) and prednisone (PN) are among the top widely prescribed oral CSs (Overman et al., 2013). PNL is the biologically active substance, whereas PN is both the prodrug

[^1]and inactive metabolite of PNL, with their interconversion mediated by $11 \beta$-hydroxysteroid dehydrogenases ( $11 \beta$-HSDs) (Raza et al., 2010). The pharmacokinetics (PK) of PNL is extremely complex, with dose dependence exhibited in both animals (Frey et al., 1980; Rocci and Jusko, 1981; Boudinot and Jusko, 1986) and humans (Rose et al., 1981). PNL binds to corticosteroid-binding globulin (CBG) with high affinity and low capacity, whereas it binds to albumin with low affinity and high capacity (Rocci et al., 1980). In humans, the dosedependent increase in plasma clearance ( $C L$ ) and volume of distribution of PNL at doses over 20 mg is attributed to the saturable binding to CBG (Rose et al., 1981; Frey and Frey, 1990; Barth et al., 1992). A decreasing trend of $C L$ and volume of distribution with dose occurred in humans at very high doses of PNL (Derendorf et al., 1985), which may be explained by saturable elimination mechanisms (e.g., saturable conversion from PNL to PN and saturable renal elimination) (Frey et al., 1980; Legler et al., 1982). The PK of PNL in rats, when calculated based on total- or free-plasma concentrations, exhibits nonlinear distribution and elimination, with decreased $C L$ and volume of distribution at steady state $\left(V_{s s}\right)$ and increased free fraction with increasing doses (Boudinot et al., 1986; Boudinot and Jusko, 1986; Huang and Jusko, 1990). Capacity-limited binding to CBG alone cannot account for the decrease in $C L$ and $V_{s s}$, as these are opposite to predictions that these parameters would increase with increased availability of free drug; thus saturable elimination and tissue distribution might be involved. The tissue distribution of PNL was assessed in rabbits, and the steady-state (SS) tissue/unbound-plasma concentration ratios were linear for all the examined tissues except the liver (Khalafallah and Jusko, 1984b); consistent results were obtained using rabbit tissue slices under in vitro conditions (Khalafallah and Jusko, 1984a).

Considering their clinical importance and the fact that CSs exert their pharmacologic actions as a direct consequence of their presence in numerous target tissues (Melby, 1977), it is important to understand the whole-body disposition characteristics of PNL. This requires the direct examination of drug concentration in tissues. Physiologically based pharmacokinetics (PBPK) modeling, which is based on physiologic tissue sizes and blood/plasma flow rates, allows characterization of tissue distribution in a diverse array of situations and also offers a way to extrapolate tissue concentrations from preclinical species to humans (Gerlowski and Jain, 1983; Sager et al., 2015).

This work characterizes the diverse complexities in disposition of PNL and PN in rats using a combination of experimental, whole-body PBPK modeling, and in silico approaches.

## Materials and Methods

Reagents and Chemicals. Prednisolone hemisuccinate sodium salt, prednisolone (purity $\geq 99 \%$ ), prednisone (purity $\geq 98 \%$ ), liquid chromatography/mass spectrometry-grade acetonitrile, methanol and high-performance liquid chromatography-grade formic acid, and phosphoric acid were purchased from Sigma-Aldrich (St Louis, MO). Prednisolone-d8 [internal standard (IS) for prednisolone, purity $\geq 97.71 \%$ ] and prednisone-d8 (IS for prednisone, purity $\geq 98 \%$ ) were obtained from Toronto Research Chemicals (Toronto, ON, Canada). Milli-Q water (Millipore Corporation, Bedford, MA) was used.

Animals. Twenty-nine healthy male Wistar rats aged 5 to 6 weeks (weighing 225-249 g) were purchased from Envigo (Chicago, IL) and housed individually in the University Laboratory Animal Facility under controlled conditions with free access to water and food. All rats were acclimated for 1 week before the experiments. The research protocol adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and was approved by the University at Buffalo Institutional Animal Care and Use Committee.

Experimental Design. The prednisolone dosing solution was prepared freshly as the hemisuccinate sodium salt in sterile saline and filtered through $0.22-\mu \mathrm{m}$ filters before use. The drug was administered subcutaneously in a volume of $1 \mathrm{ml} / \mathrm{kg}$.

PNL was first infused subcutaneously to two rats through Alzet osmotic pumps at a concentration of $336 \mathrm{mg} / \mathrm{ml}$ at a rate of $8 \mu \mathrm{l} / \mathrm{h}$ for 7 hours to achieve SS. Animals were then sacrificed by exsanguination. Whole blood was drained from the abdominal aorta into EDTAcontaining syringes. The 0.5 ml of whole blood was aliquoted, and the remaining blood was centrifuged (2000g, 15 minutes) immediately to obtain plasma samples. Various tissues [heart, liver, spleen, lung, kidney, muscle (gastrocnemus), skin, fat, brain, bone (tibia), intestine] were collected, rinsed in ice-cold PBS, and gently blotted, weighed, and immediately frozen in liquid nitrogen.

A second group of rats were given a subcutaneous bolus dose of $50 \mathrm{mg} / \mathrm{kg}$ prednisolone and sacrificed by exsanguination at $0.25,0.5,1$, $2,4,6,8,12$, and 24 hours ( $n=3$ per time point). The following procedures for tissue collection were the same as those described in the infusion study. To assess the unbound concentrations of PNL and PN in plasma, an aliquot of plasma samples was subject to ultrafiltration using Centrifree micropartition devices (Millipore Corporation) with a $30-\mathrm{kDa}$ molecular mass cutoff filter. Aliquots of plasma samples (1 ml ) were added into the ultrafiltration devices and incubated for 30 minutes at $37^{\circ} \mathrm{C}$. Then the samples were centrifuged at 2000 g for 20 minutes $\left(37^{\circ} \mathrm{C}\right)$ to obtain ultrafiltrates for measurement of free PNL plasma concentrations. Tissues were powdered under liquid nitrogen before drug extraction. All samples were stored at $-80^{\circ} \mathrm{C}$ before further analysis.

Drug Analysis. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was established to simultaneously determine the concentrations of PNL and PN in each of the collected samples. The LC-MS/MS system consisted of Shimadzu (Kyoto, Japan) modules, including a binary pump, a degasser, an autosampler, an column oven, and an Applied Biosystems (Foster City, CA) PE/ SCIEX API3000 mass spectrometer equipped with a turbo ion spray interface. Sample separations were achieved on a HydroBond AQ C8 reverse phase column $(2.1 \times 150 \mathrm{~mm}$, particle size $3 \mu \mathrm{~m}$; MAC-MOD Analytical, Inc., Chadds Ford, PA). The mobile phase consisted of eluent A [water/methanol (95:5, v/v) containing $0.1 \%$ formic acid] and eluent B [methanol containing $0.1 \%$ formic acid] and was pumped at a flow rate of $0.2 \mathrm{ml} / \mathrm{min}$ with a gradient elution. The gradient profile was: 0-0.5 minutes, $30 \% \mathrm{~B}$; a linear increase to $70 \% \mathrm{~B}$ from 0.5 to 6.5 minutes; a linear increase to $95 \%$ B over 0.1 minutes; $95 \%$ B for 5.4 minutes; a linear decrease to $30 \%$ B over 0.1 minutes; $30 \%$ B for 3.9 minutes; and stop at 16.00 minutes. The mass spectrometer was operated in the positive ion mode for the detection of ion transitions at mass-to-charge ratio 361.2/343.2 (prednisolone), 359.3/341.3 (prednisone), 369.4/351.3 (prednisolone-d8), and 367.3/349.5 (prednisone-d8). The LC-MS/MS system was controlled by Analyst software version 1.4 (Applied Biosystems SCIEX) for data acquisition and analysis.

Linearity was achieved over a concentration range of $1-2000 \mathrm{ng} / \mathrm{ml}$ for all samples. The coefficients of variation (CV\%) for intraday and interday accuracies and precisions were within $\pm 15 \%$. The lower limit of quantification (LLOQ) was $1 \mathrm{ng} / \mathrm{ml}$ for both PNL and PN. The recovery of the sample pretreatment method approached $100 \%$. The PNL and PN in various matrices during freeze-thaw cycles and longterm storage at $4^{\circ} \mathrm{C}$ or a lower temperature were known to be stable (Difrancesco et al., 2007; Methlie et al., 2013; Yao et al., 2020).

Preparation of Plasma and Ultrafiltrate Samples. Plasma and ultrafiltrate samples $(100 \mu \mathrm{l})$ were spiked with $10 \mu \mathrm{l}$ of IS working
solutions ( $300 \mathrm{ng} / \mathrm{ml}$ ) and $100 \mu \mathrm{l}$ of $4 \%$ phosphoric acid. The mixture was vortexed for 30 seconds and centrifuged at $13,000 \mathrm{~g}$ for 10 minutes $\left(4^{\circ} \mathrm{C}\right)$. A $190-\mu \mathrm{l}$ aliquot of the supernatant was then subject to solidphase extraction (SPE) using Oasis HLB 1 cc $(30 \mathrm{mg})$ cartridges (Waters Corporation, Milford, MA). Prior to extraction, each cartridge was conditioned using 1 ml of methanol and twice followed by 1 ml of water using a Vac Elut SPS24 solid-phase extraction manifold (Varian, Palo Alto, CA). The cartridges were washed twice with $500 \mu \mathrm{l}$ of water containing $5 \%(\mathrm{v} / \mathrm{v})$ methanol and eluted thrice with $500 \mu \mathrm{l}$ of methanol. The eluates were dried under nitrogen for 30 minutes and reconstituted with $100 \mu \mathrm{l}$ of methanol/water (30:70 v/v). The mixture was vortexed for 30 seconds and centrifuged ( $13,000 \mathrm{~g}, 10$ minutes, $4^{\circ} \mathrm{C}$ ). Finally, $10 \mu \mathrm{l}$ of the supernatant was injected into the LC-MS/MS for analysis.

Preparation of Whole-Blood and Tissue Samples. The PNL and PN concentrations in whole-blood and tissue samples were pretreated using a slightly modified method. Briefly, whole-blood samples were directly homogenized at $4^{\circ} \mathrm{C}$ using a PRO-200 BIO-GEN homogenizer (ProScientific, Oxford, CT), and tissue samples were homogenized in PBS at a 3-fold dilution using a bullet blender (Next Advance, Inc., Troy, NY) at $4^{\circ} \mathrm{C}$. Whole-blood and tissue homogenates $(100 \mu \mathrm{l})$ were spiked with $10 \mu \mathrm{l}$ of IS working solutions ( $300 \mathrm{ng} / \mathrm{ml}$ ) and $980 \mu$ l of methanol. The mixture was vortexed for 30 seconds and centrifuged at $14,000 \mathrm{~g}$ for 20 minutes $\left(4^{\circ} \mathrm{C}\right)$. A $900-\mu \mathrm{l}$ aliquot of the supernatant was then evaporated to dryness. The residue was reconstituted with methanol ( $50 \mu \mathrm{l}$ ) and vortexed twice for 30 seconds and then diluted with water ( $450 \mu \mathrm{l}$ ) and vortexed for another 30 seconds. A $450-\mu \mathrm{l}$ aliquot of the mixture was acidified with $4 \%$ phosphoric acid $(450 \mu \mathrm{l})$ and centrifuged at $13,000 \mathrm{~g}$ for 10 minutes $\left(4^{\circ} \mathrm{C}\right)$. An $850 \mu \mathrm{l}$ aliquot of the supernatant was subject to SPE using preconditioned Oasis PRiME HLB 1 cc ( 30 mg ) cartridges (Waters Corporation). The remaining SPE extraction procedures were similar to those described for the plasma samples except that the analytes were eluted $2 \times$ with $500 \mu$ l of acetonitrile/methanol ( $90: 10, \mathrm{v} / \mathrm{v}$ ). The eluates were dried under nitrogen for 30 minutes and reconstituted with $150 \mu \mathrm{l}$ of methanol/water (30:70, v/v). The mixture was vortexed for 30 seconds and centrifuged ( $13,000 \mathrm{~g}, 10$ minutes, $4^{\circ} \mathrm{C}$ ). Finally, $10 \mu \mathrm{l}$ of the supernatant was injected into the LC-MS/MS for analysis.

Blood-to-Plasma Ratio ( $\boldsymbol{R}_{\boldsymbol{B}}$ ). The blood-to-plasma concentration ratio ( $R_{B}$ ) was obtained using the measured blood $\left(C_{B l}\right)$ and plasma $\left(C_{p}\right)$ concentrations of PNL from the subcutaneous bolus and the subcutaneous SS infusion studies in male rats:

$$
\begin{equation*}
R_{B}=\frac{C_{B l}}{C_{p}} \tag{1}
\end{equation*}
$$

The concentration of PNL in red blood cells (RBCs) was calculated according to:

$$
\begin{equation*}
C_{R B C}=\frac{\left[C_{B l}-C_{p} \cdot(1-H c t)\right]}{H c t} \tag{2}
\end{equation*}
$$

in which $C_{R B C}$ is the concentration of PNL in RBCs, and $H c t$ is the hematocrit of male Wistar rats (0.45) (Kampfmann et al., 2012).

Correction of Tissue Concentrations. The concentrations of PNL and PN in all measured tissues were corrected for residual blood using:

$$
\begin{gather*}
C_{T}=\frac{C_{T(\text { meas })} \cdot V_{T(\text { meas })}-C_{B l} \cdot V_{T(\text { meas })} \cdot F_{v v}}{V_{T}}  \tag{3}\\
V_{T}=V_{T(\text { meas })}-V_{T(\text { meas })} \cdot F_{v v} \tag{4}
\end{gather*}
$$

in which $C_{T}$ and $C_{T(\text { meas })}$ are the residual blood-corrected and measured tissue concentrations; $V_{T(\text { meas })}$ is the measured or estimated tissue volume; $V_{T}$ is the tissue vascular-corrected volume; and $F_{v v}$ is the fractional vascular volume of blood trapped in tissues, which was obtained from the literature concentrations (Bernareggi and Rowland, 1991). The $C_{T}$ and $V_{T}$ values were used for further model analysis.

Tissue-to-Plasma Partition Coefficients. The values of $K_{p}$ were calculated and compared using several methods:

1. Calculation from the SS plasma $\left(C_{P, s s}\right)$ and tissue $\left(C_{T, s s}\right)$ concentrations in the subcutaneous infusion study. For nonelimination organs:

$$
\begin{equation*}
K_{p}=\frac{C_{T, s s}}{C_{P, s s}} \tag{5}
\end{equation*}
$$

For elimination organs (liver and kidney):

$$
\begin{align*}
K_{p, \text { Liver }} & =\frac{C_{l i v e r, s s}}{C_{P, s s}} \cdot\left(\frac{Q_{\text {liver }}+C L_{\text {int }}}{Q_{\text {liver }}}\right)  \tag{6}\\
K_{p, \text { Kidney }} & =\frac{C_{\text {kidney }, s s}}{C_{P, s s}} \cdot\left(\frac{Q_{\text {kidney }}+C L_{k}}{Q_{\text {kidney }}}\right) \tag{7}
\end{align*}
$$

in which $Q_{\text {liver }}$ and $Q_{\text {kidney }}$ are the liver and kidney blood flows, and $C L_{\text {int }}$ and $C L_{k}$ are the hepatic intrinsic and renal (metabolic) clearances.
2. Apparent $K_{p}$ values were calculated from the PBPK modelpredicted data (detailed description will follow):

$$
\begin{equation*}
K_{p}=\frac{A U C_{T, P B P K}}{A U C_{P, P B P K}} \tag{8}
\end{equation*}
$$

in which $A U C_{T, P B P K}$ and $A U C_{P, P B P K}$ are the area under the curve (AUC) of the PBPK model-predicted tissue and plasma curves. The same correction method for liver and kidney $K_{p}$ as described in eqs. 6 to 7 was applied here based on eq. 8 .
3. In silico methods implemented by the GastroPlus PBPK Simulator (version 9.6.2; Simulations Plus Inc., Lancaster, CA) were also used for $K_{p}$ prediction. Published methods (Poulin and Theil, 2002a,b; Berezhkovskiy, 2004; Rodgers and Rowland, 2006) for neutral compounds were used and are listed as Methods 1-3.

Plasma Protein Binding of PNL. The concentration-dependent plasma protein binding of PNL in various species owing to saturable binding to CBG has been well investigated previously (Sandberg et al., 1957; Rocci et al., 1980; Rocci and Jusko, 1981; Boudinot and Jusko, 1984, 1986). The binding equation is:

$$
\begin{equation*}
C_{b}=\frac{N_{C} K_{C} P_{C} \cdot C_{f}}{\left(1+K_{C} \cdot C_{f}\right)}+N_{A} K_{A} P_{A} \cdot C_{f} \tag{9}
\end{equation*}
$$

in which $C_{b}$ and $C_{f}$ are the bound- and free-plasma PNL concentrations; $N$ is the number of binding sites; $K$ is the association constant; $P$ is the plasma protein concentration; and subscripts $C$ and $A$ refer to CBG and albumin.

The total ( $C_{p}$ ) plasma concentrations can be described as:

$$
\begin{equation*}
C_{p}=\frac{N_{C} K_{C} P_{C} \cdot C_{f}}{\left(1+K_{C} \cdot C_{f}\right)}+N_{A} K_{A} P_{A} \cdot C_{f}+C_{f} \tag{10}
\end{equation*}
$$

Rearrangement of eq. 10 gives:
$C_{f}=\frac{-\left(N_{A} K_{A} P_{A}+N_{C} K_{C} P_{C}-K_{C} C_{p}+1\right)+\sqrt{\left(N_{A} K_{A} P_{A}+N_{C} K_{C} P_{C}-K_{C} C_{p}+1\right)^{2}+4\left(K_{C}+N_{A} K_{A} P_{A} K_{C}\right) C_{p}}}{2\left(K_{C}+N_{A} K_{A} P_{A} K_{C}\right)}$

The CBG-free PNL plasma concentrations $\left(C_{f C B G}\right)$ were determined as the sum of albumin-bound $\left(N_{A} K_{A} P_{A} \cdot C_{f}\right)$ and free $\left(C_{f}\right)$ PNL plasma concentrations at various total- and free-drug concentrations:

$$
\begin{equation*}
C_{f C B G}=N_{A} K_{A} P_{A} \cdot C_{f}+C_{f} \tag{12}
\end{equation*}
$$

Combining eqs. 11 and 12, the CBG-free plasma concentration can be calculated from total plasma concentration as:
$C_{f C B G}=\left(N_{A} K_{A} P_{A}+1\right)$
$\frac{-\left(N_{A} K_{A} P_{A}+N_{C} K_{C} P_{C}-K_{C} C_{p}+1\right)+\sqrt{\left(N_{A} K_{A} P_{A}+N_{C} K_{C} P_{C}-K_{C} C_{p}+1\right)^{2}+4\left(K_{C}+N_{A} K_{A} P_{A} K_{C}\right) C_{p}}}{2\left(K_{C}+N_{A} K_{A} P_{A} K_{C}\right)}$

Combining eqs. 10 and $12, C_{p}$ can be computed from $C_{f C B G}$ by:

$$
\begin{equation*}
C_{p}=C_{f C B G} \cdot\left(\frac{N_{C} K_{C} P_{C}}{\left(1+\frac{K_{C} \cdot C_{P C B G}}{\left(N_{A} K_{A} P_{A}+1\right)}\right) \cdot\left(N_{A} K_{A} P_{A}+1\right)}+1\right) \tag{14}
\end{equation*}
$$

Tissue Binding of PNL. The nonlinear tissue binding of PNL was expressed by:

$$
\begin{equation*}
C_{b T}=\frac{B_{\max } \cdot C_{u T}}{K_{d}+C_{u T}} \tag{15}
\end{equation*}
$$

in which $C_{b T}$ and $C_{u T}$ are the bound and free concentrations of PNL in tissue; $B_{\text {max }}$ is the binding capacity of the tissue ( $\mathrm{ng} / \mathrm{ml}$ ); and $K_{d}$ is the equilibrium dissociation constant. Therefore, total tissue concentration $\left(C_{T}\right)$ can be described by:

$$
\begin{equation*}
C_{T}=C_{u T}+\frac{B_{\max } \cdot C_{u T}}{K_{d}+C_{u T}} \tag{16}
\end{equation*}
$$

Assuming only CBG-free drug has access to tissues, and tissue-free concentration ( $C_{u T}$ ) is the same as the CBG-free drug concentration in tissue venous plasma ( $C_{f C B G, T V}$ ), then the tissue-to-CBG free-plasma concentration ratio ( $K_{p u}$ ) is given by:

$$
\begin{equation*}
K_{p u}=\frac{C_{T}}{C_{f C B G, T V}}=\frac{C_{T}}{C_{u T}} \tag{17}
\end{equation*}
$$

Combining eqs. 16 and 17 yields:

$$
\begin{equation*}
K_{p u}=\frac{-\left(C_{T}-K_{d}-B_{\max }\right)+\sqrt{\left(C_{T}-K_{d}-B_{\max }\right)^{2}+4 K_{d} C_{T}}}{2 K_{d}} \tag{18}
\end{equation*}
$$

PBPK Model Development and Modeling Strategy. The proposed PBPK model of prednisolone (Fig. 1) consists of two plasma/ blood compartments (arterial and venous plasma) and 12 tissue compartments (liver, kidney, lung, heart, spleen, intestine, muscle, fat, bone, skin, brain, and remainder). Tissues accounting for about $90 \%$ of total body weight were evaluated. Unmeasured tissues were lumped as a remainder compartment to attain whole-body mass balance. The model was based upon the following observations and assumptions: (1) tissue concentrations are considered to be in instant equilibrium with the CBG-free drug in venous plasma, which is assumed to be in equilibrium with the tissue-unbound concentration; (2) circulatory transport occurs by plasma/blood flow; (3) brain has two subcompartments (vascular and extravascular) with a presumed P-glycoprotein (P-gp)-mediated efflux process; (4) the distribution of PNL into fat and bone is limited to the interstitial space; (5) only CBGfree PNL is available for tissue distribution and elimination; (6) the elimination of PNL occurs from liver and kidney; (7) tissue binding of PNL is saturable; and (8) interconversion of PNL and PN was in continual rapid equilibrium. Physiologic parameters, including tissue weights and blood flow rates to different organs, were fixed to literature values in the PBPK model (Table 1) (Shah and Betts, 2012).

The model differential equations are listed below, with all initial conditions set to 0 except for the dosing depot in eq. 33.

Rates of change of drug concentration in tissues. Except for lung, liver, kidney, brain, fat, bone, arterial, and venous plasma, the mass balance in each tissue was expressed as:

$$
\begin{gather*}
V_{T, i} \cdot \frac{d C_{T, i}}{d t}=Q_{i} \cdot\left(C_{f C B G, a r t}-C_{f C B G, T V, i}\right)  \tag{19}\\
\text { where } \quad C_{f C B G, T V, i}=\frac{C_{T, i}}{K_{p u, i}} \tag{20}
\end{gather*}
$$

in which $V_{T, i}, Q_{i}, C_{T, i}$, and $K_{p u, i}$ are volume, blood flow, PNL concentration, and unbound partition coefficient for tissue $i ; C_{f C B G, T V, i}$ is the CBGfree venous plasma concentration leaving tissue $i$; and $C_{f C B G, ~ a r t ~}$ is the


Fig. 1. Whole-body PBPK model scheme for prednisolone and prednisone disposition in rats. Parameters and symbols are defined in the text and tables. Lines with arrows indicate blood flows and drug transport. Each box represents one tissue compartment as indicated by the label.

TABLE 1
Physiologic parameters of tissues in 294-g male rats

| Tissue | Volume $(V, \mathrm{ml})$ | Blood Flow $(Q, \mathrm{ml} / \mathrm{h})$ |
| :--- | :---: | :---: |
| Lung | $0.96^{a}$ | $5548.6^{b}$ |
| Brain | $1.41^{a}$ | $123.1^{b}$ |
| Brain capillary | $0.02^{a}$ |  |
| Heart | $0.87^{a}$ | $285^{b}$ |
| Intestine | $8.14^{b}$ | $1047.7^{b}$ |
| Spleen | $0.43^{a}$ | $336.8^{b}$ |
| Kidney | $1.81^{a}$ | $687.1^{b}$ |
| Muscle | $127.52^{b}$ | $1743.1^{b}$ |
| Liver | $9.94^{a}$ | $1424.4^{c}$ |
| Liver artery | NA | $39.8^{b}$ |
| Skin | $52.17^{b}$ | $376.2^{b}$ |
| Bone | $21.61^{b}$ | $115.7^{b}$ |
| Bone ISF | $4.02^{b}$ |  |
| Fat | $34.44^{b}$ | $422.8^{b}$ |
| Fat ISF | $5.85^{b}$ |  |
| Arterial blood | $5.76^{b}$ | $5548.6^{b}$ |
| Venous blood | $11.52^{b}$ | $5548.6^{b}$ |
| Remainder | $17.08^{c}$ | $371.2^{c}$ |

[^2]CBG-free PNL concentration in arterial plasma, which can be calculated from the total arterial plasma concentration $\left(C_{a r t}\right)$ using eq. 13.

Rates of change of drug concentration in the venous and arterial plasma. The total PNL concentration in venous plasma leaving tissue $i$ ( $C_{T V, i}$ ) and entering the venous and arterial plasma pools can be computed according to eqs. 14 and 20 as:

$$
\begin{equation*}
C_{T V, i}=\frac{C_{T, i}}{K_{p u, i}} \cdot\left(\frac{N_{C} K_{C} P_{C}}{\left(1+\frac{K_{C} \cdot C_{T, i}}{K_{p u, i}\left(N_{A} K_{A} P_{A}+1\right)}\right) \cdot\left(N_{A} K_{A} P_{A}+1\right)}+1\right) \tag{21}
\end{equation*}
$$

Therefore, the rates of change of PNL concentration in venous and arterial plasma pools were:

$$
\begin{align*}
V_{\text {ven }} \cdot \frac{d C_{\text {ven }}}{d t}= & \text { Input }+\left(Q_{l i v e r} \cdot C_{T V, l i v e r}+Q_{k i d n e y} \cdot C_{T V, \text { kidney }}\right. \\
& +Q_{h e a r t} \cdot C_{T V, \text { heart }}+Q_{\text {muscle }} \cdot C_{T V, \text { muscle }}+Q_{\text {fat }} \cdot C_{T V, \text { fat }} \\
& +Q_{\text {bone }} \cdot C_{T V, \text { bone }}+Q_{s k i n} \cdot C_{T V, \text { skin }} \\
& \left.+Q_{\text {remainder }} \cdot C_{T V, \text { remainder }}\right)+Q_{\text {brain }} \cdot C_{\text {brain, cap }}-Q_{l u n g} \cdot C_{v e n} \tag{22}
\end{align*}
$$

$$
\begin{equation*}
V_{a r t} \cdot \frac{d C_{a r t}}{d t}=Q_{\text {lung }} \cdot\left(C_{T V, l u n g}-C_{a r t}\right) \tag{23}
\end{equation*}
$$

Rate of change of drug concentrations in the lung can be computed as:

$$
\begin{equation*}
V_{\text {lung }} \cdot \frac{d C_{\text {lung }}}{d t}=Q_{\text {lung }} \cdot\left(C_{f C B G, v e n}-\frac{C_{\text {lung }}}{K_{\text {pu,lung }}}\right) \tag{24}
\end{equation*}
$$

in which the CBG-free venous plasma concentration of PNL ( $C_{f C B G, v e n}$ ) was computed from the total venous plasma concentration $\left(C_{v e n}\right)$ according to eq. 13 and $Q_{\text {lung }}$ is the lung blood flow corresponding to the cardiac blood flow.
Rate of change of drug concentrations in the liver can be computed as:

$$
\begin{align*}
V_{\text {liver }} \cdot \frac{d C_{\text {liver }}}{d t}= & Q_{\text {liver,art }} \cdot C_{f C B G, \text { art }}+Q_{\text {spleen }} \cdot \frac{C_{\text {spleen }}}{K_{p u, \text { spleen }}} \\
& +Q_{\text {intestine }} \cdot \frac{C_{\text {intestine }}}{K_{\text {pu,intestine }}}-Q_{\text {liver }} \cdot \frac{C_{\text {liver }}}{K_{\text {pul,liver }}}-C L_{\text {int }} \cdot \frac{C_{\text {liver }}}{K_{\text {pu,liver }}} \tag{25}
\end{align*}
$$

in which $Q_{\text {liver }}=Q_{\text {liver , art }}+Q_{\text {intestine }}+Q_{\text {spleen }}$.
Rate of change of drug concentration in the kidney is:

$$
\begin{equation*}
V_{k i d n e y} \cdot \frac{d C_{k i d n e y}}{d t}=Q_{k i d n e y} \cdot\left(C_{f C B G, \text { art }}-\frac{C_{k i d n e y}}{K_{\text {pu,kidney }}}\right)-C L_{k} \cdot \frac{C_{\text {kidney }}}{K_{\text {pu,kidney }}} \tag{26}
\end{equation*}
$$

Rates of change of drug concentration in bone and fat can be computed with:

$$
\begin{align*}
V_{\text {bone_ISF }} \cdot \frac{d C_{\text {bone }}}{d t} & =Q_{\text {bone }} \cdot\left(C_{f C B G, a r t}-\frac{C_{\text {bone }}}{K_{\text {pu bone }}}\right)  \tag{27}\\
V_{\text {fat_ISF }} \cdot \frac{d C_{\text {fat }}}{d t} & =Q_{\text {fat }} \cdot\left(C_{f C B G, a r t}-\frac{C_{f a t}}{K_{\text {pufat }}}\right) \tag{28}
\end{align*}
$$

in which $V_{\text {bone_ISF }}$ and $V_{\text {fat_ISF }}$ are the interstitial volumes of bone and fat.

The measured PNL concentrations in bone ( $C_{\text {bone(meas }}$ ) and fat ( $C_{\text {fat }}$ meas) $)$ were modeled as:

$$
\begin{align*}
C_{\text {bone(meas) })} & =\frac{C_{\text {bone }} \cdot V_{\text {bone_ISF }}}{V_{\text {bone }}}  \tag{29}\\
C_{\text {fat(meas) })} & =\frac{C_{\text {fat }} \cdot V_{\text {fat_ISF }}}{V_{\text {fat }}} \tag{30}
\end{align*}
$$

Rates of change of drug concentration in brain capillary and brain can be computed as:

$$
\begin{align*}
& V_{b r a i n ~ c a p} \cdot \frac{d C_{b r a i n ~ c a p ~}}{d t}= Q_{b r a i n} \cdot\left(C_{a r t}-C_{b r a i n ~ c a p}\right) \\
&-C L_{u p} \cdot\left(C_{f C B G, b r a i n ~ c a p}-\frac{C_{b r a i n}}{K_{p u, b r a i n}}\right) \\
&+C L_{e f f} \cdot \frac{C_{b r a i n}}{K_{p u, b r a i n}}  \tag{31}\\
& V_{b r a i n} \cdot \frac{d C_{b r a i n}}{d t}=C L_{u p} \cdot\left(C_{f C B G, b r a i n ~ c a p}-\frac{C_{b r a i n}}{K_{p u, b r a i n}}\right)-C L_{e f f} \cdot \frac{C_{b r a i n}}{K_{p u, b r a i n}} \tag{32}
\end{align*}
$$

in which $C L_{u p}$ and $C L_{\text {eff }}$ are the bidirectional uptake and unidirectional efflux clearance of PNL in brain; $C_{f C B G \text {,brain cap }}$ is the CBG-free PNL concentration in brain capillary that can be calculated from total brain capillary concentration ( $C_{b r a i n}$ cap ) using eq. 13.

The subcutaneous injection was treated as a bolus into a dosing $\operatorname{depot}\left(A_{\text {depot }}\right)$ as described by:

$$
\begin{equation*}
\frac{d A_{d e p o t}}{d t}=-k a \cdot A_{d e p o t}, \quad A_{d e p o t, 0}=D o s e \cdot F \tag{33}
\end{equation*}
$$

in which $A_{\text {depot }}$ is the amount of drug to be absorbed into the venous plasma; $k a$ is the first-order absorption rate constant; $A_{\text {depot }, 0}$ is the initial condition of $A_{\text {depot }}$; and $F$ is the bioavailability of subcutaneous dose calculated to be 0.65 from literature-reported intravenous data in rats (Huang and Jusko, 1990).

Therefore, the Input function in eq. 22 is:

$$
\begin{equation*}
\text { Input }=k a \cdot A_{\text {depot }} \tag{34}
\end{equation*}
$$

Given the rapid appearance of PN in plasma and tissues upon PNL administration and often parallel profiles, the interconversion between PNL and PN was assumed to be in continual rapid equilibrium. The concentration of PN in tissue $i\left(C_{P N, i}\right)$, including lung, kidney, muscle, skin, liver, and brain, was modeled as a linear fraction of PNL concentration in the corresponding tissue $\left(C_{P N L, i}\right)$ :

$$
\begin{equation*}
C_{P N, i}=f_{m, i} \cdot C_{P N L, i} \tag{35}
\end{equation*}
$$

in which $f_{m, i}$ is the metabolite/drug ratio of PN/PNL for tissue $i$.

The PN concentration in arterial plasma, spleen, heart, intestine, bone, and fat was fitted to a Michaelis-Menten type equation:

$$
\begin{equation*}
C_{P N, i}=\frac{P N_{\max , i} \cdot C_{P N L, i}}{P N L_{50, i}+C_{P N L, i}} \tag{36}
\end{equation*}
$$

in which $P N_{\text {max }, i}$ is the maximal PN concentration achievable, and $P N L_{50, i}$ is the PNL concentration achieving $50 \% P N_{\text {max }, i}$.

The measured PNL concentrations in arterial blood were described as:

$$
\begin{equation*}
C_{P N L, b l}=R_{B} \cdot C_{P N L, p} \tag{37}
\end{equation*}
$$

in which $C_{P N L, b l}$ and $C_{P N L, p}$ are the PNL concentrations in arterial blood and plasma.

The relationship between systemic $\left(C L_{s}\right)$ and intrinsic clearances $\left(C l_{\text {int }}\right)$ of CBG-free PNL is:

$$
\begin{equation*}
C L_{s}=\frac{Q_{\text {liver }} \cdot C L_{\text {int }}}{Q_{\text {liver }}+C L_{\text {int }}} \tag{38}
\end{equation*}
$$

A similar calculation related renal systemic clearance to kidney blood flow ( $Q_{\text {kidney }}$ ) and renal intrinsic clearance ( $C L k$ ).

Model Fitting. The blood-to-plasma concentration ratio ( $R_{B}$ ) and the metabolite/drug ratio-related parameters $\left(f_{m}, P N_{\max }\right.$, and $\left.P N L_{50}\right)$ were estimated by Orthogonal Regression (Package "onls") in R (version 3.4.3) according to eqs. 1, 35, and 36 and fixed in the PBPK model. Naïve-pooled plasma and tissue time-concentration data from all rats were analyzed jointly using the established PBPK model. The plasma protein binding parameters ( $N_{C} K_{C} P_{C}, N_{A} K_{A} P_{A}$, and $K_{C}$ ) were fixed to literature-reported values (Boudinot and Jusko, 1986), with the estimation of the subcutaneous first-order absorption rate constant ( $k a$ ), hepatic intrinsic ( $C L_{i n t}$ ) and renal ( $C L_{k}$ ) clearance, tissue binding parameters ( $K_{d}$ and $B_{\max }$ ), the bidirectional uptake ( $C L_{u p}$ ) and unidirectional efflux ( $C L_{\text {eff }}$ ) clearance of PNL in brain, and metabolite/ drug ratio for liver ( $f_{\text {m_liver }}$ ). The whole-body PBPK-model fittings involved nonlinear regression using the maximum likelihood algorithm in ADAPT 5 (Biomedical Simulations Resource, Los Angeles, CA) (D' Argenio et al., 2009). The variance model used was:

$$
\begin{equation*}
V_{i}=\left(\sigma_{1}+\sigma_{2} \cdot Y_{i}\right)^{2} \tag{39}
\end{equation*}
$$

in which $V_{i}$ represents the variance of the $i$ th data point; $Y_{i}$ is the $i$ th model-predicted concentration; and $\sigma_{1}$ and $\sigma_{2}$ are variance model parameters. Model selection was based on the goodness-of-fit criteria, which included the Akaike Information Criterion, visual inspection of the fitted profiles, and CV\% of the parameter estimates.

The Adapt code for the PBPK model is provided in the Supplemental Materials.

## Results

Distribution of PNL between Blood Fractions. Figure 2A shows the relationship between the blood-to-plasma concentration ratio $\left(R_{B}\right)$ versus plasma concentrations of PNL from both the subcutaneous bolus and subcutaneous infusion studies. The combined average $R_{B}$ value calculated using eq. 1 was $0.703 \pm 0.10(n=18)$, which was very close to the estimated $R_{B}$ value by Orthogonal Regression ( 0.71 , Table 3 ). The calculated PNL concentrations in RBCs according to eq. 2 closely match the measured free drug in plasma (Fig. 2B), indicating limited entry of PNL into RBCs.

Plasma Protein Binding of PNL. The plasma protein binding of PNL in vivo was assessed by measuring the unbound PNL concentration in plasma samples collected at each time point after subcutaneous bolus dosing using ultrafiltration. The protein binding of PNL in rats was concentration-dependent, with a lower bound fraction ( $60 \%$ ) at higher total plasma
concentrations and $90 \%$ bound at low concentrations (Fig. 3A). The protein binding parameters of PNL in rats were similar across different studies (Rocci et al., 1980; Boudinot and Jusko, $1984,1986)$ and thus were applied to relate the bound and unbound PNL plasma concentrations using eq. 9. The model simulations were in very good agreement with the observed data (Fig. 3B). Therefore, these protein binding parameter values (Table 3) were fixed in the subsequent PBPK model to calculate CBG-free drug in plasma. This assumes that tissue uptake of PNL is not restricted to free drug but includes rapid dissociation from albumin. The PNL is bound to CBG with affinity that is several orders of magnitude higher than that of albumin. There is evidence that albumin-bound drug does not retard steroid diffusion into tissues owing to the rapid dissociation from albumin (Pardridge, 1981). Our previous study showed that administration of exogenous CBG significantly decreased the apparent $C L$ and $V_{s s}$ of PNL. The PK of methylprednisolone (MPL), which does not bind to CBG, did not change in rats (Ko et al., 1995). Therefore, we assumed that only CBG-free drug has access to tissues and is subject to elimination in the current PBPK model. In contrast, the protein binding of PN was essentially linear with an average bound fraction of $55 \%$ (Fig. 3A), which is consistent with previous observations in rabbit and human plasma (Ferry and Wagner, 1987).

Application of Whole-Body PBPK Model. The tissue-to-unbound-plasma concentration ratios ( $K_{p u}$ ) decreased in all tissues with CBG-free plasma concentrations obtained using eq. 13 (calculations not shown), indicating nonlinear tissue distribution. Therefore, nonlinear $K_{p u}$ functions for each tissue were incorporated into the PBPK model in the form of eq. 18.

The whole-body PBPK model was applied to jointly fit the concentration-time data of PNL and PN from plasma and all tissues after subcutaneous bolus of $50 \mathrm{mg} / \mathrm{kg}$ PNL. The observed and model-predicted PK profiles are shown in Fig. 4. The observed PNL concentrations in arterial blood were fitted using eq. 37 (Fig. 5). In general, this model described all profiles reasonably well with slight overestimation for muscle around the maximum concentration. All model parameters were estimated precisely with reasonable CV\% values (Table 3). The absorption of PNL after subcutaneous dosing was relatively fast, with an estimated $k a$ value of 2.59 hour $^{-1}$ producing immediate high concentrations. The concentrations of PNL in tissues increased rapidly, indicating that PNL distributes quickly within blood and between blood and tissues upon dosing. The PNL PK profiles in most tissues exhibited similar patterns with a dominant early decline phase and a slow terminal phase. The model-estimated binding equilibrium dissociation constant ( $K_{d}$ ) of PNL shared by all tissues was $3.01 \mathrm{ng} / \mathrm{ml}$ with highest binding observed in muscle $\left(\operatorname{Bmax}_{1}=\right.$ $690.5 \mathrm{ng} / \mathrm{ml}$ ), followed by liver, heart, intestine, and bone $\left(\right.$ Bmax $\left._{2}=188.5 \mathrm{ng} / \mathrm{ml}\right)$ and the lowest binding observed in kidney, lung, spleen, skin, fat, and brain ( $\left.\max _{3}=50.8 \mathrm{ng} / \mathrm{ml}\right)$. Clearances of CBG-free PNL were $1789 \mathrm{ml} / \mathrm{h}$ from liver ( $C L_{i n t}$ ) and $191.2 \mathrm{ml} / \mathrm{h}$ from kidney $\left(C L_{k}\right)$. Low brain PNL concentrations ( $K_{p, \text { Brain }}=0.05$, Table 4) in contrast to the expected high permeability $(\log P=1.62)$ were well captured by incorporating a presumed unidirectional active efflux process $\left(C L_{\text {eff }}=4.58 \mathrm{ml} / \mathrm{h}\right)$ based upon the bidirectional uptake process ( $C L_{u p}=0.13 \mathrm{ml} / \mathrm{h}$ ). The much higher $C L_{\text {eff }}$ value as compared


Fig. 2. Blood partitioning of prednisolone. (A) Blood-to-plasma concentration ratio ( $R_{B}$ ) vs. plasma concentration of PNL from the subcutaneous bolus (closed squares) and subcutaneous infusion (open squares) studies. (B) Calculated RBCs (closed triangles) and measured free plasma (open triangles) prednisolone concentrations in rats after a single $50-\mathrm{mg} / \mathrm{kg}$ s.c. dose of PNL. The dashed line indicates the mean value of all points.
with that of $C L_{u p}$ indicates that the limited brain distribution of PNL is strongly affected by the active efflux process. The PK profiles of PNL in fat and bone were reasonably characterized by assuming only the interstitial space was available for distribution.

Plasma and tissue PN concentrations at each time point after dosing were well characterized simply as a fraction of PNL concentration reflecting their rapid equilibrium (Fig. 4). The metabolite/drug ratio-related parameters $\left(f_{m}, P N_{\max }\right.$ and $P N L_{50}$ ) were fixed in the PBPK model to the estimated values from the individual organ data (Table 2). Orthogonal regression of the PN versus PNL relationships listed in Table 2 resulted in reasonable fittings but with inexplicable high relative standard error values. Using ordinary leastsquares regression in Adapt yielded CV\% values that were usually less than $30 \%$ but were less reliable fittings. Within the assessed concentration range, the conversion from PNL to PN, as defined by the PN/PNL ratio, exhibited saturable characteristics in plasma, heart, intestine, spleen, bone, and fat, with the lowest $P N L_{50}$ (PNL concentration at half-maximal saturation) in fat and bone and the highest in intestine. Although linear PN/PNL ratios were found for lung, kidney, muscle, skin, liver, brain, and liver, the linear ratio gave the highest $f_{m}$ for kidney ( 0.076 , Table 2) and the lowest for liver (0.01, Table 3).

The PNL $K_{p}$ values obtained using different methods are listed in Table 4. For methods based on in vivo data, the $K_{p}$ for liver and kidney were corrected for tissue elimination. The uncorrected liver and kidney $K_{p}$ values are also listed. The time- and concentration-averaged $K_{p}$ values calculated from
the PBPK model-fitted AUC values were generally around 1 and similar to those obtained from the SS infusion study and the in silico methods for most tissues except for brain and liver. The $K_{p, \text { Brain }}$ obtained by in silico methods were more than 10 -fold higher than those obtained from the in vivo data. The corrected $K_{p, L i v e r}$ (3.67) obtained from the SS infusion study was higher than those calculated by the other methods.

## Discussion

The PBPK model incorporating nonlinear plasma and tissue binding well characterized the disposition characteristics of PNL and its major metabolite PN in plasma and 11 tissues of rats after SS infusion and a $50-\mathrm{mg} / \mathrm{kg}$ s.c. bolus dose of PNL. The blood partitioning of PNL ( $R_{B}=0.703 / 0.71$ ) is comparable to that of dexamethasone (DEX) ( $R_{B}=0.664$ ) (Fig. 2A) (Song et al., 2020). The RBC uptake of PNL was limited to the unbound drug in plasma (Fig. 2B), as also found in humans (Araki et al., 1966) and rabbits (Khalafallah and Jusko, 1984b). Initial modeling was attempted based on blood PNL concentrations, but fittings were much better as presented. The model thus assumes that CBG-free drug is available for distribution from both plasma and RBCs.

Moderate binding to cellular constituents may account for tissue distribution of PNL, a neutral molecule. The tissue-to-unbound-plasma concentration ratios $\left(K_{p u}\right)$ declined with higher free-plasma concentrations, and the slow terminal decline of plasma and tissue concentrations required nonlinear tissue binding for best fittings. Preliminary model fittings without considering nonlinear tissue binding resulted


Fig. 3. Plasma protein binding of prednisolone and prednisone. (A) Bound fractions vs. total concentrations of prednisolone and prednisone. (B) Relationship of bound vs. unbound concentrations of prednisolone. Red open circles are PNL, and black closed circles are PN data. The curve depicts model simulations using eq. 9 with protein binding parameters values listed in Table 3.


Fig. 4. The prednisolone and prednisone concentration-time profiles in plasma and tissues after $50-\mathrm{mg} / \mathrm{kg}$ s.c. bolus dose of PNL in rats. Red open circles are PNL, and black closed circles are PN data. Curves depict PBPK model fittings of PNL (solid line) and PN (dashed line).
in more rapid decline of the terminal phases in all tissues to below the LLOQ by 4 hours after dosing. The rabbit exhibited linear distribution of PNL into tissues except liver; but these studies were performed over a small range of free-plasma concentrations ( $66-1213 \mathrm{ng} / \mathrm{ml}$ ) (Khalafallah and Jusko, 1984b).

The moderate lipid solubility ( $\log \mathrm{P}=1.62$ ) and molecular weight (360.4) allow for rapid equilibrium of PNL between blood and tissues, as indicated by the rapid increase in PNL concentrations in tissues (Figs. 4 and 5). Similar PNL profiles across tissues allowed use of one universal tissue binding equilibrium dissociation constant $\left(K_{d}\right)$ and three groups of binding capacities ( $B_{\max }$ ). The GRs, which are ubiquitously expressed in tissues and most abundant in the liver (Ballard et al., 1974), may contribute to the tissue binding of PNL. We have shown that the in vitro $K_{d}$ value for the PNL-GR interaction in liver is about 61 nM (Boudinot et al., 1986). Also, $11 \beta$-HSD, which interconverts biologically
active GCs (cortisol and PNL) with their inactive counterparts (cortisone and PN), may also contribute to tissue binding. There are two distinct $11 \beta$-HSD enzymes, of which $11 \beta$-HSD1 is a bidirectional enzyme but primarily an activator of GCs, whereas $11 \beta$-HSD2 unidirectionally inactivates GCs (Raza et al., 2010). The affinity constant $\left(K_{d}\right)$ of the weaker binding cortisol to human $11 \beta$-HSD2 is $25-55 \mathrm{nM}$ (Chapman et al., 2013). The PBPK model-estimated $K_{d}$ of PNL ( $3.01 \mathrm{ng} / \mathrm{ml}=$ $8.4 \mathrm{nM})$ is close to this and the GR $K_{d}$ value. Furthermore, using a highly purified rat plasma membrane free of CBG and GR, the presence of a GC-responsive site mediating highaffinity active uptake of PNL was demonstrated in rat liver (Lackner et al., 1998). Thus, there are several possible explanations for the nonlinear tissue distribution of PNL.

Several tissues, including brain, fat, and bone, exhibited relatively little drug uptake consistent with our finding for DEX (Song et al., 2020). PNL is a substrate for P-gp, which is highly expressed at the blood-brain barrier and hampers brain

## Arterial Blood



Fig. 5. The prednisolone concentration-time profiles in blood after $50-\mathrm{mg} / \mathrm{kg}$ s.c. bolus dose of PNL in rats. Red open circles are PNL data. Curves depict PBPK model fittings.
exposure of steroids (Karssen et al., 2002; Yates et al., 2003). Despite the moderate lipid solubility of PNL, the in vivo $K_{p, B r a i n}$ values ( $0.05 / 0.03$ ) were much lower than in silico values and those for other tissues. This difference was accounted for in the PBPK model by incorporating P-gp-mediated active efflux in brain. Although this phenomenon may reflect lower binding in the brain, our preliminary modeling without the P-gp-mediated efflux process showed an approximate 20 -fold overestimation of brain exposure even though the $B \max$ value was estimated to be low. This was also seen for DEX in rats ( $K_{p, B r a i n}=0.06$ ) (Song et al., 2020), another P-gp substrate (Ueda et al., 1992; Schinkel et al., 1995).
The concentrations of PNL in fat, in which drug uptake usually depends on lipid solubility, were low ( $K_{p, F a t}=0.17$ ), as was also found for DEX ( $K_{p, F a t}=0.15$ ) (Song et al., 2020). These results are in line with findings that the SS fat $K_{p}$ of PNL in rabbits was 0.13 (Khalafallah and Jusko, 1984b). This explains why volumes of distribution of PNL only increased modestly in obese men (Milsap et al., 1984) and rats (Nichols et al., 1989). Further, CBG is expressed in rat white adipose tissue, and the CBG layer surrounding white adipose tissue cells near the plasma membrane may act as a protective barrier limiting the access of GCs to adipocytes (del Mar Grasa et al., 2001).

The relatively low concentrations of PNL in bone ( $K_{p, \text { Bone }}=$ 0.22 ) are consistent with observations for DEX ( $K_{p, \text { Bone }}=0.23$ ) (Song et al., 2020). Why bone has such low values is not clear. Nevertheless, knowledge of factors responsible for steroid access to bone may be important for understanding their deleterious effects (Lukert and Raisz, 1990). The limited distribution of PNL into fat and bone was well described by assuming only interstitial space is accessible.

Hepatic and renal metabolism and renal excretion are responsible for PNL clearance (Caspi and Pechet, 1958; Vermeulen and Caspi, 1958; Glenn, 1959; Bailey and West, 1967; Rocci et al., 1981). The model-estimated CBG-free hepatic intrinsic clearance was high ( $1789 \mathrm{ml} / \mathrm{h}$ ), accounting for rapid conversion of PNL to PN. Using eq. 38 and the hepatic blood flow ( $1424 \mathrm{ml} / \mathrm{h}$ ), this converts to a systemic clearance of $793 \mathrm{ml} / \mathrm{h}$. For kidney, the systemic clearance

TABLE 2
PN/PNL ratios as determined by Orthogonal Regression in each tissue ${ }^{a}$

|  | Parameters ${ }^{\mathrm{b}}(\mathrm{RSE} \%)$ |  |  |
| :--- | :---: | :---: | :---: |
| Tissue | $P N_{\text {max }}(\mathrm{ng} / \mathrm{ml})$ | $P N L_{50}(\mathrm{ng} / \mathrm{ml})$ | $f_{m}$ |
| Fat | $166(41.9)$ | $283(202)$ |  |
| Bone | $108(28.4)$ | $314(173)$ |  |
| Spleen | $230(48.2)$ | $794(252)$ |  |
| Heart | $338(63.3)$ | $2575(251)$ |  |
| Intestine | $589(62.8)$ | $3174(231)$ | $0.076(39.5)$ |
| Plasma | $249(47.2)$ | $1276(224)$ | $0.016(7.7)$ |
| Kidney |  | $0.059(26.7)$ |  |
| Lung |  | $0.064(17.2)$ |  |
| Brain |  | $0.060(28.3)$ |  |
| Skin |  |  |  |
| Muscle |  |  |  |
| $f_{m}$, linear PN/PNL ratio estimated according to eq. 35; $P N_{\text {max }}$ and $P N L_{50}$, the |  |  |  |
| maximal PN conc. achievable and the PNL conc. at which half of the maximal PN |  |  |  |
| conc. is achieved estimated based on eq. 36. |  |  |  |

(CLs) is $149 \mathrm{ml} / \mathrm{h}$. The overall systemic clearance is thus $942 \mathrm{ml} / \mathrm{h}$, which is comparable to the value of $1129 \mathrm{ml} / \mathrm{h}$ for CBG-free PNL clearance in rats (Boudinot and Jusko, 1986; Boudinot et al., 1986). These previous studies showed similar plasma concentration profiles of PNL and PN after a $50-\mathrm{mg} / \mathrm{kg}$ i.v. dose to ours. The liver thus accounts for $90 \%$ of PNL disposition, and the kidney accounts for $10 \%$. However, these clearance values do not account for PNL and PN interconversion because recycling augments the PNL AUC and thus underestimates the true clearance (Ebling and Jusko, 1986). There is evidence of an enterohepatic circulation (EHC) of GCs, which might partially explain their appreciable liver clearance. The intestinal absorption and biliary excretion of DEX in rats were reduced by dicyclomine, a cholinergic blocking agent, and consequently resulted in a decrease in AUC (Ogiso et al., 1985), indicating the involvement of EHC in DEX disposition. The possibility for EHC in rats might also apply to PNL (Mueller and Potter,

TABLE 3
PBPK model parameter estimates after a single subcutaneous dose of $50-\mathrm{mg} / \mathrm{kg}$ PNL in rats

| Parameter (Unit) | Definition | Estimate (CV\%) |
| :---: | :---: | :---: |
| $\begin{aligned} & N_{C} P_{C} \\ & \quad(\mathrm{ng} / \mathrm{ml}) \end{aligned}$ | Binding capacity of CBG | 297.8 (Fixed) |
| $K_{C}(\mathrm{ml} / \mathrm{ng})$ | Association constant of CBG | 0.0046 (Fixed) |
| $\begin{gathered} N_{A} P_{A} \\ (\mathrm{ng} / \mathrm{ml}) \end{gathered}$ | Binding capacity of albumin | 164,342 <br> (Fixed) |
| $K_{A}(\mathrm{ml} / \mathrm{ng})$ | Association constant of albumin | $\begin{aligned} & 1.02 \times 10^{-5} \\ & \text { (Fixed) } \end{aligned}$ |
| $B_{m a x}$ ( $\mathrm{ng} / \mathrm{ml}$ ) | Binding capacity of muscle | 690.5 (12.5) |
| $B_{m a x}$ ( $\mathrm{ng} / \mathrm{ml}$ ) | Binding capacity of liver, heart, intestine, bone | 188.5 (11.6) |
| Bmax $_{3}$ ( $\mathrm{ng} / \mathrm{ml}$ ) | Binding capacity of kidney, lung, skin, spleen, fat, brain | 50.77 (10.8) |
| $K_{d}(\mathrm{ng} / \mathrm{ml})$ | Dissociation constant for PNL-tissue interaction | 3.01 (23.8) |
| $k a\left(\mathrm{~h}^{-1}\right)$ | Subcutaneous first-order absorption rate constant | 2.59 (5.1) |
| $C L_{\text {int }}(\mathrm{ml} / \mathrm{h})$ | CBG-free hepatic intrinsic clearance | 1789 (16.3) |
| $C L_{k}(\mathrm{ml} / \mathrm{h})$ | CBG-free renal clearance | 191.2 (40.3) |
| $C L_{u p}(\mathrm{ml} / \mathrm{h})$ | Brain bidirectional uptake clearance | 0.13 (22.6) |
| $C L_{\text {eff }}(\mathrm{ml} / \mathrm{h})$ | Brain unidirectional efflux clearance | 4.58 (25.9) |
| $f_{m-l i v e r}$ | Metabolite/drug ratio of PN/PNL for liver | 0.01 (30.1) |
| $R_{B}{ }^{a}$ | Blood-to-plasma conc. ratio | 0.71 (Fixed) |

[^3]TABLE 4
Summary of prednisolone tissue partition coefficients by several methods

| Tissue | PBPK | Infusion ${ }^{\text {a }}$ | Method 1 | Method 2 | Method 3 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $K_{p, L u n g}$ | 1.00 | $1.21 \pm 0.47$ | 0.86 | 0.7 | 0.53 |
| $K_{p, \text { Brain }}$ | 0.05 | $0.03 \pm 0.003$ | 1.24 | 0.89 | 0.63 |
| $K_{p, \text { Kidney }}$ | 1.02 | $0.22 \pm 0.05$ | 0.79 | 0.66 | 0.47 |
| $K_{p, \text { Kidney }}($ uncorrected) | 0.79 | $0.17 \pm 0.04$ |  |  |  |
| $K_{p, \text { Heart }}$ | 1.17 | $0.92 \pm 0.19$ | 0.74 | 0.64 | 0.47 |
| $K_{p, \text { Intestine }}$ | 1.17 | $1.37 \pm 0.78$ | NA ${ }^{\text {b }}$ | $\mathrm{NA}^{\text {b }}$ | $\mathrm{NA}^{\text {b }}$ |
| $K_{p, \text { Spleen }}$ | 1.01 | $0.47 \pm 0.16$ | 0.67 | 0.64 | 0.46 |
| $K_{p, \text { Muscle }}$ | 1.88 | $0.32 \pm 0.02$ | 0.67 | 0.6 | 0.36 |
| $K_{p, L i v e r}$ | 1.31 | $3.67 \pm 0.19$ | 0.77 | 0.63 | 0.48 |
| $K_{p, L i v e r}$ (uncorrected) | 0.58 | $1.63 \pm 0.08$ |  |  |  |
| $K_{p, \text { Skin }}$ | 1.01 | $0.57 \pm 0.30$ | 0.81 | 0.63 | 0.46 |
| $K_{p, \text { Bone }}$ | 0.22 | $0.29 \pm 0.13$ | $\mathrm{NA}^{\text {c }}$ | $\mathrm{NA}^{\text {c }}$ | $\mathrm{NA}^{\text {c }}$ |
| $K_{p, F a t}$ | $0.17$ | $0.14 \pm 0.05$ | 0.51 | 0.4 | 0.42 |
| $K_{p, \text { Remainder }}$ | 1.00 (Fixed) |  |  |  |  |

NA, not applicable.
${ }^{a}$ Calculated based on SS infusion study with $n=2$ (S.D. provided).
${ }^{b}$ Tissues associated with gastro-intestinal tract were lumped together.
${ }^{c}$ Different bone marrows were listed separately in software.
1981), although total radioactivity was measured in their study, which would include PNL metabolites.
As shown in Fig. 4, the appearance of PN in each tissue is very rapid upon PNL administration with similar times to reach maximum concentrations observed for both compounds. Modeling PN concentration simply as a fraction of PNL described the PN data reasonably well. This supports the assumption that the interconversion between PNL and PN is in continual rapid equilibrium. The interconversion of PNL and PN is mediated by $11 \beta$-HSDs, of which $11 \beta$-HSD1 is widely distributed with high expression in the liver and also in adipose tissue, muscle, bone, synovium, gonadal tissue, decidua, ocular tissues, and the CNS. The $11 \beta$-HSD2 is largely restricted to the mineralocorticoid-target tissues and the placenta (Raza et al., 2010). There has been evidence showing that the different tissue ratios of PN/PNL are mainly due to the tissue-specific expression and activity of $11 \beta$-HSDs (Escher et al., 1994). The conversion from PNL to PN is mediated by $11 \beta-\mathrm{HSD} 2$, and some tissues exhibit $11 \beta$-HSD2 saturation. The nonlinear PN/ PNL ratio in rat plasma is consistent with observations in dogs (Frey et al., 1980) and humans (Legler et al., 1982). The $11 \beta$ HSD2 has tissue-specific distribution with highest enzyme activity in kidney (Monder, 1991; Yau et al., 1991), which may explain the appreciable and nonsaturable production of PN in kidney. Our liver concentrations of PN were very low. This could be explained by the high $11 \beta$-HSD1 expression in the liver and also agrees with findings in rats (Escher et al., 1994) that the PN/PNL ratio in liver was far lower than in other tissues. Liver perfusions were carried out in rabbits in which the extraction ratio of PNL was $50 \%$, but no measurable PN was formed (Hale and Benet, 1991). Redistribution of PN between various tissues cannot be ruled out owing to the likely high permeability of this compound, which partly necessitated our metabolite modeling approach.

The $K_{p}$ predictions using three in silico methods were similar and generally comparable to those obtained from in vivo data except for liver and brain (Table 4). The difference between liver $K_{p}$ before (1.63) and after (3.67) metabolic correction can be attributed to substantial hepatic extraction of PNL and moderate partitioning. Similar findings of moderate liver partitioning were found for DEX ( $K_{p}=5.06$ ) (Song et al., 2020) and MPL ( $K_{p}=13.5$ ) (Ayyar et al., 2019). The extensive accumulation of

GCs within liver may be largely attributed to the abundant expression of GRs (Ballard et al., 1974). Also, the GC importermediated active uptake of PNL, DEX, and MPL into highly purified rat liver plasma membranes free of CBGs and GRs (Lackner et al., 1998) may be another factor contributing to their high $K_{p}$ values. However, the PBPK model-derived $K_{p}$ (1.31) and the in silico $K_{p}(0.48-0.77)$ for liver were 2.8 - to 10 -fold smaller than those obtained with the SS infusion. The plasma concentrations of PNL after the $50-\mathrm{mg} / \mathrm{kg}$ bolus s.c. dose (Fig. 4) are substantially higher than those from $(1032.5 \mathrm{ng} / \mathrm{ml})$ the infusion study. These nonlinear $K_{p}$ differences agree with observations in rabbits that the tissue distribution of PNL is saturable in liver (Khalafallah and Jusko, 1984b). The in silico methods assume that tissue distribution is largely determined by neutral and phospholipid drug partitioning. They do not consider the roles of transport proteins, complex binding of drugs in liver, and active efflux in brain.

Limitations of the current study are that only one bolus dose level of PNL was used, and the subcutaneous bioavailability ( $F$ ) was based on the intravenous data from another study (Huang and Jusko, 1990). However, the 50$\mathrm{mg} / \mathrm{kg}$ dose of subcutaneous PNL yielded a wide range of plasma and tissue concentrations spanning several orders of magnitude. The calculated $F$ appeared reliable in that the same rat strain (male Wistar rats) with comparable body weights were used, and the intravenous AUC values were consistent across studies (Boudinot and Jusko, 1986; Boudinot et al., 1986; Huang and Jusko, 1990). The metabolic interconversion rates between PNL and PN were observed to be in rapid equilibrium. It would be necessary to directly give PN to assess its clearance parameters (Ebling and Jusko, 1986). Through incorporating nonlinear plasma and tissue binding, the current PBPK model was able to reasonably capture the PK profiles of PNL and PN in all tissues. Although the late low concentrations of PNL in muscle were overpredicted, these values are complicated by being close to the LLOQ of $1 \mathrm{ng} / \mathrm{ml}$, account for less than $0.1 \%$ of early concentrations, and are the latest values in individual animals after subcutaneous dosing and subject to diverse sources of variation. The similarity of PNL and PN profiles across tissues supports the general behavior of all tissues as modeled.

The metabolic and pharmacokinetic properties of PNL and PN are generally similar in rat and human, making this PBPK model applicable to human and perhaps other species. In particular, as described for humans in the Introduction, this includes nonlinear binding to CBG in plasma, reversible metabolism with PNL dominating over PN in a similar ratio in plasma, metabolism by liver and kidney, involvement of CYP3A in liver (Pichard et al., 1992; Penzak et al., 2005), and $11 \beta$-HSDs in various tissues, a similar apparent $V_{s s} /$ body weight in rat ( $1.31 \mathrm{l} / \mathrm{kg}$ ) (Huang and Jusko, 1990) and human ( $1.04 \mathrm{l} / \mathrm{kg}$ ) (Czock et al., 2005) implying similar tissue binding. There is also similar binding to glucocorticoid receptors in both species. Differences would include metabolic rates and possible EHC only in the rat. Because corticosteroids generally have similar PK properties in various species, receptors in most tissues, and multiple therapeutic and adverse actions, this PBPK model may provide greater insights into their pharmacodynamic complexities.

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## Authorship Contributions

Participated in research design: Li, DuBois, Jusko.
Conducted experiments: Li, DuBois.
Performed data analysis: Li, Jusko.
Wrote or contributed to the writing of the manuscript: Li, DuBois, Almon, Jusko.

## References

Araki Y, Yokota O, Kato T, Kashima M, and Miyazaki T (1966) Dynamics of synthetic corticosteroids in man, in Steroid Dynamics (Pincus P and Nakao JTT eds) pp 463-480, Academic Press, New York.
Ayyar VS, Song D, DuBois DC, Almon RR, and Jusko WJ (2019) Modeling corticosteroid pharmacokinetics and pharmacodynamics, part I: determination and prediction of dexamethasone and methylprednisolone tissue binding in the rat. J Pharmacol Exp Ther 370:318-326.
Bailey E and West HF (1967) Prednisolone metabolism and rheumatoid arthritis. Lancet 2:1231-1232.
Ballard PL, Baxter JD, Higgins SJ, Rousseau GG, and Tomkins GM (1974) General presence of glucocorticoid receptors in mammalian tissues. Endocrinology 94: 998-1002.
Barth J, Damoiseaux M, Möllmann H, Brandis KH, Hochhaus G, and Derendorf H (1992) Pharmacokinetics and pharmacodynamics of prednisolone after intravenous and oral administration. Int J Clin Pharmacol Ther Toxicol 30:317-324.
Berezhkovskiy LM (2004) Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. J Pharm Sci 93:1628-1640.
Bergmann TK, Barraclough KA, Lee KJ, and Staatz CE (2012) Clinical pharmacokinetics and pharmacodynamics of prednisolone and prednisone in solid organ transplantation. Clin Pharmacokinet 51:711-741.
Bernareggi A and Rowland M (1991) Physiologic modeling of cyclosporin kinetics in rat and man. J Pharmacokinet Biopharm 19:21-50.
Boudinot FD, D'Ambrosio R, and Jusko WJ (1986) Receptor-mediated pharmacodynamics of prednisolone in the rat. J Pharmacokinet Biopharm 14:469-493.
Boudinot FD and Jusko WJ (1984) Plasma protein binding interaction of prednisone and prednisolone. J Steroid Biochem 21:337-339.
Boudinot FD and Jusko WJ (1986) Dose-dependent pharmacokinetics of prednisolone in normal and adrenalectomized rats. J Pharmacokinet Biopharm 14:453-467.
Caspi E and Pechet MM (1958) Metabolism of 1-dehydrosteroids in man. I. Isolation of six urinary products after the administration of prednisolone. J Biol Chem $\mathbf{2 3 0}$ : 843-851.
Chapman K, Holmes M, and Seckl J (2013) 11ß-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. Physiol Rev 93: 1139-1206.
Coutinho AE and Chapman KE (2011) The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol 335:2-13.
Czock D, Keller F, Rasche FM, and Häussler U (2005) Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. Clin Pharmacokinet 44:61-98.
D' Argenio D, Schumitzky A, and Wang X (2009) Adapt 5 user's guide: pharmacokinetic/pharmacodynamic systems analysis software, BMSR, University of Southern California, Los Angeles, CA.
del Mar Grasa M, Cabot C, Adán C, de Matteis R, Esteve M, Cinti S, Fernández JA, López, Remesar X, and Alemany A (2001) Corticosteroid-binding globulin synthesis and distribution in rat white adipose tissue. Mol Cell Biochem 228:25-31.

Derendorf H, Rohdewald P, Möllmann H, Rehder J, Barth J, and Neveling D (1985) Pharmacokinetics of prednisolone after high doses of prednisolone hemisuccinate. Biopharm Drug Dispos 6:423-432.
Difrancesco R, Frerichs V, Donnelly J, Hagler C, Hochreiter J, and Tornatore KM (2007) Simultaneous determination of cortisol, dexamethasone, methylprednisolone, prednisone, prednisolone, mycophenolic acid and mycophenolic acid glucuronide in human plasma utilizing liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 859:42-51.
Ebling WF and Jusko WJ (1986) The determination of essential clearance, volume, and residence time parameters of recirculating metabolic systems: the reversible metabolism of methylprednisolone and methylprednisone in rabbits. J Pharmacokinet Biopharm 14:557-599.
Escher G, Frey FJ, and Frey BM (1994) 11 beta-Hydroxysteroid dehydrogenase accounts for low prednisolone/prednisone ratios in the kidney. Endocrinology 135: 101-106.
Ferry JJ and Wagner JG (1987) The nonlinear pharmacokinetics of prednisone and prednisolone. II. Plasma protein binding of prednisone and prednisolone in rabbit and human plasma. Biopharm Drug Dispos 8:261-272.
Frey BM and Frey FJ (1990) Clinical pharmacokinetics of prednisone and prednisolone. Clin Pharmacokinet 19:126-146.
Frey FJ, Frey BM, Greither A, and Benet LZ (1980) Prednisolone clearance at steady state in dogs. J Pharmacol Exp Ther 215:287-291.
Gerlowski LE and Jain RK (1983) Physiologically based pharmacokinetic modeling: principles and applications. J Pharm Sci 72:1103-1127.
Glenn EM (1959) In vitro and in vivo metabolism of prednisolone: studies concerning its biological effectiveness. Endocrinology 64:373-378.
Hale VG and Benet LZ (1991) Prednisolone and prednisone exhibit linear extraction in the perfused rabbit liver. Drug Metab Dispos 19:87-93.
Huang ML and Jusko WJ (1990) Nonlinear pharmacokinetics and interconversion of prednisolone and prednisone in rats. J Pharmacokinet Biopharm 18:401-421.
Kadmiel M and Cidlowski JA (2013) Glucocorticoid receptor signaling in health and disease. Trends Pharmacol Sci 34:518-530.
Kampfmann I, Bauer N, Johannes S, and Moritz A (2012) Differences in hematologic variables in rats of the same strain but different origin. Vet Clin Pathol 41: 228-234.
Karssen AM, Meijer OC, van der Sandt IC, De Boer AG, De Lange EC, and De Kloet ER (2002) The role of the efflux transporter P-glycoprotein in brain penetration of prednisolone. J Endocrinol 175:251-260.
Khalafallah N and Jusko WJ (1984a) Determination and prediction of tissue binding of prednisolone in the rabbit. J Pharm Sci 73:362-366.
Khalafallah N and Jusko WJ (1984b) Tissue distribution of prednisolone in the rabbit. J Pharmacol Exp Ther 229:719-725.
Ko HC, Almon RR, and Jusko WJ (1995) Effect of corticosteroid binding globulin on the pharmacokinetics of prednisolone in rats. Pharm Res 12:902-904.
Lackner C, Daufeldt S, Wildt L, and Alléra A (1998) Glucocorticoid-recognizing and -effector sites in rat liver plasma membrane. Kinetics of corticosterone uptake by isolated membrane vesicles. III. Specificity and stereospecificity. J Steroid Biochem Mol Biol 64:69-82.
Legler UF, Frey FJ, and Benet LZ (1982) Prednisolone clearance at steady state in man. J Clin Endocrinol Metab 55:762-767.
Lukert BP and Raisz LG (1990) Glucocorticoid-induced osteoporosis: pathogenesis and management. Ann Intern Med 112:352-364.
Melby JC (1977) Clinical pharmacology of systemic corticosteroids. Annu Rev Pharmacol Toxicol 17:511-527.
Methlie P, Hustad SS, Kellmann R, Almås B, Erichsen MM, Husebye E, and Løvås K (2013) Multisteroid LC-MS/MS assay for glucocorticoids and androgens, and its application in Addison's disease. Endocr Connect 2:125-136.
Milsap RL, Plaisance KI, and Jusko WJ (1984) Prednisolone disposition in obese men. Clin Pharmacol Ther 36:824-831.
Monder C (1991) Heterogeneity of 11 beta-hydroxysteroid dehydrogenase in rat tissues. J Steroid Biochem Mol Biol 40:533-536.
Mueller UW and Potter JM (1981) Enterohepatic circulation of prednisolone in rats. Res Commun Chem Pathol Pharmacol 32:195-206.
National Research Council (2011) Guide for the Care and Use of Laboratory Animals, National Academies Press, Washington, DC.
Nichols AI, D'Ambrosio R, Pyszczynski NA, and Jusko WJ (1989) Pharmacokinetics and pharmacodynamics of prednisolone in obese rats. J Pharmacol Exp Ther 250: 963-970.
Oakley RH and Cidlowski JA (2011) Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. J Biol Chem 286:3177-3184.
Ogiso T, Iwaki M, and Ohtori A (1985) Effect of dicyclomine on intestinal absorption, disposition and biliary excretion of dexamethasone. J Pharmacobiodyn 8:41-49.
Overman RA, Yeh JY, and Deal CL (2013) Prevalence of oral glucocorticoid usage in the United States: a general population perspective. Arthritis Care Res (Hoboken) 65:294-298.
Pardridge WM (1981) Transport of protein-bound hormones into tissues in vivo. Endocr Rev 2:103-123.
Penzak SR, Formentini E, Alfaro RM, Long M, Natarajan V, and Kovacs J (2005) Prednisolone pharmacokinetics in the presence and absence of ritonavir after oral prednisone administration to healthy volunteers. J Acquir Immune Defic Syndr 40: 573-580.
Pichard L, Fabre I, Daujat M, Domergue J, Joyeux H, and Maurel P (1992) Effect of corticosteroids on the expression of cytochromes P450 and on cyclosporin A oxidase activity in primary cultures of human hepatocytes. Mol Pharmacol 41:1047-1055.
Poulin P and Theil FP (2002a) Prediction of pharmacokinetics prior to in vivo studies. 1. Mechanism-based prediction of volume of distribution. J Pharm Sci 91:129-156.

Poulin P and Theil FP (2002b) Prediction of pharmacokinetics prior to in vivo studies. II. Generic physiologically based pharmacokinetic models of drug disposition. J Pharm Sci 91:1358-1370.

Raza K, Hardy R, and Cooper MS (2010) The 11beta-hydroxysteroid dehydrogenase enzymes--arbiters of the effects of glucocorticoids in synovium and bone. Rheumatology (Oxford) 49:2016-2023.
Rhen T and Cidlowski JA (2005) Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med 353:1711-1723.
Rocci ML Jr., Johnson NF, and Jusko WJ (1980) Serum protein binding of prednisolone in four species. J Pharm Sci 69:977-978.
Rocci ML Jr. and Jusko WJ (1981) Dose-dependent protein binding and disposition of prednisolone in rabbits. J Pharm Sci 70:1201-1204.
Rocci ML Jr., Szefler SJ, Acara M, and Jusko WJ (1981) Prednisolone metabolism and excretion in the isolated perfused rat kidney. Drug Metab Dispos 9:177-182.
Rodgers T and Rowland M (2006) Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J Pharm Sci 95:1238-1257.
Rose JQ, Yurchak AM, and Jusko WJ (1981) Dose dependent pharmacokinetics of prednisone and prednisolone in man. J Pharmacokinet Biopharm 9:389-417.
Saag KG, Koehnke R, Caldwell JR, Brasington R, Burmeister LF, Zimmerman B, Kohler JA, and Furst DE (1994) Low dose long-term corticosteroid therapy in rheumatoid arthritis: an analysis of serious adverse events. Am J Med 96: 115-123.
Sager JE, Yu J, Ragueneau-Majlessi I, and Isoherranen N (2015) Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications, and model verification. Drug Metab Dispos 43:1823-1837.
Sandberg AA, Slaunwhite WR Jr., and Antoniades HN (1957) The binding of steroids and steroid conjugates to human plasma proteins. Recent Prog Horm Res 13: 209-260, NaN-267.
Schinkel AH, Wagenaar E, van Deemter L, Mol CA, and Borst P (1995) Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and
pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Invest 96:1698-1705.
Shah DK and Betts AM (2012) Towards a platform PBPK model to characterize the plasma and tissue disposition of monoclonal antibodies in preclinical species and human. J Pharmacokinet Pharmacodyn 39:67-86.
Song D, Sun L, DuBois DC, Almon RR, Meng S, and Jusko WJ (2020) Physiologically based pharmacokinetics of dexamethasone in rats. Drug Metab Dispos 48:811-818.
Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T, and Hori R (1992) Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. J Biol Chem 267:24248-24252.
Vermeulen A and Caspi E (1958) The metabolism of prednisolone by homogenates of rat liver. J Biol Chem 233:54-56.
Yao Q, Guo Y, Xue J, Kong D, Li J, Tian X, Hao C, and Zhou T (2020) Development and validation of a LC-MS/MS method for simultaneous determination of six glucocorticoids and its application to a pharmacokinetic study in nude mice. J Pharm Biomed Anal 179:112980.
Yates CR, Chang C, Kearbey JD, Yasuda K, Schuetz EG, Miller DD, Dalton JT, and Swaan PW (2003) Structural determinants of P-glycoprotein-mediated transport of glucocorticoids. Pharm Res 20:1794-1803.
Yau JL, Van Haarst AD, Moisan MP, Fleming S, Edwards CR, and Seckl JR (1991) 11 beta-Hydroxysteroid dehydrogenase mRNA expression in rat kidney. Am $J$ Physiol 260:F764-F767.

Address correspondence to: Dr. William J. Jusko, Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, NY 14214-8033. E-mail: wjjusko@buffalo.edu

# Physiologically-Based Pharmacokinetic Modeling Involving Nonlinear Plasma and Tissue 

 Binding: Application to Prednisolone and Prednisone in RatsXiaonan Li, Debra C. DuBois, Richard R. Almon, and William J. Jusko
The Journal of Pharmacology and Experimental Therapeutics
MS \#JPET-AR-2020-000191

## ADAPT 5 code for PBPK model



C\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#C

Subroutine SYMBOL Implicit None

Include 'globals.inc'
Include 'model.inc'

CC


```
C Enter as Indicated C
```



```
NDEqs = 16 ! Enter # of Diff. Eqs.
NSParam = 12 ! Enter # of System Parameters.
NVparam = 2 ! Enter # of Variance Parameters.
NSecPar = 0 ! Enter # of Secondary Parameters.
NSecOut = 0 ! Enter # of Secondary Outputs (not used).
Ieqsol = 1 ! Model type: 1 - DIFFEQ, 2 - AMAT, 3 - OUTPUT only.
Descr = 'no Kns, 3 Bmax,nonlinear bone fat fm'
```

CC

C Enter Symbol for Each System Parameter (eg. Psym(1)='Kel') C


```
PSym(1) = 'BW (g)'
PSym(2) = 'Bmax1' !liver,bone,gut,heart
PSym(3) = 'Bmax2' !kidney, brain,spleen, skin,adipose,lung
PSym(4) = 'ROther'
```

```
Psym(5) = 'Ka'
Psym(6) = 'CLint'
Psym(7) = 'KD'
Psym(8) = 'CLk'
Psym(9) = 'CLup'
Psym(10) = 'CLeff'
Psym(11) = 'fm_liver'
Psym(12) = 'BmaxM'
```

CC

C Enter Symbol for Each Variance Parameter \{eg: PVsym(1)='Sigma'\} C

PVsym(1) ='Intercept'
PVsym(2) ='Sigma'
CC

C Enter Symbol for Each Secondary Parameter \{eg: PSsym(1)='CLt'\} C


C
Return
End
C\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#
Subroutine DIFFEQ(T,X,XP)
Implicit None
Include 'globals.inc'
Include 'model.inc'
Real*8 T, X (MaxNDE), XP (MaxNDE)
Integer i
CC

C Enter Differential Equations Below \{e.g. XP(1) = $-\mathrm{P}(1) * \mathrm{X}(1)$ \} C

C ADAPT model parameters
Real*8 Ka, BW,RH, RK, RBrain, RM, RSp, RGut
Real*8 RAd, RSk, ROther, Rlung, RLiver, RBone
Real*8 Bmax_H, Bmax_K, Bmax_Brain, BmaxM, Bmax1
Real*8 Bmax_Ad, Bmax_Sk ,Bmax_Sp,Bmax_liver, Bmax2
Real*8 Bmax_Gut, Bmax_lung, Bmax_Bone, KD
Real*8 CLup, CLeff,fd_Bone,fd_Ad,KD1,KD_M,Rb

Real*8 CLK12,CLK10,CLK20,CLK21,CLkidney, CLint, CLk
Real*8 PSABPNL,PSABPN, PSBAPNL, PSBAPN
Real*8 RHPN, RKPN, RBrainPN, RMPN, RSpPN, RGutPN
Real*8 RAdPN, RSkPN, ROtherPN, RlungPN, RLiverPN, RBonePN
C Fixed parameters
Real*8 Q, QH, QK, QBrain, QM, QSp, QGut, QHA
Real*8 QAd, QSk, QOther,QBone
Real*8 Vven, Vart, VH, VK, VBrain, VM, VSp, VGut, VLiver
Real*8 VAd, VSk, VOther,Vlung,VBone,Vbrcap,VISF_Bone,VISF_Ad
Real*8 NKPA, NKPCBG, KCBG,VISFM_Bone,VM_Bone
Real*8 KnsBone,KnsH,KnsK,KnsBrain, KnsM, KnsSp, KnsGut, KnsAd, KnsSk
Real*8 KnsLung,KnsLiver
C Local state variables
Real*8 CHeart, CKidney, CBrain, CMuscle, CSpleen, CGut, CFat, CSkin
Real*8 COther, CLiver, CLung, Cart, Cven, CBone, Aab, Cbrcap, Cfbrcap
Real*8 CHeartPN, CKidneyPN, CBrainPN, CMusclePN, CSpleenPN, CGutPN
Real*8 CFatPN,CSkinPN
Real*8 COtherPN, CLiverPN, CLungPN, CartPN, CvenPN, CBonePN
Real*8 Cfart, Cfven, CfPN, CfvenPN, Cfbrcapp, Cfvenp, Cfartp
C Local derivatives of state variables
Real*8 ddt_CHeart, ddt_CKidney, ddt_CBrain, ddt_CMuscle
Real*8 ddt_CSpleen, ddt_CGut, ddt_CFat, ddt_CSkin, ddt_COther
Real*8 ddt_CLiver, ddt_CLung, ddt_Cart,ddt_Cven,ddt_Aab,ddt_CBone
Real*8 ddt_CHeartPN, ddt_CKidneyPN, ddt_CBrainPN, ddt_CMusclePN
Real*8 ddt_CSpleenPN, ddt_CGutPN, ddt_CFatPN, ddt_CSkinPN
Real*8 ddt_CLiverPN, ddt_CLungPN, ddt_CartPN,ddt_CvenPN
Real*8 ddt_COtherPN,ddt_CBonePN,ddt_Cbrcap

C Assign ADAPT model parameter values to local variables

| BW | $=P(1) \quad$ ! ave BW 293.65 g |
| :--- | :--- |
| Bmax1 | $=P(2)$ |
| Bmax2 | $=P(3)$ |
| ROther | $=P(4)$ |
| Ka | $=P(5)$ |
| CLint | $=P(6)$ |
| KD | $=P(7)$ |
| CLk | $=P(8)$ |
| CLup | $=P(9)$ |
| CLeff | $=P(10)$ |
| BmaxM | $=P(12)$ |

C Protein binding parameters
NKPA=1. 67
NKPCBG=1.37
$\mathrm{KCBG}=0.0046$ ! $\mathrm{mL} / \mathrm{ng}$
C unbound PNL concentration in venous plasma
Cfvenp $=0.5^{*}(-($ NKPA + NKPCBG $-K C B G * X(2)+1)$
$+(($ NKPA + NKPCBG-KCBG*X $(2)+1) * * 2$
$+4 *($ KCBG $+N K P A * K C B G) * X(2)) * * 0.5)$
/ (KCBG+NKPA*KCBG)
C CBG-unbound PNL concentration in venous plasma
Cfven $=(N K P A+1) *$ Cfvenp

C unbound PNL concentration in arterial plasma
Cfartp $=0.5$ * ( $-($ NKPA + NKPCBG-KCBG*X (4) +1)
$\$+((N K P A+N K P C B G-K C B G * X(4)+1) * * 2$
$\$+4 *($ KCBG + NKPA*KCBG $) * X(4)) * * 0.5)$
$\$ /($ KCBG + NKPA*KCBG)

C CBG-unbound PNL concentration in arterial plasma Cfart $=($ NKPA +1$) *$ Cfartp

C unbound PNL concentration in brain capillary
Cfbrcapp $=0.5^{*}(-($ NKPA + NKPCBG-KCBG*X $(16)+1)$
$\$+((N K P A+N K P C B G-K C B G * X(16)+1) * * 2$
$\$+4 *($ KCBG + NKPA*KCBG $) * \mathrm{X}(16)) * * 0.5)$
\$ / (KCBG+NKPA*KCBG)
C CBG-unbound PNL concentration in brain capillary Cfbrcap $=(N K P A+1) * C f b r c a p p$

C Nonlinear Kpu in tissues
Rlung $=0.5$ * ( $(\mathrm{X}(3)-\mathrm{KD}-\mathrm{Bmax} 2)$
$\$+(4 * K D * X(3)+(X(3)-K D-B m a x 2) * * 2) * * 0.5) / K D$
RH=0.5* (- (X (5) -KD-Bmax1)
\$ + (4*KD*X(5)+(X(5)-KD-Bmax1)**2)**0.5)/KD
$R K=0.5 *(-(X(6)-K D-B m a x 2)$
$\$+(4 * K D * X(6)+(X(6)-K D-B m a x 2) * * 2) * * 0.5) / K D$
RBrain=0.5* (-(X (7) -KD-Bmax2)
$\$+(4 * K D * X(7)+(X(7)-K D-B m a x 2) * * 2) * * 0.5) / K D$
$R M=0.5$ * $(-(X(8)-K D-B m a x M)$
$\$+(4 * K D * X(8)+(X(8)-K D-B m a x M) * * 2) * * 0.5) / K D$
RSp $=0$. 5* $^{*}(-(\mathrm{X}(9)-\mathrm{KD}-\mathrm{Bmax} 2)$
$\$+(4 * K D * X(9)+(X(9)-K D-B m a x 2) * * 2) * * 0.5) / K D$
RGut $=0.5^{*}(-(\mathrm{X}(10)-\mathrm{KD}-$ Bmax1)
\$ $+(4 * K D * X(10)+(X(10)-K D-B m a x 1) * * 2) * * 0.5) / K D$
RAd $=0.5^{*}(-(\mathrm{X}(11)-\mathrm{KD}-\mathrm{Bmax} 2)$
$\$+(4 * K D * X(11)+(X(11)-K D-B m a x 2) * * 2) * * 0.5) / K D$
RSk $=0.5^{*}(-(\mathrm{X}(12)-\mathrm{KD}-\mathrm{Bmax} 2)$
$\$+(4 * K D * X(12)+(X(12)-K D-B m a x 2) * * 2) * * 0.5) / K D$
RLiver=0.5*(-(X (13)-KD-Bmax1)
\$ $+(4 * K D * X(13)+(X(13)-K D-B m a x 1) * * 2) * * 0.5) / K D$
RBone $=0.5^{*}(-(X(14)-K D-B m a x 1)$
$\$+(4 * K D * X(14)+(X(14)-K D-B m a x 1) * * 2) * * 0.5) / K D$

C Fixed parameters: blood flow Q's in mL/hr, Ref: Shah

```
        Q = 5548.6
    QH = 285
    QK = 687.1
    QBrain = 123.1
    QM = 1743.1
    QSp = 336.8
    QGut = 1047.7
    QHA = 39.8
    QAd = 422.8
    QSk = 376.2
    QBone = 115.7
    QOther = 371.2
C Fixed parameters: V's in mL corrected by Fvv (Bernareggi)
    Vven = 11.52
    Vart = 5.76
    VH}=0.8
    VK = 1.81
    VM = 127.52
    VSp = 0.43
    VGut = 8.14
    Vliver = 9.94
    VAd = 34.44
    VSk = 52.17
    VOther = 17.08
    VLung = 0.96
    VBone = 21.61
    Vbrcap = 0.02
    VBrain = 1.41
    VISF_Bone=4.02 ! Shah:Fv=0.186
    VISF_Ad=5.85 ! Shah:Fv=0.17
C Assign code state variable values to local variables: Organ/Tissue
Concentrations (ng/mL)
C PNL
            Aab=X(1)
            Cven=X(2)
            CLung=X (3)
            Cart=X(4)
            CHeart=X(5)
            CKidney=X(6)
            CBrain=X(7)
            CMuscle=X(8)
            CSpleen=X(9)
            CGut=X(10)
            CFat=X(11)
            CSkin=X(12)
            CLiver=X(13)
            CBone=X(14)
            COther=X(15)
            Cbrcap=X(16)
```

C Model diffrential equations
C 1. Dosing depot $d d t \_A a b=-K a * A a b$

C 2. Venous plasma Pool
ddt_Cven $=($ Ka*Aab
$+\mathrm{QH}^{*}(\mathrm{CHeart} / \mathrm{RH}+(\mathrm{NKPCBG} *(\mathrm{CHeart} / \mathrm{RH}) /(\mathrm{NKPA}+1))$
/(1+KCBG* (CHeart/RH) /(NKPA+1)))

+ QK* (CKidney/RK+(NKPCBG* (CKidney/RK)/(NKPA+1))
/(1+KCBG* (CKidney/RK) /(NKPA+1)))
+ QBrain*Cbrcap
+ QM* (CMuscle/RM+ (NKPCBG* (CMuscle/RM) /(NKPA+1))
/(1+KCBG* (CMuscle/RM) /(NKPA+1)))
+ (QSp+QHA+QGut)
*(CLiver/RLiver+(NKPCBG*(CLiver/RLiver) /(NKPA+1))
/(1+KCBG* (CLiver/RLiver)/(NKPA+1)))
+ QAd*(CFat/RAd+ (NKPCBG* (CFat/RAd)/(NKPA+1))
/(1+KCBG* (CFat/RAd) / (NKPA+1)) )
+QSk *(CSkin/RSk+(NKPCBG*(CSkin/RSk)/(NKPA+1))
/(1+KCBG*(CSkin/RSk) /(NKPA+1)))
+ QBone* (CBone/RBone+ (NKPCBG* (CBone/RBone) /(NKPA+1))
/(1+KCBG* (CBone/RBone)/(NKPA+1)))
+ QOther* (COther/ROther+(NKPCBG* (COther/ROther) /(NKPA+1))
/(1+KCBG* (COther/ROther) /(NKPA+1)))
- Q*Cven) / Vven

C 3. Lung
ddt_CLung $=$ Q * ( Cfven- CLung/ RLung ) / VLung

C 4. Arterial plasma Pool
ddt_Cart $=$ Q * ((CLung/RLung $+($ NKPCBG* $(C L u n g / R L u n g) /(N K P A+1))$
ㅊ $\left./\left(1+\operatorname{KCBG}^{*}(C L u n g / R L u n g) /(N K P A+1)\right)-\operatorname{Cart}\right) / \operatorname{Vart}$

C 5. Heart
ddt_CHeart $=$ QH * ( Cfart - CHeart / RH ) / VH
C 6. Kidney
ddt_CKidney $=(Q K *(C f a r t-C K i d n e y / R K)-C L k * C K i d n e y / R K) ~ / ~ V K ~$

C 16. Brain capillary
ddt_Cbrcap $=$ (QBrain *(Cart-Cbrcap)-CLup*(Cfbrcap-CBrain/RBrain)
x +CLeff*CBrain / RBrain)/Vbrcap
C 7. Brain
ddt_CBrain $=$ (CLup* ( Cfbrcap-CBrain/RBrain )
x $\mathbf{x}$-CLeff*CBrain / RBrain)/ VBrain

C 8. Muscle
ddt_CMuscle =QM * ( Cfart - CMuscle/ RM ) / VM

```
C 9. Spleen
                ddt_CSpleen = QSp * ( Cfart - CSpleen / RSp ) / VSp
C 10. Gut
                                ddt_CGut = QGut * ( Cfart - CGut/ RGut ) / vGut
C 11. Fat
                ddt_CFat = QAd * ( Cfart - CFat / RAd ) / VISF_Ad
C 12. Skin
        ddt_CSkin = QSk * ( Cfart - CSkin/ RSk ) / VSk
C 13. Liver
    ddt_CLiver = ( QSp*CSpleen/RSp + QHA*Cfart + QGut*CGut/RGut
    x - (QSp+QHA+QGut+CLint)*CLiver/RLiver)/ VLiver
C 14.Bone
    ddt_CBone = QBone*(Cfart-CBone/RBone)/VISF_Bone
C 15. Other
    ddt_COther = QOther * ( Cfart - COther/ ROther ) / VOther
C Assign local state variable derivative values to code variables
    XP (1) =ddt_Aab
    XP (2) =ddt_Cven
    XP (3) =ddt_CLung
    XP (4) =ddt_Cart
    XP (5) =ddt_CHeart
    XP (6) =ddt_CKidney
    XP (7) =ddt_CBrain
    XP (8)=ddt_CMuscle
    XP (9) =ddt_CSpleen
    XP (10) =ddt_CGut
    XP (11) =ddt_CFat
    XP (12)=ddt_CSkin
    XP (13) =ddt_CLiver
    XP (14) =ddt_CBone
    XP (15) =ddt_Cother
    XP (16) =ddt_Cbrcap
C---------------------------------------------------------------------------
C-------------------------------------------------------------------------------
C
```

```
    Return
    End
C######################################################################C
    Subroutine OUTPUT(Y,T,X)
    Implicit None
    Include 'globals.inc'
    Include 'model.inc'
Real*8 Y(MaxNOE),T,X(MaxNDE)
Real*8 PNmax_art,PNL50_art,fm_lung,PNmax_heart,PNL50_heart,fm_k
Real*8 fm_sk,PNmax_spleen,PNL50_spleen,PNmax_gut,PNL50_gut
Real*8 fm_brain,fm_muscle,PNmax_bone,PNL50_bone,VAd,VM_Bone
Real*8 PNmax_fat,PNL50_fat,fm_liver
Real*8 VBone,VISF_Bone,VISF_Ad,VISFM_Bone,Rb
CC
C-------------------------------------------------------------------------------
C Enter Output Equations Below {e.g. Y(1) = X(1)/P(2) }
C-----c--------------------------------------------------------------------------
    fm_liver=P(11)
    PNmax_art = 249.3
    PNL50_art = 1276
    fm_k = 0.076
    PNmax_heart = 338
    PNL50_heart = 2575
    PNmax_fat= 166
    PNL50_fat= 283
    PNmax_spleen= 230
    PNL50_spleen= 794
    PNmax_gut = 589
    PNL50_gut = 3173
    fm_sk=0.064
    fm_lung = 0.016
    fm_brain = 0.06
    fm_muscle=0.06
    PNmax_bone = 107.5
    PNL50_bone = 314.5
    Rb=0.71
    VAd = 34.44
    VBone = 21.61
    VISF_Bone=4.02 ! Shah:Fv=0.186
    VISF_Ad=5.85 ! Shah:Fv=0.17
C PNL
Y(1:8)=X(3:10)
Y(9) =X(11)*VISF_Ad/VAd
```

```
        Y(10:11) =X (12:13)
        Y(12)=X(14)*VISF_Bone/VBone
C PN
        Y(13) = fm_lung*X(3) !CLungPN
        Y(14) = PNmax_art*X(4)/(PNL50_art+X(4)) !CartPN
        Y(15) = PNmax_heart*X(5)/(PNL50_heart+X(5)) !CHeartPN
        Y(16) = fm_k*X(6) !CKidneyPN
        Y(17) = fm_brain*X(7) !CBrainPN
        Y(18) = fm_muscle*X(8) !CMusclePN
        Y(19) = PNmax_spleen*X(9)/(PNL50_spleen+X(9)) !CSpleenPN
        Y(20) = PNmax_gut*X(10) /(PNL50_gut+X(10)) !CGutPN
        Y(21) = PNmax_fat*X(11) /(PNL50_fat+X(11)) !CFatPN
        Y(22) = fm_sk*X(12) !CSkinPN
        Y(23) = fm_liver*X(13) !CLiverPN
    Y(24) = PNmax_bone*X(14)/(PNL50_bone+X(14)) !CBonePN
c PNL in arterial blood
    Y(25) = X(4)*Rb
```


C\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#
Subroutine VARMOD (V,T,X,Y)
Implicit None
Include 'globals.inc'
Include 'model.inc'
Real*8 V(MaxNOE), T,X(MaxNDE), Y(MaxNOE)
Real*8 Sigma,Intercept
CC

C Enter Variance Model Equations Below C
$C \quad\{e . g . V(1)=(P V(1)+P V(2) * Y(1)) * * 2\} \quad C$

Intercept $=P V(1)$
Sigma=PV(2)
$V(1: 25)=(P V(1)+P V(2) * Y(1: 25)) * * 2$


C
Return
End

```
                            Subroutine COVMOD(Pmean, ICmean, PC)
C Defines any covariate model equations (MLEM, ITS)
            Implicit None
            Include 'globals.inc'
            Include 'model.inc'
            Real*8 PC(MaxNCP)
            Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)
CC
C------------------------------------------------------------------------------------
C Enter # of Covariate Parameters C
C----C----------------------------------------------------------------------------
            NCparam = 0 ! Enter # of Covariate Parameters.
CC
C----------------------------------------------------------------------------------
C Enter Symbol for Covariate Params {eg: PCsym(1)='CLRenal'} C
C-----c------------------------------------------------------------------------------
CC
C------------------------------------------------------------------------------------
C For the Model Params. that Depend on Covariates Enter the Equation C
C {e.g. Pmean(1) = PC(1)*R(2) } C
C----C-------------------------------------------------------------------------
C------------------------------------------------------------------------------------
C-------------------------------------------------------------------------------------
C
    Return
    End
C######################################################################C
    Subroutine POPINIT(PmeanI,ICmeanI,PcovI,ICcovI, PCI)
C Initial parameter values for population program parameters (ITS, MLEM)
    Implicit None
    Include 'globals.inc'
    Include 'model.inc'
    Integer I,J
    Real*8 PmeanI (MaxNSP+MaxNDE), ICmeanI (MaxNDE)
    Real*8 PcovI (MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcovI (MaxNDE,MaxNDE)
    Real*8 PCI (MaxNCP)
CC
C----------------------------------------------------------------------------------------
C Enter Initial Values for Population Means C
C { e.g. PmeanI(1) = 10.0 } C
C-----C----------------------------------------------------------------------------
```

```
CC
C-----------------------------------------------------------------------------------
C Enter Initial Values for Pop. Covariance Matrix (Lower Triang.) C
C { e.g. PcovI (2,1) = 0.25 } C
C-----C---------------------------------------------------------------------------
CC
C---------------------------------------------------------------------------------
C Enter Values for Covariate Model Parameters C
C { e.g. PCI(1) = 2.0 } C
C----C------------------------------------------------------------------------
C------------------------------------------------------------------------------
C--------------------------------------------------------------------------------
C
    Return
    End
C######################################################################C
    Subroutine PRIOR(Pmean,Pcov,ICmean,ICcov)
C Parameter mean and covariance values for MAP estimation (ID,NPD,STS)
    Implicit None
    Include 'globals.inc'
    Include 'model.inc'
    Integer I,J
    Real*8 Pmean (MaxNSP+MaxNDE), ICmean(MaxNDE)
    Real*8 Pcov(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcov(MaxNDE,MaxNDE)
```

CC

C Enter Nonzero Elements of Prior Mean Vector C
C \{ e.g. Pmean(1) = 10.0 \} C

CC

C Enter Nonzero Elements of Covariance Matrix (Lower Triang.) C
C \{ e.g. $\operatorname{Pcov}(2,1)=0.25$ \} C



C
Return
End
C\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#C
Subroutine SPARAM(PS,P,IC)

Implicit None

Include 'globals.inc'

Real*8 PS (MaxNSECP), P(MaxNSP+MaxNDE), IC (MaxNDE)


Return
End
C\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#C

Subroutine AMAT (A)
Implicit None
Include 'globals.inc'
Include 'model.inc'

Integer $I, J$
Real*8 A(MaxNDE, MaxNDE)
DO $I=1$, Ndeqs Do $J=1$, Ndeqs $A(I, J)=0.0 D 0$ End Do
End Do

CC

C Enter non zero elements of state matrix $\{e \cdot g . A(1,1)=-P(1)\} \quad C$



C
Return
End
C\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#\#C


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    https://doi.org/10.1124/jpet.120.000191.
    S This article has supplemental material available at jpet.aspetjournals.org.

[^1]:    ABBREVIATIONS: AUC, area under the curve; $11 \beta$-HSD, $11 \beta$-hydroxysteroid dehydrogenase; CBG, corticosteroid-binding globulin; CL, clearance; CS, corticosteroid; CV\%, coefficients of variation; DEX, dexamethasone; EHC, enterohepatic circulation; GR, glucocorticoid receptor; IS, internal standard; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LLOQ, lower limit of quantification; MPL, methylprednisolone; PBPK, physiologically based pharmacokinetics; P-gp, P-glycoprotein; PK, pharmacokinetics; PN, prednisone; PNL, prednisolone; RBC, red blood cell; SPE, solid-phase extraction; SS, steady state; $V_{\text {ss }}$, volume of distribution at SS.

[^2]:    NA, not applicable.
    ${ }^{a}$ Tissue vascular-corrected volume $\left(V_{T}\right)$ calculated based on experiment value using eq. 4.
    ${ }^{b}$ Calculated based on the value from Shah and Betts (2012).
    ${ }^{c}$ Calculated value assuming 1) $1 \mathrm{ng} / \mathrm{ml}$ tissue density, volume for remainder compartment = body weight - summation of volume for all measured tissues; 2) blood flow for remainder compartment = cardiac output - summation of blood flow for all measured tissues; 3) liver blood flow = summation of blood flow of liver artery, spleen, and intestine).

[^3]:    ${ }^{a} R_{B}$ was estimated by Orthogonal Regression according to eq. 1.

