DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

Title Page

In Silico Prediction of the Absorption and Disposition of Cefadroxil in Humans using an Intestinal Permeability Method Scaled from Humanized *PepT1* Mice

Yongjun Hu, David E. Smith

Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA (Y.H., D.E.S.)

Running Title Page

Simulation of Cefadroxil Absorption and Disposition in Human

Address correspondence to: Dr. David E. Smith, University of Michigan, College of Pharmacy, 428 Church Street, Ann Arbor, Michigan 48109-1065, USA. Telephone: 734-647-1431; Facsimile: 734-615-6162; E-mail: smithb@umich.edu

Text	2 7pages
Tables	6
Figures	8
References	52
Abstract	250 words
Introduction	786 words
Discussion	1356 words

ABBREVIATIONS: ACAT, advanced compartmental and transit; AIC, Akaike information criterion; BW, body weight; CL, clearance; FDA, Food and Drug Administration; P_{eff}, effective permeability; PepT1, peptide transporter 1; Vd, apparent volume of distribution; V1, central compartment volume of distribution; WT, wildtype

ABSTRACT

It is difficult to predict the pharmacokinetics and plasma concentration-time profiles of new chemical entities in humans based on animal data. Some pharmacokinetic parameters, such as clearance and volume of distribution, can be scaled allometrically from rodents, mammals and non-human primates with good success. However, it is far more challenging to predict the oral pharmacokinetics of experimental drug candidates. In the present study, we used *in situ* estimates of intestinal permeability, obtained *in silico* and from rat, wildtype (WT) and humanized PepT1 (*huPepT1*) mice, to predict the systemic exposure of cefadroxil, an orally administered model compound, under a variety of conditions. Using GastroPlus® simulation software, we found that the C_{max} and $AUC_{0\text{-}t}$ of cefadroxil were better predicted using intestinal permeability estimates (both segmental and jejunal) from *huPepT1* than from WT mice, and that intestinal permeabilities based on *in silico* and rat estimates gave worse predictions. We also observed that accurate predictions were possible for cefadroxil during oral dose escalation (i.e., 5, 15 and 30 mg/kg cefadroxil), a drug-drug interaction study (i.e., 5 mg/kg oral cefadroxil plus 45 mg/kg oral cephalexin), and during an oral multiple dose study [i.e., 500 mg (6.7 mg/kg) cefadroxil every six hours]. Finally, the great majority of cefadroxil was absorbed in duodenal and jejunal segments of the small intestine after a 5 mg/kg oral dose. Thus, by combining a humanized mouse model and *in silico* software, the present study offers a novel strategy for better translating preclinical pharmacokinetic data to oral drug exposure during first-in-human studies.

Introduction

The translation of animal pharmacokinetics and plasma concentration-time profiles to humans is critical for the safe and effective development of new chemical entities. Allometric scaling is a valuable approach in predicting, from preclinical studies, primary pharmacokinetic parameters of candidate drugs in humans such as clearance (CL) and volume of distribution (Vd) (Tang and Mayersohn, 2006). However, other pharmacokinetic parameters, such as absorption rate constant (Ka), bioavailability (F), and related effects on maximum plasma concentration (Cmax) and systemic exposure (AUC) of candidate drugs, are more unpredictable. This unpredictability is due, in part, to species differences in intestinal physiology, along with differences among species in the quantity and quality of intestinal transporters and/or enzymes that impact systemic availability.

Oral drug absorption is a complex process and, as a result, its predictive modeling and simulation continue to be a challenge in humans. Thus, several mechanistic approaches have emerged to better predict oral absorption and bioavailability (Huang et al., 2009), including quasi-equilibrium models, steady-state models, and dynamic models, differing largely by their dependence of spatial and temporal variables. Dynamic models, developed and extended from the mid-1990s, include the compartmental absorption and transit (CAT) model (Yu et al., 1996), the Grass model (Grass, 1997), the gastrointestinal transit absorption (GITA) model (Sawamoto et al., 1997), the advanced compartmental absorption and transit (ACAT) model (Yu and Amidon, 1999), and the advanced dissolution, absorption and metabolism (ADAM) model (Jamei et al., 2009). All of these models treat the gastrointestinal tract as a series of linked

sequential compartments in which drug absorption occurs from each compartment as a function of time.

The ACAT model, as implemented in Gastroplus™ software, takes into account physicochemical factors (e.g., pKa, solubility, permeability), physiological factors (e.g., gastric emptying, intestinal transit, presystemic metabolism and transport) and formulation factors (e.g., dosage form and dose) in predicting oral drug absorption. In doing so, Gastroplus™ was successful in predicting the oral absorption profiles of several drugs in which transporters and/or enzymes were involved (Tubic et al., 2006; Bolger et al., 2009; Abuasal et al., 2012), as well as in predicting food effects (Henze et al., 2018), formulation effects (Cvijic et al., 2018) and drug-drug interactions (Chung and Kesisoglou, 2018; Pedersen et al., 2017). However, even though Gastroplus™ has a function for the "optimization of select parameters," accuracy in predicting plasma concentration-time profiles of a drug is still limited by the quality of data that is parameterized into the program (as is other programs). In particular, one must rely on the fidelity of *in silico* estimates for some parameters such as intestinal permeability or obtain these estimates experimentally from *in vitro* Caco-2 cells or parallel artificial membrane permeability assays (PAMPA), or from *in situ* intestinal perfusions of mice or rats. The ability of intestinal permeability to predict oral bioavailability is made even more difficult by differences between species, regional differences along the length of the small and large intestines, and by the presence and potential saturability of enzymes and transporters (Cao et al., 2006).

Membrane transporters have demonstrated an essential role in the absorption, distribution, metabolism and excretion of many drugs, although sometimes they are accompanied by species differences in functional activity and specificity (Hu and Smith, 2016; Hu et al., 2012; Chu et al., 2013). To overcome these species differences, humanized mouse models were developed in

which the human genomic DNA was introduced into mice lacking the target gene, thereby avoiding overlapping functional activities between the endogenous murine gene and the human transgene (Hu et al., 2014; Cheung and Gonzalez, 2008). For example, previous oral dose escalation studies by our group (Hu and Smith, 2016) demonstrated a linear AUC (or C_{max}) relationship with cefadroxil dose in wildtype mice. However, in humanized *PepT1* (*huPepT1*) mice as well as in human subjects (Garrigues et al., 1991), a nonlinear relationship was observed between AUC (or C_{max}) and cefadroxil dose. *In situ* jejunal perfusions indicated that this species difference was due to the greater affinity (i.e., lower K_m) of cefadroxil for human PepT1, as compared to mouse PepT1, such that saturable intestinal absorption occurred in *huPepT1* (and human subjects) but not wildtype mice. Humanized mouse models have also been generated in order to overcome species differences in drug metabolism, disposition and regulation (Cheung and Gonzalez, 2008; Liu et al., 2015; Patterson et al., 2008; Ma et al., 2007; Miksys et al., 2005).

In the present study, we hypothesized that the *in situ* intestinal permeability of cefadroxil obtained from *huPepT1* mice, as compared to *in silico* or rat values, would better predict the *in vivo* plasma concentration-time profiles of cefadroxil in humans. This approach was successfully applied to the *in vivo* performance of cefadroxil in humans after oral dose escalation, an oral drug-drug-interaction study, and after multiple oral dosing.

Materials and Methods

Physicochemical Properties of Cefadroxil. Details on the physicochemical properties of cefadroxil, including dose and dosage form information, are shown in Table 1. The values provided were based on literature information, by default values provided in GastroPlus™ v9.5 (Simulations Plus, Lancaster, CA, USA), and by the ADMET Predictor v8.5 (Simulations Plus).

***In Vivo* Pharmacokinetics of Intravenous Cefadroxil.** The plasma concentration-time profiles of cefadroxil in mice, following an 11 nmol/g (4 mg/kg) intravenous dose, were reported previously (Hu and Smith, 2016) and fit to a two-compartment body model using Phoenix WinNonlin v8.0 (Princeton, NJ) and a weighing scheme of $1/y^2$. Other models (e.g., one- and three-compartment body models) were tested but were found less suitable as judged by Akaike's information criterion. The goodness of fit was evaluated by r^2, the standard error of parameter estimates, and by visual inspection of the residual plots. The total clearance and central volume of distribution values were then adjusted for humans using an allometric scaling approach (as described below).

Intestinal Permeability of Cefadroxil. The effective permeability (P_{eff}) of cefadroxil in human intestine was unknown and, as a result, four different methods were used to estimate this parameter. They included values based on: 1) *in silico* human P_{eff} of 0.35 x 10^{-4} cm/sec (ADMET Predictor), 2) rat P_{eff} of 0.75 x 10^{-4} cm/sec (Caldwell et al., 2004), 3) wildtype mouse P_{eff} and 4) humanized *PepT1* mouse P_{eff} (Hu and Smith, 2016). The rodent values were then adjusted for humans using an allometric scaling approach (as described below).

Allometric Scaling. The predicted values of cefadroxil clearance (CL) and volume of distribution (Vd) were estimated in human subjects by Equation 1 (Santella and Henness, 1982; Hosea et al., 2009; Ito and Houston, 2005) and Equation 2 (Sanoh et al., 2015; Huh et al., 2011):

$$CL_{human} = CL_{rodent} \bullet (BW_{human} / BW_{rodent})^{0.72} \quad (1)$$

$$Vd_{human} = Vd_{rodent} \bullet (BW_{human} / BW_{rodent})^{0.89} \quad (2)$$

where BW is body weight of human (70 kg) and rodent (0.25 kg for rat and 0.02 kg for mouse). The final estimates for CL and Vd are listed in Table 2.

Given that the absorption rate constant (Ka) = $2 \bullet P_{eff} / R$ (Yu et al., 1996), and assuming that the absorption rate constant was the same between human subjects and humanized *PepT1* mice (i.e., $Ka_{human} = Ka_{huPepT1}$), the predicted P_{eff} in human subjects ($P_{eff.human}$) was estimated as:

$$P_{eff.human} = P_{eff.huPepT1} \bullet (R_{human} / R_{huPepT1}) \quad (3)$$

where R is the intestinal radius. Here, the jejunal P_{eff} of cefadroxil was obtained using the method presented in the Supplemental text (see Table S1 and Figure S1), with P_{eff} values in other regions of the mouse intestines being estimated accordingly (see Table S2). The results were then scaled allometrically to human subjects (Table 3).

Finally, in those studies in which cefadroxil was co-administered with cephalexin, a PepT1 inhibitor, the predicted P_{eff} in human subjects [$P_{eff.human + CPX}$] was estimated as:

$$P_{eff.human + CPX} = P_{eff.huPepT1 + CPX} \bullet (R_{human} / R_{huPepT1}) \quad (4)$$

Here, the P_{eff} value of cefadroxil was obtained during *in situ* jejunal perfusions of *huPepT1* mice when coperfused with 10 mM cephalexin (Hu and Smith, 2016). P_{eff} values in other regions of the mouse intestines were then estimated accordingly (see Supplemental text and Table S2), and scaled allometrically to human subjects (Table 3).

A flow chart outlining our overall approach is shown in Figure 1.

Parameter Sensitivity Analysis (PSA). Accurate input parameters are crucial for obtaining meaningful predictions of the oral performance of cefadroxil using the ACAT model. Therefore, several physiological (i.e., intestinal transit time, length, radius, pH, permeability and fluid volume) and pharmacokinetic (i.e., clearances and volumes of distribution) properties of cefadroxil were examined to determine which parameters, if any, might most influence the *in silico* predictions. Specifically, the effect of parameter sensitivity on the maximum plasma concentration (C_{max}) and area under the plasma concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) was determined for cefadroxil at a human dose of 5 mg/kg. Test factors used in the PSA were scaled by10-fold in each direction.

***In Silico* Predictions of Oral Cefadroxil Performance.** All simulations for the plasma concentration-time profiles of cefadroxil were performed using GastroPlus v9.5 software. The ACAT model conditions included "human-physical-fasted" and "Opt logD Model SA/V 6.1." Input parameters produced by the ADMET Predictor v8.5 remained unchanged except for that of allometric scaling. Cefadroxil was administered as immediate release solution in 250 mL of

water, regardless of dose. The oral plasma concentration-time data in humans were obtained from the literature after dose escalation of cefadroxil (5, 15, 30 mg/kg) and during a drug-drug interaction study of 5 mg/kg cefadroxil + 45 mg/kg cephalexin (Garrigues et al., 1991), and from an oral multiple dose study of 500 mg (6.7 mg/kg) cefadroxil every six hours (Santella and Henness, 1982). Population estimates (i.e., mean, 90% confidence interval and 95% probability) were also obtained from 25 bootstrap analyses, and the precited values compared to observed values in humans for the maximum plasma concentration (C_{max}) and area under the plasma concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}).

Results

Parameter Sensitivity Analysis (PSA). As shown in Figure 2A, the C_{max} of cefadroxil was most sensitive to changes in CL, V1 and P_{eff}, followed by modest changes caused by small intestinal radius, and by little to no change by the other physiological parameters. Figure 2B showed that cefadroxil AUC_{0-t} was most sensitive to changes in CL, with modest changes caused by small intestinal radius, transit time and P_{eff}. Collectively, it appeared that intestinal permeability had the greatest effect on cefadroxil, in which intestinal permeability was positively correlated with drug exposure, resulting in 3.5- and 3.0-fold changes in C_{max} and AUC_{0-t}, respectively, over a 100-fold range of P_{eff} values.

Effect of Species-Dependent Intestinal Permeability on Predicting the Systemic Oral Exposure of Cefadroxil in Human Subjects. Predicted plasma concentration-time profiles of cefadroxil in human subjects were generated using *in silico* estimates of intestinal P_{eff}, rat P_{eff} (jejunal), wildtype mouse P_{eff} (jejunal vs. segmental) and humanized *PepT1* mouse P_{eff} (jejunal vs. segmental) at an oral cefadroxil dose of 5 mg/kg. As shown in Figures 3A and 3B, the predicted plasma concentrations of cefadroxil were substantially lower than that observed in humans when using *in silico* or rat P_{eff} estimates, respectively. In fact, the percent error of predicted vs. observed was on the order of 51% for C_{max} and 37% for AUC_{0-t} when using these two approaches (Table 4). Although P_{eff} estimates from wildtype mice (segmental or jejunum) also poorly predicted the plasma concentration-time profiles of cefadroxil (Figure 3C or Figure 3D), much better predictions were observed when P_{eff} estimates from *huPepT1* mice (jejunal or segmental) were applied, in which the percent error was about 28% for C_{max} and about 10%

AUC_{0-t} (Figure 3E or Figure 3F). Based on these results, mouse P_{eff} was further analyzed after oral cefadroxil doses of 15 and 30 mg/kg. As shown in Figure 4 and Table 4, P_{eff} values from *huPepT1* mice continued to provide substantially better predictions of cefadroxil system exposure than from wildtype mice, for both of the higher dose levels. In fact, the correlation between observed and predicted C_{max} (Figure 5A) or AUC_{0-t} (Figure 5B) of cefadroxil at the 5, 15 and 30 mg/kg oral doses was more congruent when P_{eff} was based on *huPepT1* as compared to wildtype mice.

Comparison of Segmental versus Jejunal Permeability Approach on Predicting the Systemic Oral Exposure of Cefadroxil in Human Subjects. At the onset, it was unclear as to whether the plasma concentration-time profiles of cefadroxil would be better predicted by P_{eff} values based from the jejunum alone or from all segments of the small and large intestines (i.e., duodenum, jejunum, ileum and colon). As a result, we performed simulations based on both approaches and found that neither approach had a clear advantage over the other in the dose range studied (Table 4). In fact, there was no difference between the segmental vs. jejunal approach in predicting C_{max} (Figure 6A) or AUC_{0-t} (Figure 6B) for both wildtype and *huPepT1* mice, as demonstrated by all slopes being within 10% of unity. This finding was consistent with the great majority of cefadroxil being absorbed from regions having similar P_{eff} values, such as that observed in the duodenum and jejunum (Figure 7).

Population Analysis of the Drug-Drug Interaction Study for Cefadroxil ± Cephalexin in Human Subjects. The *huPepT1* P_{eff} approach was further evaluated for its ability to predict the plasma concentration-time profiles of 5 mg/kg oral cefadroxil in human subjects when

administered in the presence of 45 mg/kg oral cephalexin, a PepT1 inhibitor (Garrigues et al., 1991). As shown in Table 5, the % error for C_{max} and AUC_{0-t} values of cefadroxil were < 20% of that in human subjects when based on either the segmental or jejunal P_{eff} approach. Moreover, as shown in Figures 8A (segmental) and 8B (jejunal), the 90% confidence intervals for the plasma concentration-time profiles of cefadroxil overlapped with most of the observed data in human subjects.

Population Analysis of the Plasma Concentration-Time Profiles for Cefadroxil in Human Subjects after Oral Multiple Dosing. Since the therapeutic efficacy of cefadroxil (and most drugs in general) is assessed using steady-state plasma concentrations, the *huPepT1* P_{eff} approach was further evaluated for its ability to predict the plasma concentration-time profiles of cefadroxil in human subjects when administered 500 mg (6.7 mg/kg) orally every 6 hours for 24 hours (i.e., 4 doses) (Santella and Henness, 1982). As shown in Table 6, the % error for C_{max} and AUC_{0-t} values of cefadroxil were < 23% and < 5%, respectively, of that in human subjects when based on either the segmental or jejunal P_{eff} approach. In addition, as shown in Figures 8C (segmental) and 8D (jejunal), the 90% confidence intervals for the plasma concentration-time profiles of cefadroxil during multiple dose sampling appeared to overlap with most of the observed data in human subjects.

Discussion

Cefadroxil, a PepT1 substrate, is a first generation aminocephalosporin that has good patient compliance and a relatively broad spectrum of antibacterial activity (Pfeffer et al., 1977; Tanrisever and Santella, 1986). At equivalent oral doses, cefadroxil has a greater drug exposure and a longer serum half-life as compared to cephalexin and cephradine (Pfeffer et al., 1977). Importantly, cefadroxil is rapidly and almost completely absorbed following oral administration, with more than 90% of drug being excreted unchanged in the urine over 24 hr (Garrigues et al., 1991). However, because of the nonlinear absorption reported by these same authors for cefadroxil in humans, the drug's maximum plasma concentration (C_{max}) and systemic exposure (AUC) are more difficult to predict, in contrast to drugs that exhibit linear pharmacokinetics. Although cefadroxil is relatively safe with few severe adverse reactions (PDR.net, 2018), accurate predictions of C_{max} and AUC are extremely valuable, in general, in determining efficacy and safety for first-in-man clinical trials, especially for those drugs that are administered orally (FDA Guidance, 2005).

Clearance (CL) and volume of distribution (Vd) have been successfully predicted in humans by interspecies allometric scaling (Martinez et al., 2006; Mahmood et al., 2006; Mahmood, 2002; Mahmood, 1999). However, it is still problematic to translate the absorption rate constant (Ka) and oral bioavailability (F) from preclinical studies to clinical trials (Musther et al., 2014), especially for drugs that have transporter-mediated intestinal uptake. Indeed, some investigators have attempted, but with limited success, to correlate rat and human jejunal permeabilities, and to then extend these correlations to fraction absorbed and oral bioavailability (Fagerholm et al., 1996; Cao et al., 2006). Although differences in carrier-mediated transport were noted in one study as a significant factor in reducing the confidence in predictions (Fagerholm et al., 1996),

differences in presystemic drug metabolism were identified as a limiting factor in the other study (Cao et al., 2006). Moreover, such comparisons are challenging because of disparities between relevant gene expression profiles obtained during *in vitro* and *in vivo* conditions (Sun et al., 2002), and because of differences between species in drug capacity (V_{max}) and affinity (K_m) of related transporters (Hu et al., 2012; Song et al., 2017).

Humanized mouse models have been developed in an attempt to improve the predictability of pharmacokinetics, metabolic contributions, drug toxicity and receptor response when translating results from animals to human subjects (Scheer and Wilson, 2015; Katoh et al, 2004; Gonzalez and Yu, 2006; Scheer and Wolf, 2013). In particular, our laboratory generated humanized PepT1 (*huPepT1*) mice (Hu et al., 2014) and demonstrated that the correlation between systemic exposure (or C_{max}) of cefadroxil with oral dose escalation in humans was more similar to that of *huPepT1* mice as compared to wildtype animals (Hu and Smith, 2016). This current study extended these results and addressed the ability of *huPepT1* mouse intestinal permeability to predict the oral dose nonlinear pharmacokinetics of cefadroxil in humans without the need for using transport parameters (i.e., V_{max} and K_m) scaled for humans. In doing so, we made the following major observations: 1) the C_{max} and $AUC_{0\text{-}t}$ of cefadroxil were better predicted using intestinal permeability estimates (both segmental and jejunal) from *huPepT1* than from wildtype mice; 2) intestinal permeabilities based on *in silico* and rat estimates gave worse predictions; 3) accurate predictions were possible for cefadroxil during oral dose escalation, a drug-drug interaction with cephalexin, and during multiple oral dosing; and 4) the great majority of cefadroxil was absorbed in the duodenal and jejunal segments of the small intestine.

CL and V1 showed the greatest effect on C_{max} and $AUC_{0\text{-}t}$ (Figure 2) and, as a result, these two parameters were optimized in our analysis (Table 2). P_{eff} also showed a significant effect on

the plasma concentration-time profile of orally administered cefadroxil, indicating that an accurate assessment of this parameter was essential for improved predictions (Figure 2). Thus, we placed a significant effort on how to best estimate intestinal permeability, first by comparing *in silico* and rodent estimates, and then by comparing jejunal versus multiple intestinal segments.

It should be appreciated that the substitution of human PepT1 for mouse PepT1 had no effect on the total clearance (or renal clearance) of cefadroxil since this parameter did not differ between wildtype and *huPepT1* mice after both low (i.e., 11 nmol/g or 4 mg/kg) and high (528 nmol/g or 192 mg/kg) intravenous bolus administrations of drug (Hu and Smith, 2016). Moreover, renal PepT1 plays a very minor role in the tubular reabsorption of cefadroxil in kidney, accounting for only 5% of this process as compared to 95% being reabsorbed by PepT2 (Shen et al., 2007). Based on the Vss of CEF, the drug would be restricted to extracellular fluid (ECF). Given Vd (ECF) = $7 + 8 \cdot fu = 7 + 8 \cdot 0.8$, the Vss equals 13.4 L (fu for CEF is 0.8; Shen et al., 2007). Based on 75 kg human, the 0.185 L/kg value (V1 + V2, Table 2) equals 13.9 L.

There is scant information on the *in vitro* - *in vivo* extrapolation (IVIVE) of scaling factors for intestinal transport proteins, especially with respect to kinetic data (e.g., V_{max} and K_m) describing the active uptake and oral absorption of transporter substrates or drugs. Literature-obtained relative expression factors (i.e., human protein expression divided by Caco-2 protein expression for a given transporter) have been reported to range from 0.4 to 5.1 for P-glycoprotein and from 1.1 to 90 for breast cancer resistance protein (Harwood et al., 2016). This variability, especially from different laboratories, has made it difficult to apply IVIVE for successful pharmacokinetic outcomes in human subjects. This difficulty may be due to a variety of reasons, including that of *in vitro* accuracy and reproducibility of cell culture systems, culture conditions, inconsistent intestinal expression of transporters and expression quantification, and post-

translational effects on transporter activity. As shown by these same authors (Harwood et al., 2016), a 4.3-fold increase (optimization) in the V_{max} of P-glycoprotein was required to account for the drug-drug interaction between orally administered digoxin and rifampin in eight healthy volunteers. Because of the difficulty in scaling kinetic data such as V_{max}, whether estimated *in vitro* from cell cultures or PAMPA, or *in situ* from single-pass intestinal perfusions, we elected to use a concentration-dependent permeability approach and to then allometrically scale the results from mouse to human based on intestinal radius.

During our analysis, we found that *in silico*, rat and wildtype mouse estimates of jejunal permeability were inadequate predictors of cefadroxil oral pharmacokinetics (Figures 3 and 4). However, segmental and jejunal estimates of *huPepT1* mouse permeability both gave improved estimates of the plasma concentration-time profiles of orally administered cefadroxil (Figures 3 and 4), the former approach being more physiologically correct. However, it may not make much of a difference in this specific case because the duodenal and jejunal permeabilities were similar for cefadroxil (Table 3), representing intestinal regions where most of the drug was predicted to be absorbed (Figure 7). It was also observed, using *huPepT1* mouse permeabilities (both segmental and jejunal), that the population predictions of oral cefadroxil pharmacokinetics were well characterized during a drug-drug interaction study with cephalexin (Figures 8A and B), and during multiple oral dosing of cefadroxil (Figures 8C and D). Thus, our approach in applying concentration-dependent permeabilities based on *huPepT1* mice gave improved predictions of oral cefadroxil pharmacokinetics (i.e., C_{max} and $AUC_{0\text{-}t}$) under nonlinear conditions and for a number of study designs. One caveat is that we assumed, in our analyses, that the absorption rate constant of cefadroxil was the same between human subjects and humanized *PepT1* mice (i.e., $Ka_{human} = Ka_{huPepT1}$). We feel this is a reasonable assumption since valacyclovir, another PepT1

substrate therapeutic, had similar Ka values in humans (0.68 hr^{-1}) and *huPepT1* (0.86 hr^{-1}) mice (Epling et al., 2018).

In summary, the current studies have demonstrated that simulation software (i.e., GastroPlus), in combination with intestinal permeability estimates from *huPepT1* mice, can be used to predict the oral pharmacokinetic behavior of a therapeutic agent in humans without the need for "artificial" scaling of V_{max}. Moreover, our approach was applied for the first time to the nonlinear intestinal absorption of a model PepT1 substrate, cefadroxil. This approach may have great practical value in the accurate prediction of the plasma concentration-time profiles during oral single and multiple dosing, and for drug-drug interaction studies of new chemical entities in humans that are primarily absorbed in the intestines by PepT1. The possibility of extending this approach to compounds which are absorbed by other intestinal uptake and/or efflux transporters would have to be tested and validated experimentally.

Author Contributions

Participated in research design: Hu, Smith

Conducted experiments: Hu

Performed data analysis: Hu

Wrote or contributed to the writing of the manuscript: Hu, Smith

References

Abuasal BS, Bolger MB, Walker DK, Kaddoumi A. In silico modeling for the nonlinear absorption kinetics of UK-343,664: a P-gp and CYP3A4 substrate. Mol Pharm. 2012;9(3):492-504.

Bolger MB, Lukacova V, Woltosz WS. Simulations of the nonlinear dose dependence for substrates of influx and efflux transporters in the human intestine. AAPS J. 2009;11(2):353-363.

Caldwell GW, Masucci JA, Yan Z, Hageman W. Allometric scaling of pharmacokinetic parameters in drug discovery: can human CL, Vss and t1/2 be predicted from in-vivo rat data? Eur J Drug Metab Pharmacokinet. 2004;29(2):133-143.

Cao X, Gibbs ST, Fang L, Miller HA, Landowski CP, Shin HC, Lennernas H, Zhong Y, Amidon GL, Yu LX, Sun D. Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model. Pharm Res. 2006;23(8):1675-1686.

Cheung C, Gonzalez FJ. Humanized mouse lines and their application for prediction of human drug metabolism and toxicological risk assessment. J Pharmacol Exp Ther. 2008;327(2):288-299.

Chu X, Bleasby K, Evers R. Species differences in drug transporters and implications for translating preclinical findings to humans. Expert Opin Drug Metab Toxicol. 2013;9(3):237-252.

Chung J, Kesisoglou F. Physiologically Based Oral Absorption Modelling to Study Gut-Level Drug Interactions. Journal of Pharmaceutical Sciences. 2018;107(1):18-23.

Cvijic S, Ibric S, Parojcic J, Djuris J. An in vitro - in silico approach for the formulation and characterization of ranitidine gastroretentive delivery systems. Journal of Drug Delivery Science and Technology. 2018;45:1-10.

Epling D, Hu Y, Smith DE. Evaluating the intestinal and oral absorption of the prodrug valacyclovir in wildtype and huPepT1 transgenic mice. Biochem Pharmacol. 2018 [Epub ahead of print].

FDA. Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. https://wwwfdagov/downloads/drugs/guidances/ucm078932pdf. 2005:30.

Fagerholm U, Johansson M, Lennernäs H. Comparison Between Permeability Coefficients in Rat and Human Jejunum. Pharmaceutical Research. 1996;13(9):1336-1342.

Garrigues TM, Martin U, Peris-Ribera JE, Prescott LF. Dose-dependent absorption and elimination of cefadroxil in man. Eur J Clin Pharmacol. 1991;41(2):179-183.

Gonzalez FJ, Yu AM. Cytochrome P450 and xenobiotic receptor humanized mice. Annu Rev Pharmacol Toxicol. 2006;46:41-64.

Grass GM. Simulation models to predict oral drug absorption from in vitro data. Advanced Drug Delivery Reviews. 1997;23(1):199-219.

Harwood MD, Achour B, Neuhoff S, Russell MR, Carlson G, Warhurst G, Rostami-Hodjegan A. In vitro-in vivo extrapolation scaling factors for intestinal p-glycoprotein and breast cancer resistance protein: Part II. the Impact of cross-laboratory variations of intestinal transporter relative expression factors on predicted drug disposition. Drug Metab Dispos. 2016;44(3):476-480.

Henze LJ, Griffin BT, Christiansen M, Bundgaard C, Langguth P, Holm R. Exploring gastric emptying rate in minipigs: Effect of food type and pre-dosing of metoclopramide. Eur J Pharm Sci. 2018;118:183-190.

Hosea NA, Collard WT, Cole S, Maurer TS, Fang RX, Jones H, Kakar SM, Nakai Y, Smith BJ, Webster R, Beaumont K. Prediction of human pharmacokinetics from preclinical information: comparative accuracy of quantitative prediction approaches. J Clin Pharmacol. 2009;49(5):513-533.

Hu Y, Chen X, Smith DE. Species-dependent uptake of glycylsarcosine but not oseltamivir in Pichia pastoris expressing the rat, mouse, and human intestinal peptide transporter PEPT1. Drug Metab Dispos. 2012;40(7):1328-1335.

Hu Y, Smith DE. Species differences in the pharmacokinetics of cefadroxil as determined in wildtype and humanized PepT1 mice. Biochem Pharmacol. 2016;107:81-90.

Hu Y, Xie Y, Wang Y, Chen X, Smith DE. Development and characterization of a novel mouse line humanized for the intestinal peptide transporter PEPT1. Mol Pharm. 2014;11(10):3737-3746.

Huang W, Lee SL, Yu LX. Mechanistic approaches to predicting oral drug absorption. AAPS J. 2009;11(2):217-224.

Huh Y, Smith DE, Feng MR. Interspecies scaling and prediction of human clearance: comparison of small- and macro-molecule drugs. Xenobiotica. 2011;41(11):972-987.

Ito K, Houston JB. Prediction of human drug clearance from in vitro and preclinical data using physiologically based and empirical approaches. Pharm Res. 2005;22(1):103-112.

Jamei M, Marciniak S, Feng K, Barnett A, Tucker G, Rostami-Hodjegan A. The Simcyp population-based ADME simulator. Expert Opin Drug Metab Toxicol. 2009;5(2):211-223.

Katoh M, Matsui T, Nakajima M, Tateno C, Kataoka M, Soeno Y, Horie T, Iwasaki K, Yoshizato K, Yokoi T. Expression of human cytochromes P450 in chimeric mice with humanized liver. Drug Metab Dispos. 2004;32(12):1402-1410.

Liu Z, Megaraj V, Li L, Sell S, Hu J, Ding X. Suppression of pulmonary CYP2A13 expression by carcinogen-induced lung tumorigenesis in a CYP2A13-humanized mouse model. Drug Metab Dispos. 2015;43(5):698-702.

Ma X, Shah Y, Cheung C, Guo GL, Feigenbaum L, Krausz KW, Idle JR, Gonzalez FJ. The PREgnane X receptor gene-humanized mouse: a model for investigating drug-drug interactions mediated by cytochromes P450 3A. Drug Metab Dispos. 2007;35(2):194-200.

Mahmood I. Prediction of clearance in humans from in vitro human liver microsomes and allometric scaling. A comparative study of the two approaches. Drug Metabol Drug Interact. 2002;19(1):49-64.

Mahmood I. Prediction of clearance, volume of distribution and half-life by allometric scaling and by use of plasma concentrations predicted from pharmacokinetic constants: a comparative study. J Pharm Pharmacol. 1999;51(8):905-910.

Mahmood I, Martinez M, Hunter RP. Interspecies allometric scaling. Part I: prediction of clearance in large animals. J Vet Pharmacol Ther. 2006;29(5):415-423.

Martinez M, Mahmood I, Hunter RP. Interspecies allometric scaling: prediction of clearance in large animal species: part II: mathematical considerations. J Vet Pharmacol Ther. 2006;29(5):425-432.

Miksys SL, Cheung C, Gonzalez FJ, Tyndale RF. Human CYP2D6 and mouse CYP2Ds: organ distribution in a humanized mouse model. Drug Metab Dispos. 2005;33(10):1495-1502.

Musther H, Olivares-Morales A, Hatley OJ, Liu B, Rostami Hodjegan A. Animal versus human oral drug bioavailability: do they correlate? Eur J Pharm Sci. 2014;57:280-291.

Patterson D, Graham C, Cherian C, Matherly LH. A humanized mouse model for the reduced folate carrier. Mol Genet Metab. 2008;93(2):95-103.

Pedersen JM, Khan EK, Bergström CAS, Palm J, Hoogstraate J, Artursson P. Substrate and method dependent inhibition of three ABC-transporters (MDR1, BCRP, and MRP2). European Journal of Pharmaceutical Sciences. 2017;103:70-76.

Pfeffer M, Jackson A, Ximenes J, de Menezes JP. Comparative human oral clinical pharmacology of cefadroxil, cephalexin, and cephradine. Antimicrob Agents Chemother. 1977;11(2):331-338.

PDR.net 2018. http://www.pdr.net/drug-summary/Cefadroxil-Tablets-cefadroxil-3008.2894.

Sanoh S, Naritomi Y, Fujimoto M, Sato K, Kawamura A, Horiguchi A, Sugihara K, Kotake Y, Ohshita H, Tateno C, Horie T, Kitamura S, Ohta S. Predictability of plasma concentration-time curves in humans using single-species allometric scaling of chimeric mice with humanized liver. Xenobiotica. 2015;45(7):605-614.

Santella PJ, Henness D. A review of the bioavailability of cefadroxil. J Antimicrob Chemother. 1982;10 Suppl B:17-25.

Sawamoto T, Haruta S, Kurosaki Y, Higaki K, Kimura T. Prediction of the plasma concentration profiles of orally administered drugs in rats on the basis of gastrointestinal transit kinetics and absorbability. J Pharm Pharmacol. 1997;49(4):450-457.

Scheer N, Wilson ID. A comparison between genetically humanized and chimeric liver humanized mouse models for studies in drug metabolism and toxicity. Drug Discov Today. 2016;21(2):250-263.

Scheer N, Wolf CR. Xenobiotic receptor humanized mice and their utility. Drug Metab Rev. 2013;45(1):110-121.

Shalaeva M, Kenseth J, Lombardo F, Bastin A. Measurement of dissociation constants (pKa values) of organic compounds by multiplexed capillary electrophoresis using aqueous and cosolvent buffers. J Pharm Sci. 2008;97(7):2581-2606.

Shen H, Ocheltree SM, Hu Y, Keep RF, Smith DE. Impact of genetic knockout of PEPT2 on cefadroxil pharmacokinetics, renal tubular reabsorption, and brain penetration in mice. Drug Metab Dispos. 2007;35(7):1209-1216.

Song F, Hu Y, Jiang H, Smith DE. Species Differences in Human and Rodent PEPT2-Mediated Transport of Glycylsarcosine and Cefadroxil in Pichia Pastoris Transformants. Drug Metab Dispos. 2017;45(2):130-136.

Sun D, Lennernas H, Welage LS, Barnett JL, Landowski CP, Foster D, Fleisher D, Lee KD, Amidon GL. Comparison of human duodenum and Caco-2 gene expression profiles for 12,000 gene sequences tags and correlation with permeability of 26 drugs. Pharm Res. 2002;19(10):1400-1416.

Tang H, Mayersohn M. A global examination of allometric scaling for predicting human drug clearance and the prediction of large vertical allometry. J Pharm Sci. 2006;95(8):1783-1799.

Tanrisever B, Santella PJ. Cefadroxil. A review of its antibacterial, pharmacokinetic and therapeutic properties in comparison with cephalexin and cephradine. Drugs. 1986;32 Suppl 3:1-16.

Tubic M, Wagner D, Spahn-Langguth H, Bolger MB, Langguth P. In silico modeling of non-linear drug absorption for the P-gp substrate talinolol and of consequences for the resulting pharmacodynamic effect. Pharm Res. 2006;23(8):1712-1720.

Yang B, Smith DE. In silico absorption analysis of valacyclovir in wildtype and Pept1 knockout mice following oral dose escalation. Pharm Res. 2017;34(11):2349-2361.

Yu LX, Lipka E, Crison JR, Amidon GL. Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. Adv Drug Deliv Rev. 1996;19(3):359-376.

Yu LX, Amidon GL. A compartmental absorption and transit model for estimating oral drug absorption. Int J Pharm. 1999;186(2):119-125.

Footnotes

This work was supported by the National Institutes of Health National Institute of General Medical Sciences [Grant R01-GM115481 to D.E.S].

TABLE 1

Predicted physicochemical properties of cefadroxil

Property	Cefadroxil	Source
Molecular formula	$C_{16}H_{17}N_3O_5S$	
Molecular weight	363.39	
Predicted logP (neutral)	-2.08	ADMET Predictor V8.5
pka1	2.55	Shaleva et al, 2008
pKa2	7.21	
pka3	9.71	
Aqueous solubility	2.68 mg/mL (pH 5.13)	ADMET Predictor V8.5
Diffusion coefficient	0.72×10^{-5} cm^2/sec	ADMET Predictor V8.5
Mean precipitation time	900 sec	GastroPlus Default
Drug particle density	1.2 g/mL	GastroPlus Default
Particle size (radius)	25.0 μm	GastroPlus Default
Dosage form (human)	Solution	
Dose volume (human)	250 mL	

TABLE 2

Observed and allometric scaling of primary pharmacokinetic parameters of cefadroxil

Parameter	Mouse (observed)	% CV	Mouse (optimized)	% Error	Human (predicted)
CL (L/hr/kg)	0.92	10.5	0.88	-4.3	0.079
CLD (L/hr/kg)	0.38	29.8	-	-	0.041
V1 (L/kg)	0.18	20.6	0.16	15.5	0.063
V2 (L/kg)	0.31	42.9	-	-	0.122

Plasma concentration-time profiles were fit to a two-compartment disposition model in mice after an 11 nmol/g (4 mg/kg) intravenous bolus dose of cefadroxil (Yu and Smith, 2016).

CL, total plasma clearance such that K10=CL/V1; CLD, clearance between the central and peripheral compartments such that CLD=CL12=CL21, K12=CL12/V1 and K21=CL21/V2; V1, volume of distribution in central compartment; V2, volume of distribution in peripheral compartment.

TABLE 3

Allometric scaling of cefadroxil P_{eff} to humans when estimated by the *in situ* permeability of small and large intestines from wildtype (WT) and humanized (hu) PepT1 mice

	WT	hu	WT	hu	WT	hu	WT	hu	hu
	5 mg/kg		15 mg/kg		30 mg/kg		500 mg (6.7 mg/kg)		CEF + CEP
Stomach	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Duo	3.95	1.69	2.19	0.96	1.40	0.58	3.46	1.49	0.35
Jej 1	4.48	1.24	2.49	0.70	1.58	0.42	3.93	1.09	0.26
Jej 2	4.32	1.19	2.40	0.68	1.53	0.41	3.78	1.05	0.25
Ile 1	1.95	0.80	1.08	0.45	0.69	0.27	1.71	0.71	0.17
Ile 2	1.86	0.76	1.03	0.43	0.66	0.26	1.63	0.67	0.16
Ile 3	1.73	0.71	0.96	0.40	0.61	0.24	1.52	0.63	0.15
Caecum	0.01	0.20	0.01	0.11	0.01	0.07	0.01	0.17	0.04
Asc Colon	0.02	0.24	0.01	0.13	0.01	0.08	0.02	0.21	0.05

P_{eff}, intestinal permeability in units of cm/sec (x 10^{-4}).

Intestinal segments include Duo, duodenum; Jej, jejunum; Ile, ileum; Asc colon, ascending colon. The P_{eff} values of cefadroxil in humans were predicted after single oral doses of 5, 15 and 30 mg/kg, after 500 mg (6.7 mg/kg) oral doses every six hours, and after the drug-drug interaction of 5 mg/kg cefadroxil plus 45 mg/kg cephalexin (CEF + CEP). The method for obtaining these values was described in the Supplemental Material.

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

TABLE 4

Comparison of predicted and observed pharmacokinetic parameters of cefadroxil in humans when estimated by *in silico* and allometric scaling methods of *in situ* intestinal permeability from wildtype (WT) and humanized (hu) PepT1 mice

Dose (mg/kg)	P_{eff} Method	C_{max} (μg/mL)			AUC_{0-t} (μg•hr/mL)		
		Observed	Predicted	% Error	Observed	Predicted	% Error
5	In Silico	14.7	7.1	-51.7		28.4	-37.5
	Rat Jej		7.2	-51.1		28.7	-36.8
	WT Seg		30.2	106	45.5	55.1	21.1
	WT Jej		30.7	109		55.6	22.3
	hu Seg		19.1	29.8		49.2	8.3
	hu Jej		18.6	26.4		50.5	11.0
15	WT Seg	33.9	71.8	112		158	25.4
	WT Jej		73.7	117		163	29.5
	hu Seg		37.1	9.5	126	119	-5.2
	hu Jej		37.6	10.9		125	-0.4
30	WT Seg	53.8	110	104		287	20.1
	WT Jej		91.0	69.0		307	28.6
	hu Seg		53.0	-1.5	239	196	-17.8
	hu Jej		56.2	4.5		209	-12.6

Jej, P_{eff} based on jejunal permeability; Seg, P_{eff} based on segmental permeabilities of duodenum, jejunum, ileum and colon. Observed values were obtained from the literature (Garrigues et al., 1991).

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

TABLE 5

Population analysis for drug-drug interaction of 5 mg/kg cefadroxil + 45 mg/kg cephalexin in human subjects when estimated by the intestinal permeability from *huPepT1* mice

Parameter	Observed	Segmental P_{eff}			Jejunal P_{eff}		
		Predicted	% CV	% Error	Predicted	% CV	% Error
C_{max} (μg/mL)	8.4	6.8	27.4	-19.3	7.5	16.4	-10.5
AUC_{0-t} (μg•hr/mL)	36.8	29.7	31.4	-19.2	30.6	21.7	-16.8

Observed values were obtained from the literature (Garrigues et al., 1991).

TABLE 6

Population analysis for multiple dosing regimen of 500 mg (6.7 mg/kg) cefadroxil administered orally every six hours in human subjects when estimated by the intestinal permeability from *huPepT1* mice

		Segmental P_{eff}			Jejunal P_{eff}		
Parameter	Observed	Predicted	% CV	% Error	Predicted	% CV	% Error
C_{max} (μg/mL)	15.9	19.5	26.3	22.9	19.5	26.3	22.4
AUC_{0-t} (μg•hr/mL)	223	225	31.3	-1.0	237	34.0	4.2

Observed values were obtained from the literature (Santella and Henness, 1982).

Figure legends

Figure 1. Schematic strategy of simulations.

Figure 2. Sensitivity of predicted C_{max} (panel A) and AUC_{0-t} (panel B) to input parameters after a human oral dose of 5 mg/kg cefadroxil. Parameters were changed by multiplying the initial input values with scaling factors in the range of 0.1 to 10. Vol, volume; K12 and K21 are the distribution rate constants between the central and peripheral comparments, respectively; SITT, small intestine transit time; Len, length; Rad, radius; Duo, duodenum; Jej, jejunum; CL, clearance; V1, volume of distribution for central compartment; Peff, effective permeability.

Figure 3. Model predicted plasma concentration-time profiles of cefadroxil (CEF) after a human oral dose of 5 mg/kg using permeability estimates (P_{eff}) obtained *in silico* (panel A), and from rats (panel B), wildtype (panels C and D) and humanized (panels E and F) mice. Both segmental (Seg) and jejunal (Jej) approaches were applied in mice. Human data were obtained from the literature (Garrigues et al., 1991). WT, wildtype mice; hu, humanized *PepT1* mice.

Figure 4. Model predicted plasma concentration-time profiles of cefadroxil (CEF) after human oral doses of 15 and 30 mg/kg using permeability estimates (P_{eff}) obtained from wildtype (panels A, B, E and F) and humanized (panels C, D, G and H) mice. Both segmental (Seg) and jejunal (Jej) approaches were applied. Human data were obtained from the literature (Garrigues et al., 1991). WT, wildtype mice; hu, humanized *PepT1* mice.

Figure 5. Correlation between the segmental (Seg P_{eff}) and jejunal permeability (Jej P_{eff}) estimates in predicting the C_{max} (panel A) and AUC_{0-t} (panel B) of cefadroxil after human oral doses of 5, 15 and 30 mg/kg. The dotted line represents a slope of unity. WT, wildtype mice; hu, humanized *PepT1* mice.

Figure 6. Correlation between the observed and predicted C_{max} (panel A) and the observed and predicted AUC_{0-t} (panel B) of cefadroxil after human oral doses of 5, 15 and 30 mg/kg. The pharmacokinetic parameters were predicted using jejunal permeability (Jej P_{eff}), as estimated from wildtype (WT) and humanized (hu) *PepT1* mice. The dotted line represents a slope of unity. Human data were obtained from the literature (Garrigues et al., 1991).

Figure 7. Contribution of specific intestinal regions in the absorption of cefadroxil after a human oral dose of 5 mg/kg. Oral absorption was predicted using both segmental (Seg P_{eff}) and jejunal permeability (Jej P_{eff}), as estimated from wildtype (panels A and B, respectively) and humanized (panels C and D, respectively) mice. WT, wildtype mice; hu, humanized *PepT1* mice.

Figure 8. Population analysis of the predicted plasma concentration-time profiles of cefadroxil during a drug-drug interaction study of 5 mg/kg oral cefadroxil plus 45 mg/kg oral cephalexin (panels A and B) (human data were obtained from Garrigues et al., 1991), and during a multiple dose study of 500 mg (6.7 mg/kg) cefadroxil administered orally every 6 hours (q6h) (human data were obtained from Santella and Hennes, 1982) (panels C and D). Analyses were performed using segmental (Seg P_{eff}) and jejunal permeability (Jej P_{eff}), as estimated from humanized (hu) *PepT1* mice.

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

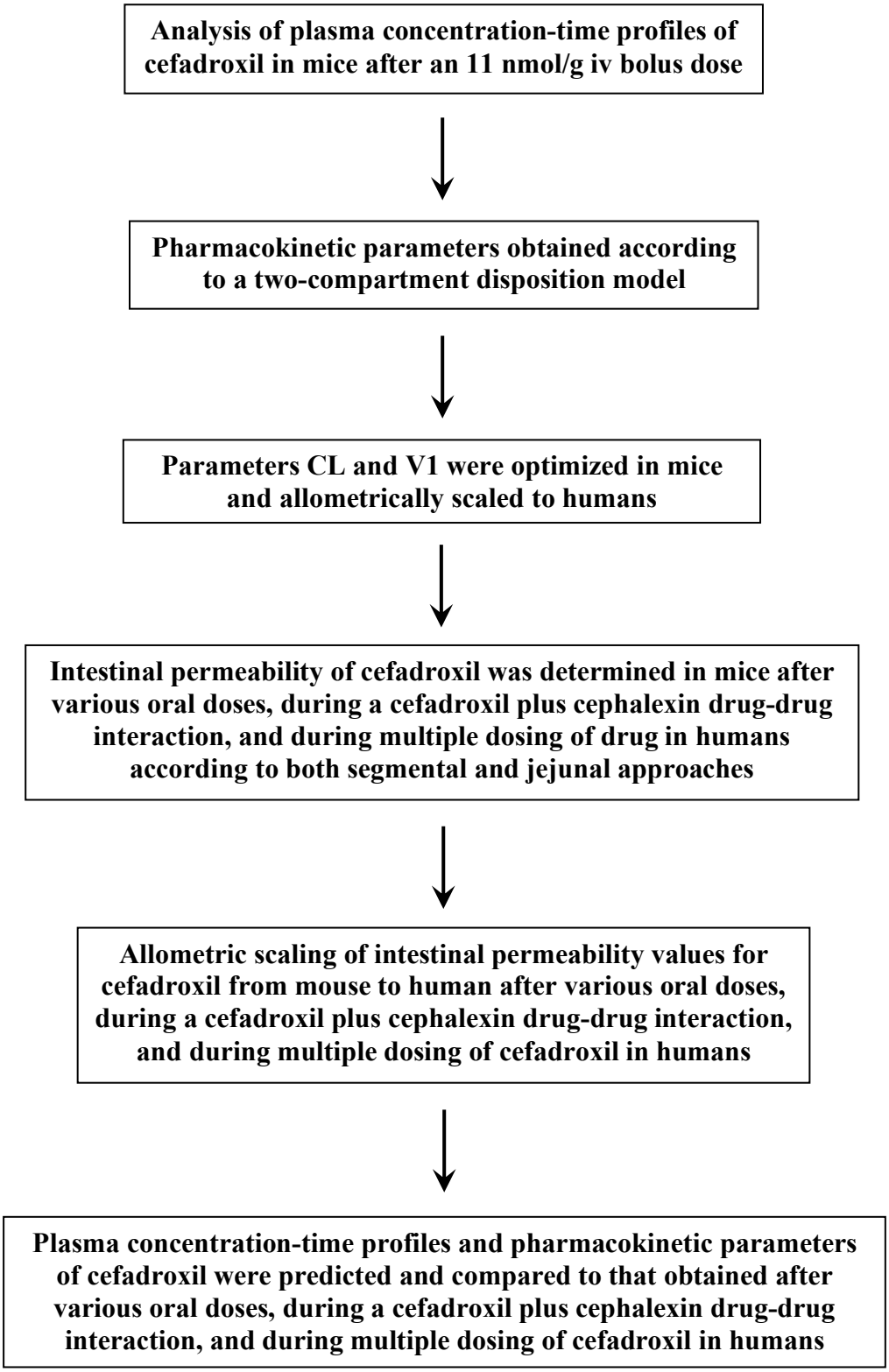


Figure 1

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

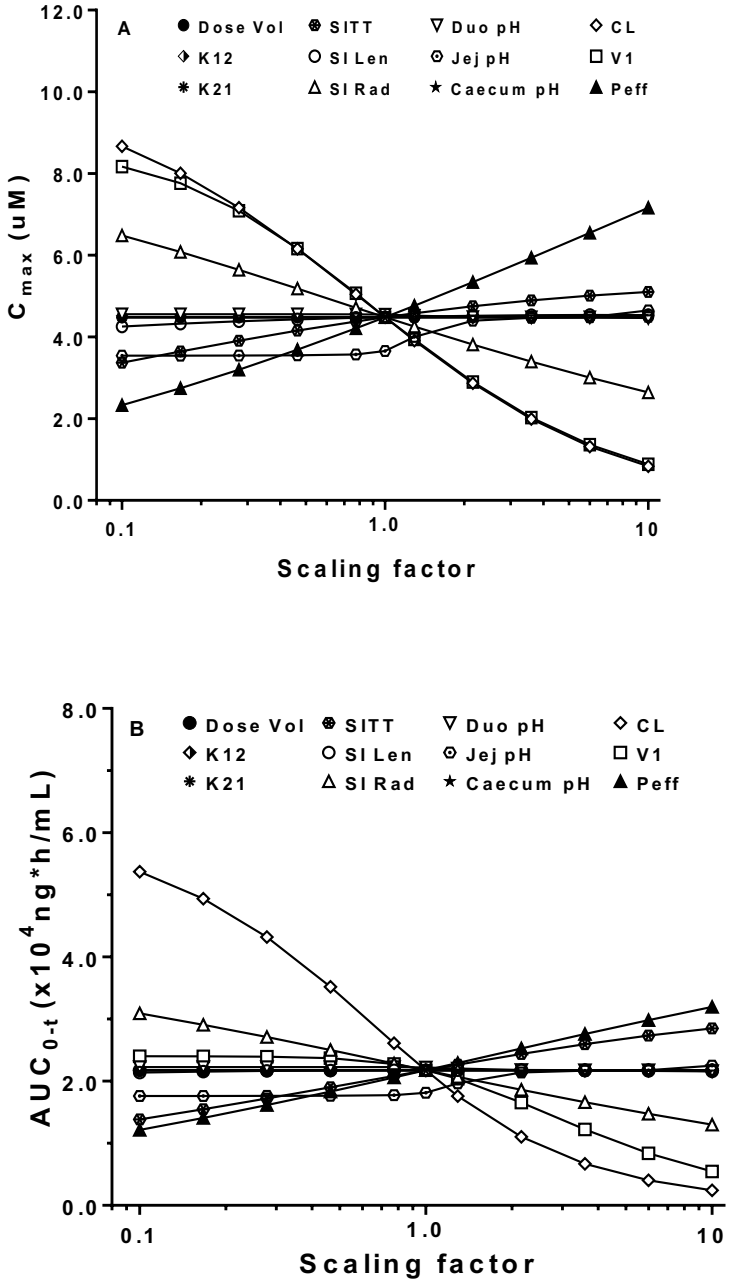


Figure 2

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

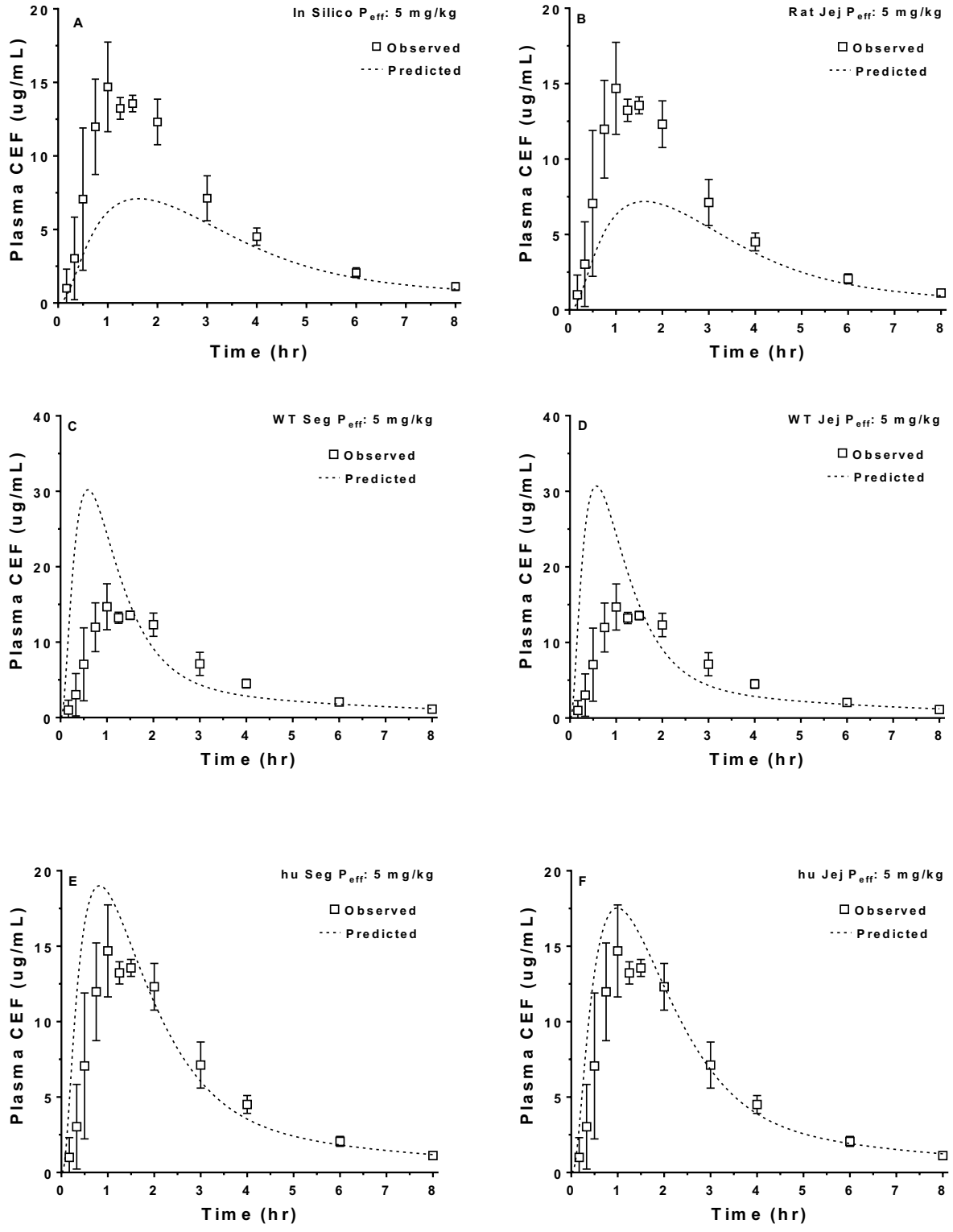


Figure 3

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

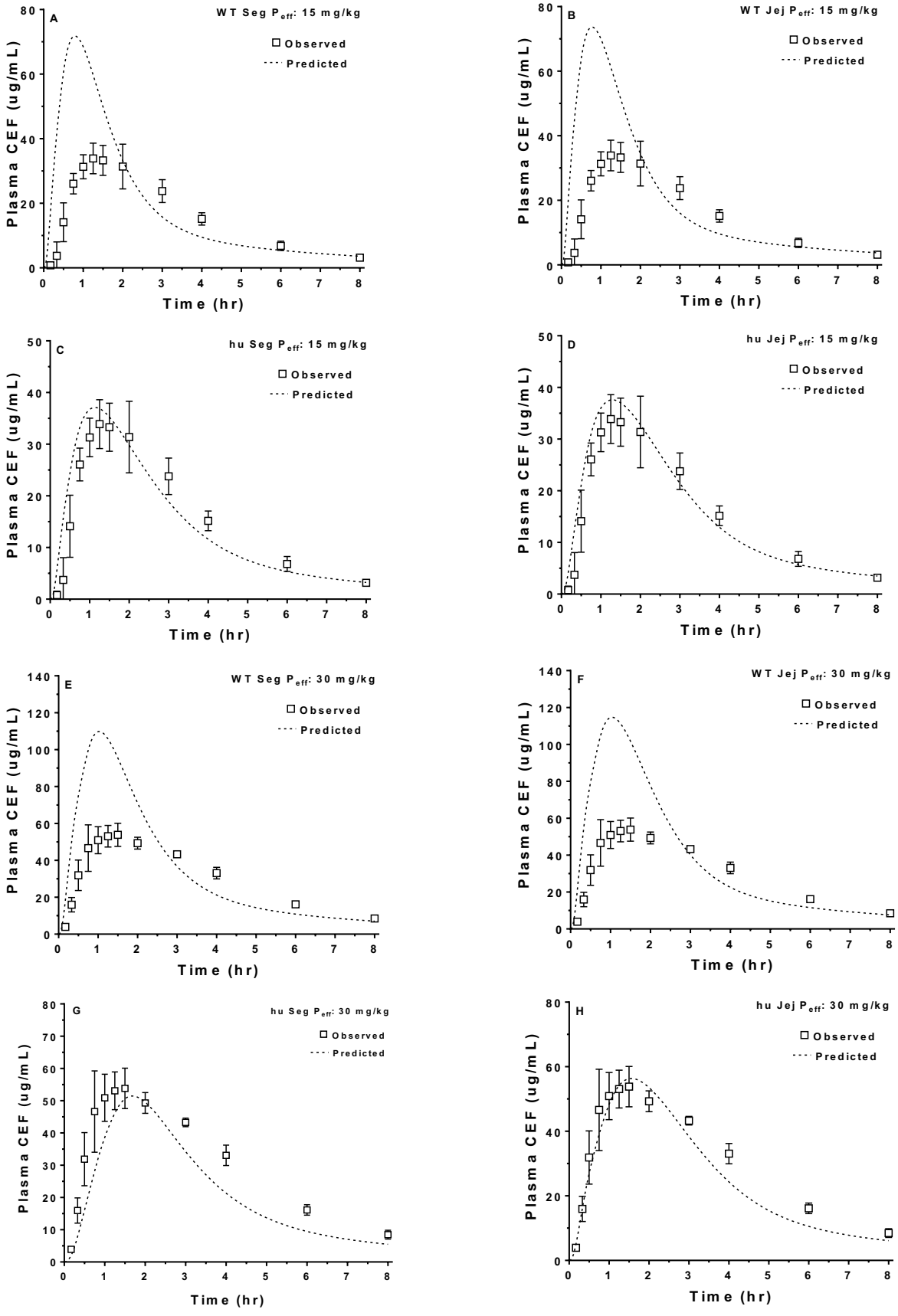


Figure 4

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

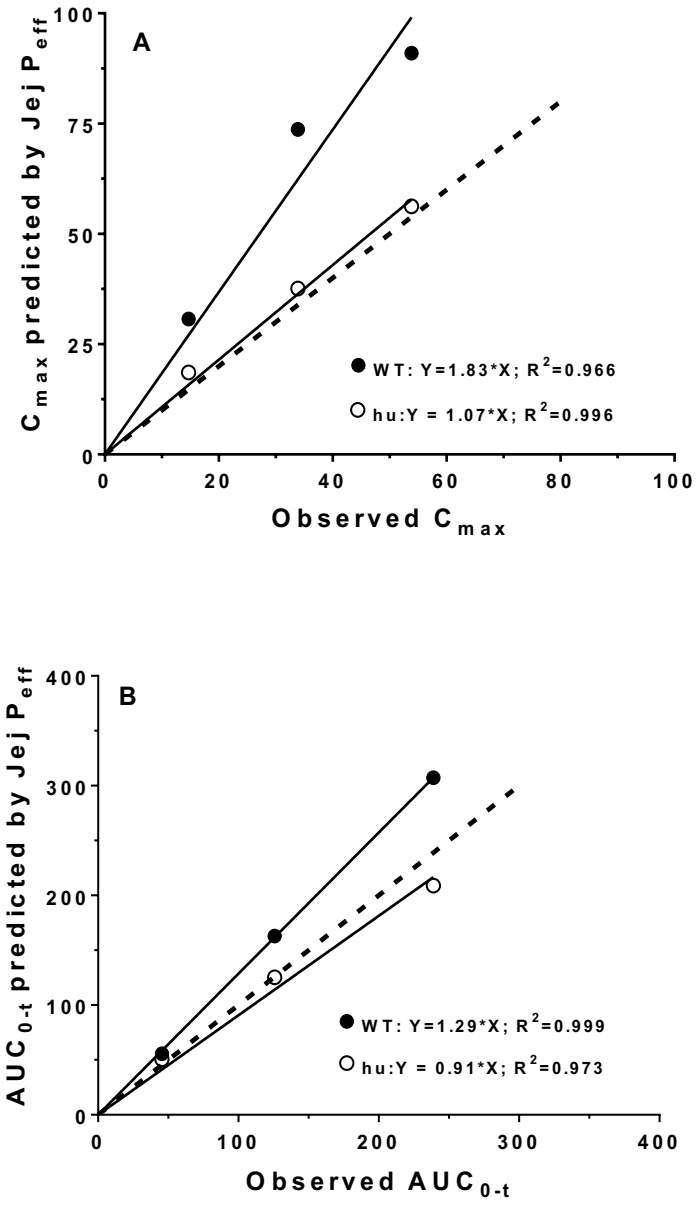


Figure 5

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

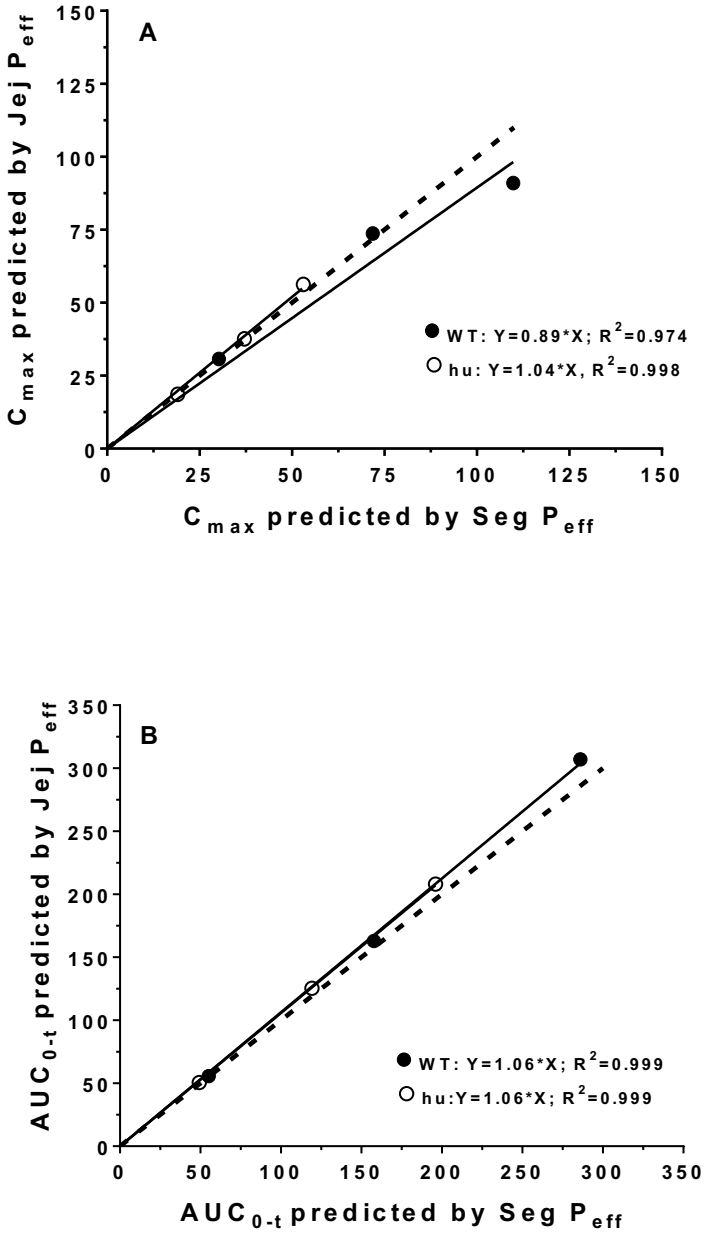
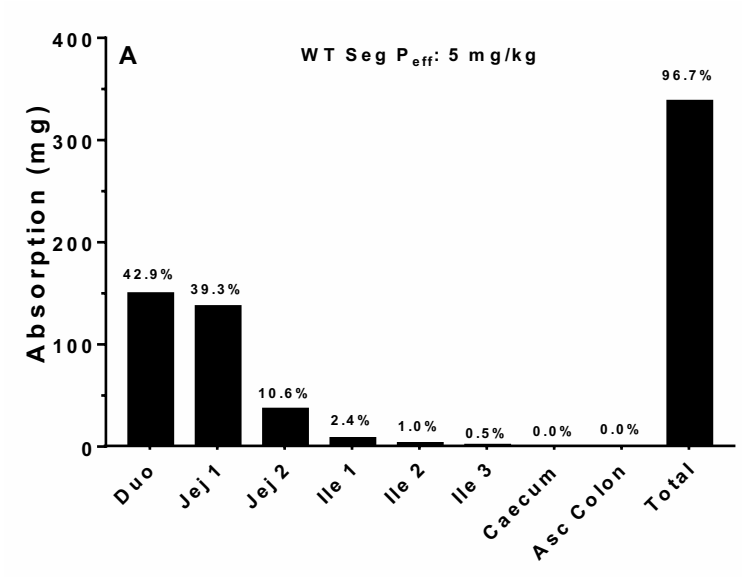
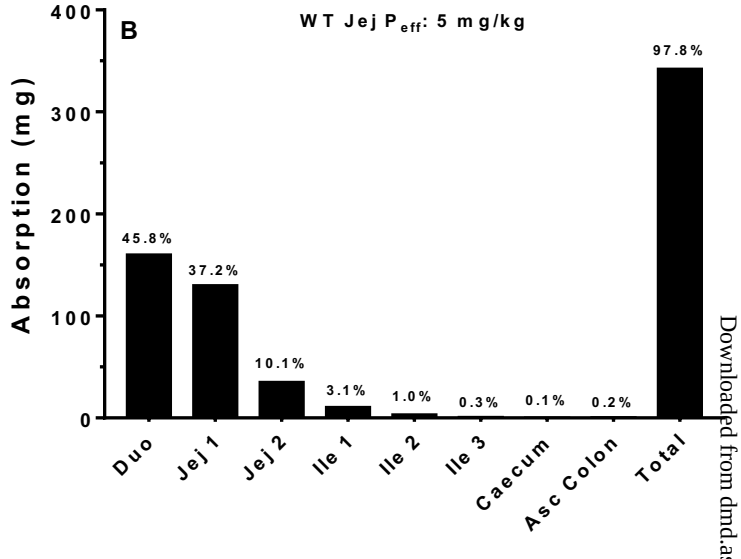
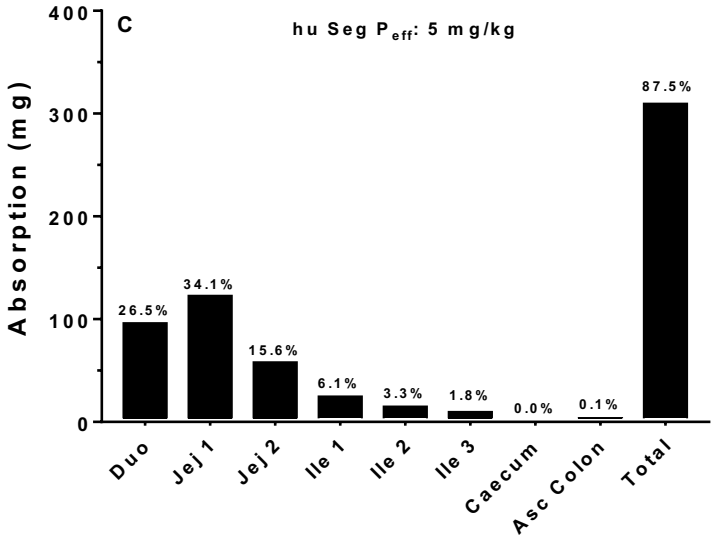


Figure 6

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.







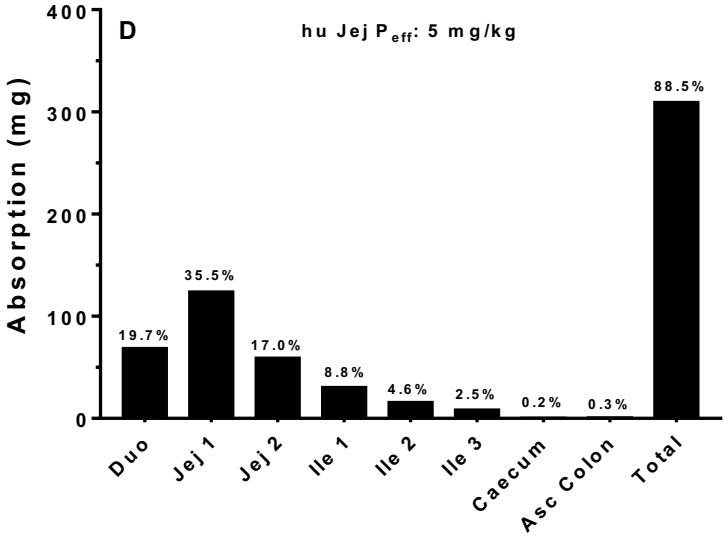


Figure 7

DMD Fast Forward. Published on December 28, 2018 as DOI: 10.1124/dmd.118.084236
This article has not been copyedited and formatted. The final version may differ from this version.

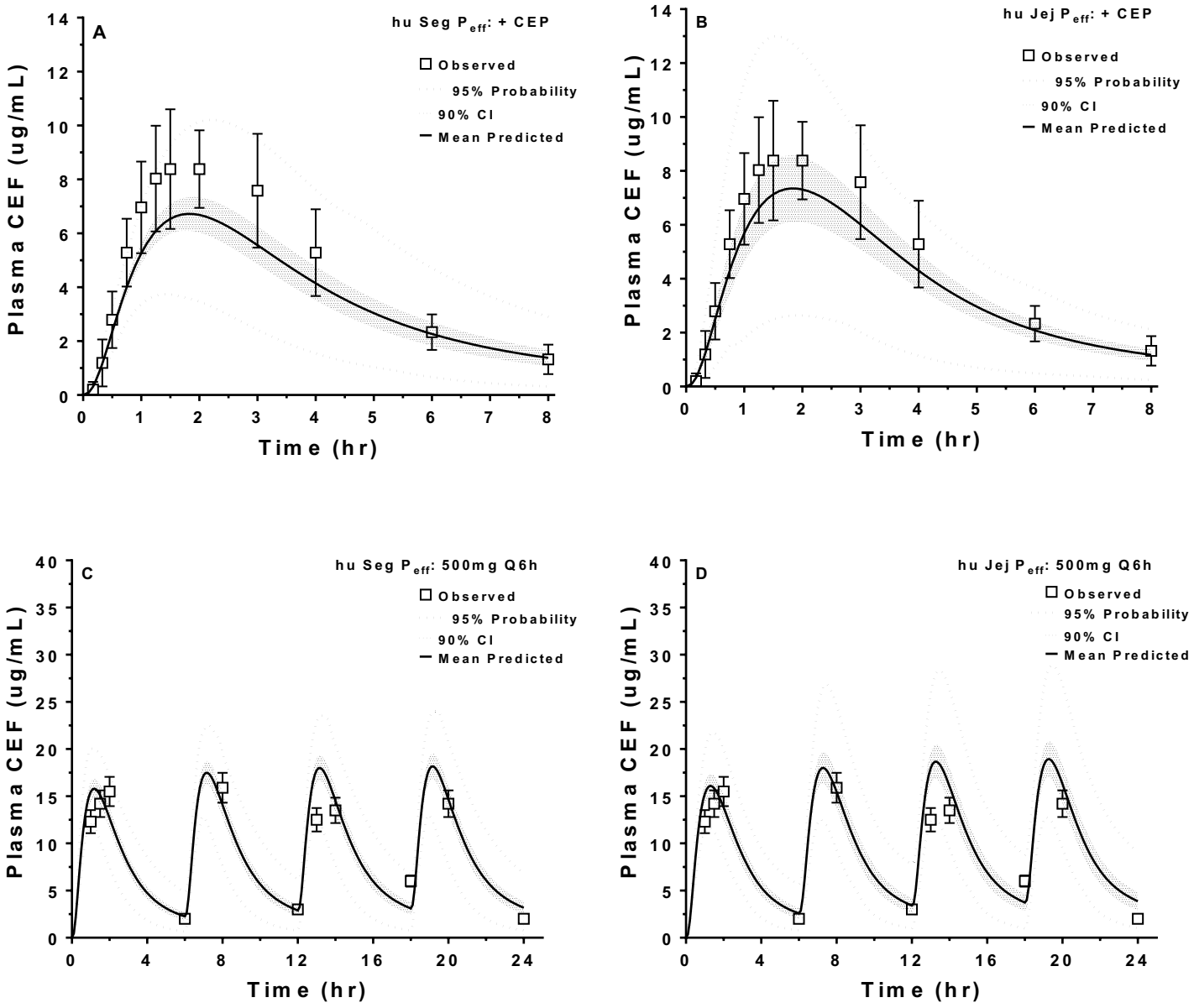


Figure 8