

## Title Page

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

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## Running Title Page

### Simulation of Cefadroxil Absorption and Disposition in Human

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**ABBREVIATIONS:** ACAT, advanced compartmental and transit; AIC, Akaike information criterion; BW, body weight; CL, clearance; FDA, Food and Drug Administration;  $P_{\text{eff}}$ , effective permeability; PepT1, peptide transporter 1;  $V_d$ , apparent volume of distribution;  $V_1$ , 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  $AUC_{0-t}$  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 ( $P_{\text{eff}}$ ) 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  $P_{\text{eff}}$  of  $0.35 \times 10^{-4}$  cm/sec (ADMET Predictor), 2) rat  $P_{\text{eff}}$  of  $0.75 \times 10^{-4}$  cm/sec (Caldwell et al., 2004), 3) wildtype mouse  $P_{\text{eff}}$  and 4) humanized *PepT1* mouse  $P_{\text{eff}}$  (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):

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

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

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

$$P_{\text{eff, human}} = P_{\text{eff, huPepT1}} \cdot (R_{\text{human}} / R_{\text{huPepT1}}) \quad (3)$$

where R is the intestinal radius. Here, the jejunal  $P_{\text{eff}}$  of cefadroxil was obtained using the method presented in the Supplemental text (see Table S1 and Figure S1), with  $P_{\text{eff}}$  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 cephalixin, a *PepT1* inhibitor, the predicted  $P_{\text{eff}}$  in human subjects [ $P_{\text{eff, human} + \text{CPX}}$ ] was estimated as:



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

Here, the  $P_{\text{eff}}$  value of cefadroxil was obtained during *in situ* jejunal perfusions of *huPepT1* mice when coperfused with 10 mM cephalixin (Hu and Smith, 2016).  $P_{\text{eff}}$  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 ( $C_{\text{max}}$ ) and area under the plasma concentration-time curve from time zero to the last measurable concentration ( $\text{AUC}_{0-t}$ ) was determined for cefadroxil at a human dose of 5 mg/kg. Test factors used in the PSA were scaled by 10-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 cephalixin (Garrigues et al., 1991), and from an oral multiple dose study of 500 mg (6.7 mg/kg) cefadroxil every six hours (Santella and Hennes, 1982). Population estimates (i.e., mean, 90% confidence interval and 95% probability) were also obtained from 25 bootstrap analyses, and the predicted 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  $P_{\text{eff}}$ , 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  $P_{\text{eff}}$ . 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  $P_{\text{eff}}$  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  $P_{\text{eff}}$ , rat  $P_{\text{eff}}$  (jejunal), wildtype mouse  $P_{\text{eff}}$  (jejunal vs. segmental) and humanized *PepT1* mouse  $P_{\text{eff}}$  (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  $P_{\text{eff}}$  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  $P_{\text{eff}}$  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  $P_{\text{eff}}$  estimates from *huPepT1* mice (jejunal or segmental) were applied, in which the percent error was about 28% for  $C_{\max}$  and about 10%

AUC<sub>0-t</sub> (Figure 3E or Figure 3F). Based on these results, mouse P<sub>eff</sub> was further analyzed after oral cefadroxil doses of 15 and 30 mg/kg. As shown in Figure 4 and Table 4, P<sub>eff</sub> 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<sub>max</sub> (Figure 5A) or AUC<sub>0-t</sub> (Figure 5B) of cefadroxil at the 5, 15 and 30 mg/kg oral doses was more congruent when P<sub>eff</sub> 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<sub>eff</sub> 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<sub>max</sub> (Figure 6A) or AUC<sub>0-t</sub> (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<sub>eff</sub> 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<sub>eff</sub> 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  $P_{\text{eff}}$  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*  $P_{\text{eff}}$  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 Hennes, 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  $P_{\text{eff}}$  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 aminoccephalosporin 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 cephalixin 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 ( $K_a$ ) 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  $AUC_{0-t}$  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  $AUC_{0-t}$  (Figure 2) and, as a result, these two parameters were optimized in our analysis (Table 2).  $P_{\text{eff}}$  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  $V_{ss}$  of CEF, the drug would be restricted to extracellular fluid (ECF). Given  $V_d(\text{ECF}) = 7 + 8 \cdot f_u = 7 + 8 \cdot 0.8$ , the  $V_{ss}$  equals 13.4 L ( $f_u$  for CEF is 0.8; Shen et al., 2007). Based on 75 kg human, the 0.185 L/kg value ( $V_1 + V_2$ , 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  $AUC_{0-t}$ ) 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.,  $Ka_{\text{human}} = Ka_{\text{huPepT1}}$ ). We feel this is a reasonable assumption since valacyclovir, another *PepT1*

substrate therapeutic, had similar  $K_a$  values in humans ( $0.68 \text{ hr}^{-1}$ ) and *huPepT1* ( $0.86 \text{ hr}^{-1}$ ) 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

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## 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 <sub>16</sub> H <sub>17</sub> N <sub>3</sub> O <sub>5</sub> S	
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 x 10 <sup>-5</sup> cm <sup>2</sup> /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  $K_{10} = CL/V_1$ ; CLD, clearance between the central and peripheral compartments such that  $CLD = CL_{12} = CL_{21}$ ,  $K_{12} = CL_{12}/V_1$  and  $K_{21} = CL_{21}/V_2$ ; V1, volume of distribution in central compartment; V2, volume of distribution in peripheral compartment.

TABLE 3

Allometric scaling of cefadroxil  $P_{\text{eff}}$  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

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

Intestinal segments include Duo, duodenum; Jej, jejunum; Ile, ileum; Asc colon, ascending colon.

The  $P_{\text{eff}}$  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 cephalixin (CEF + CEP). The method for obtaining these values was described in the Supplemental Material.

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 <sub>eff</sub> Method	C <sub>max</sub> (µg/mL)			AUC <sub>0-t</sub> (µg•hr/mL)		
		Observed	Predicted	% Error	Observed	Predicted	% Error
5	In Silico	14.7	7.1	-51.7	45.5	28.4	-37.5
	Rat Jej		7.2	-51.1		28.7	-36.8
	WT Seg		30.2	106		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	126	158	25.4
	WT Jej		73.7	117		163	29.5
	hu Seg		37.1	9.5		119	-5.2
	hu Jej		37.6	10.9		125	-0.4
30	WT Seg	53.8	110	104	239	287	20.1
	WT Jej		91.0	69.0		307	28.6
	hu Seg		53.0	-1.5		196	-17.8
	hu Jej		56.2	4.5		209	-12.6

Jej, P<sub>eff</sub> based on jejunal permeability; Seg, P<sub>eff</sub> based on segmental permeabilities of duodenum, jejunum, ileum and colon. Observed values were obtained from the literature (Garrigues et al., 1991).

TABLE 5

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

Parameter	Observed	Segmental P <sub>eff</sub>			Jejunal P <sub>eff</sub>		
		Predicted	% CV	% Error	Predicted	% CV	% Error
C <sub>max</sub> (μg/mL)	8.4	6.8	27.4	-19.3	7.5	16.4	-10.5
AUC <sub>0-t</sub> (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

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

Observed values were obtained from the literature (Santella and Hennes, 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 compartments, 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 ( $P_{\text{eff}}$ ) 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.

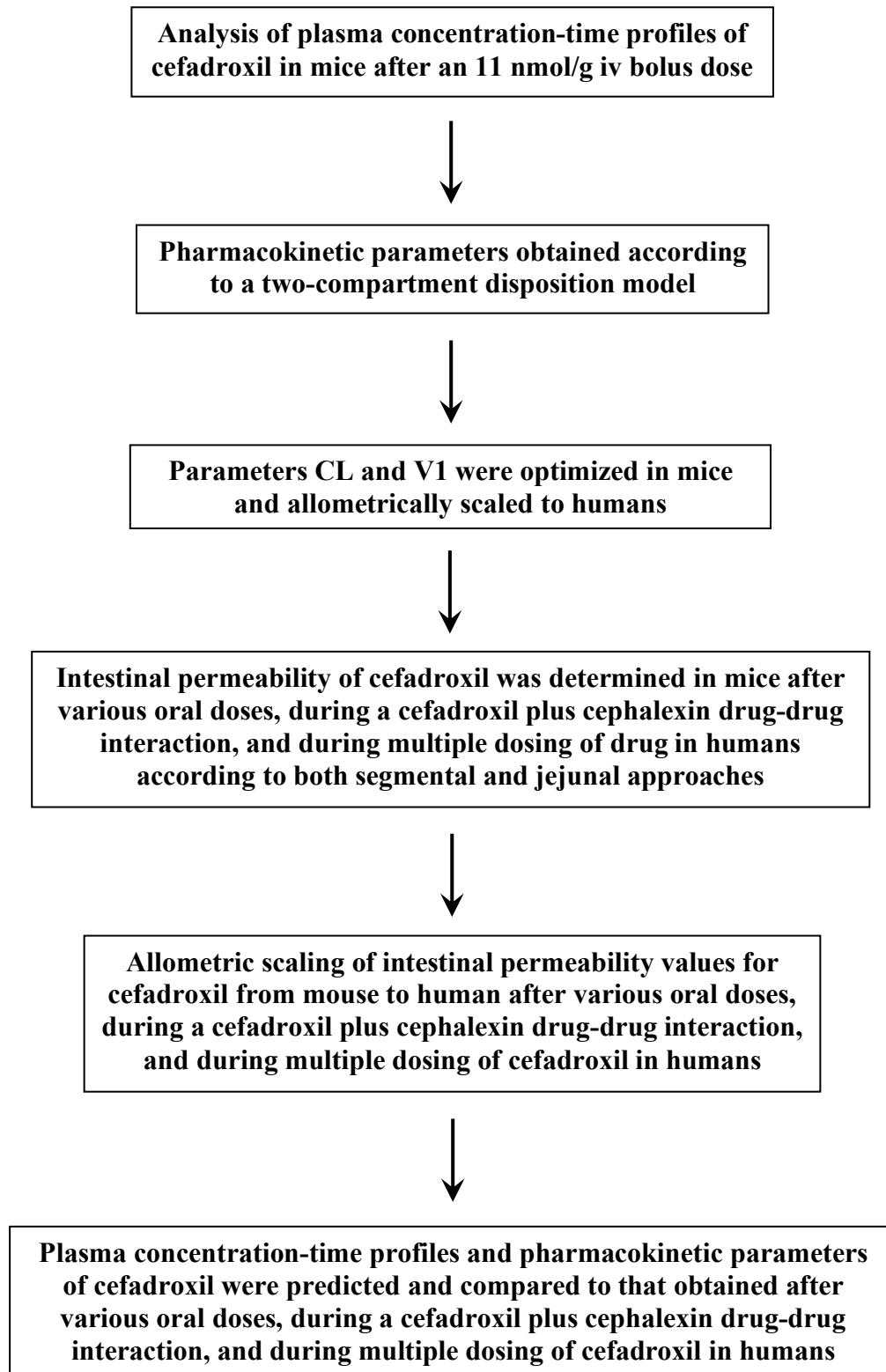
**Figure 4.** Model predicted plasma concentration-time profiles of cefadroxil (CEF) after human oral doses of 15 and 30 mg/kg using permeability estimates ( $P_{\text{eff}}$ ) obtained from wildtype (panels A, B, E and F) and humanized (panels C, D, G and H) mice. Both segmental (Seg) and jejunal (Jej) 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  $P_{\text{eff}}$ ) and jejunal permeability (Jej  $P_{\text{eff}}$ ) estimates in predicting the  $C_{\text{max}}$  (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  $C_{\text{max}}$  (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  $P_{\text{eff}}$ ), 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  $P_{\text{eff}}$ ) and jejunal permeability (Jej  $P_{\text{eff}}$ ), 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 cephalixin (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  $P_{\text{eff}}$ ) and jejunal permeability (Jej  $P_{\text{eff}}$ ), as estimated from humanized (hu) *PepT1* mice.



**Figure 1**

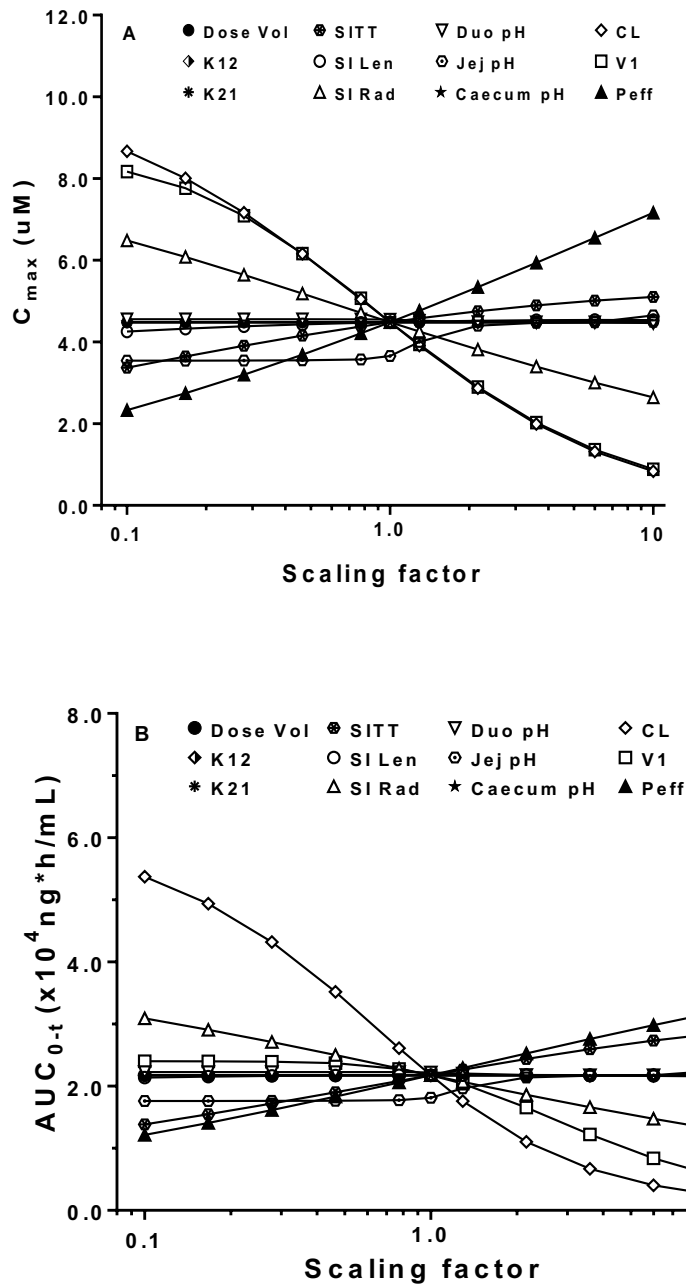


Figure 2

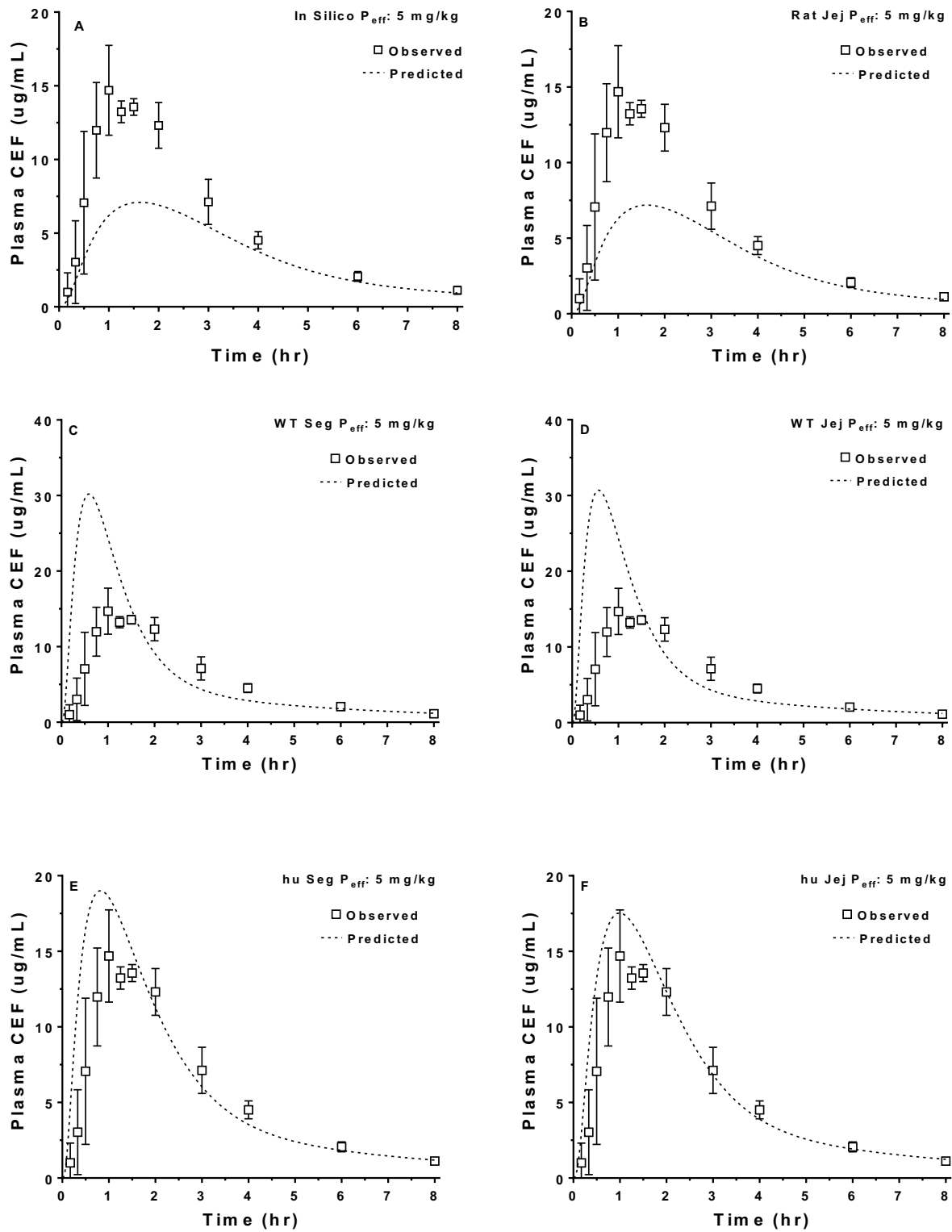


Figure 3

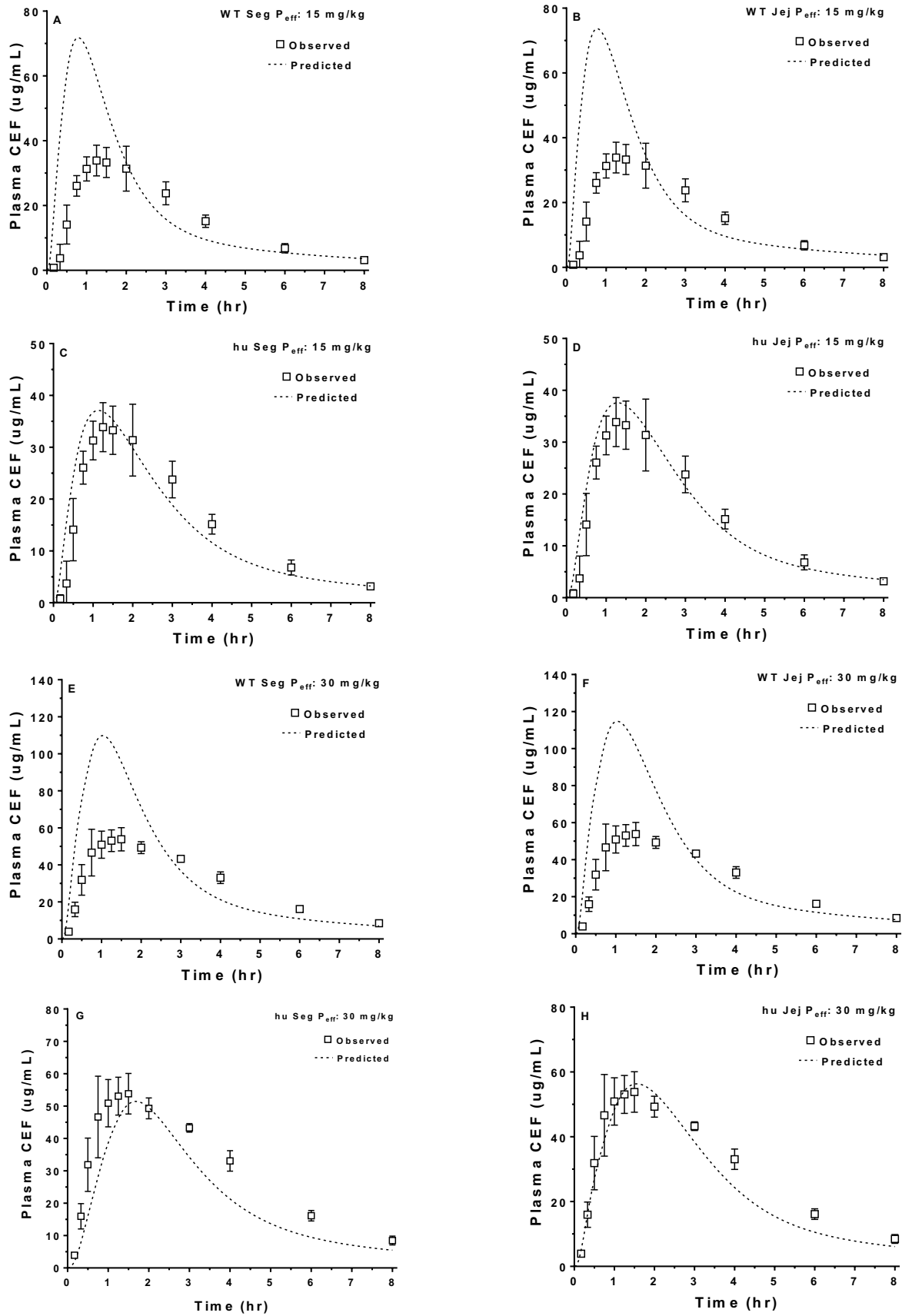


Figure 4

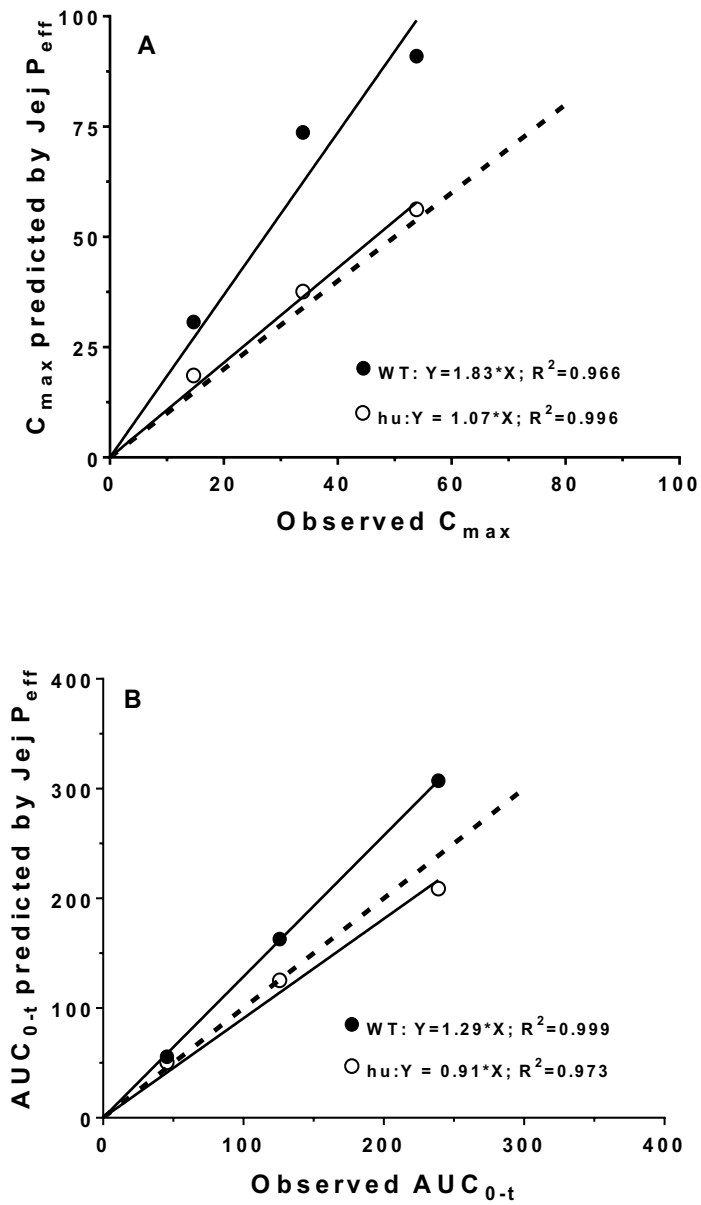


Figure 5



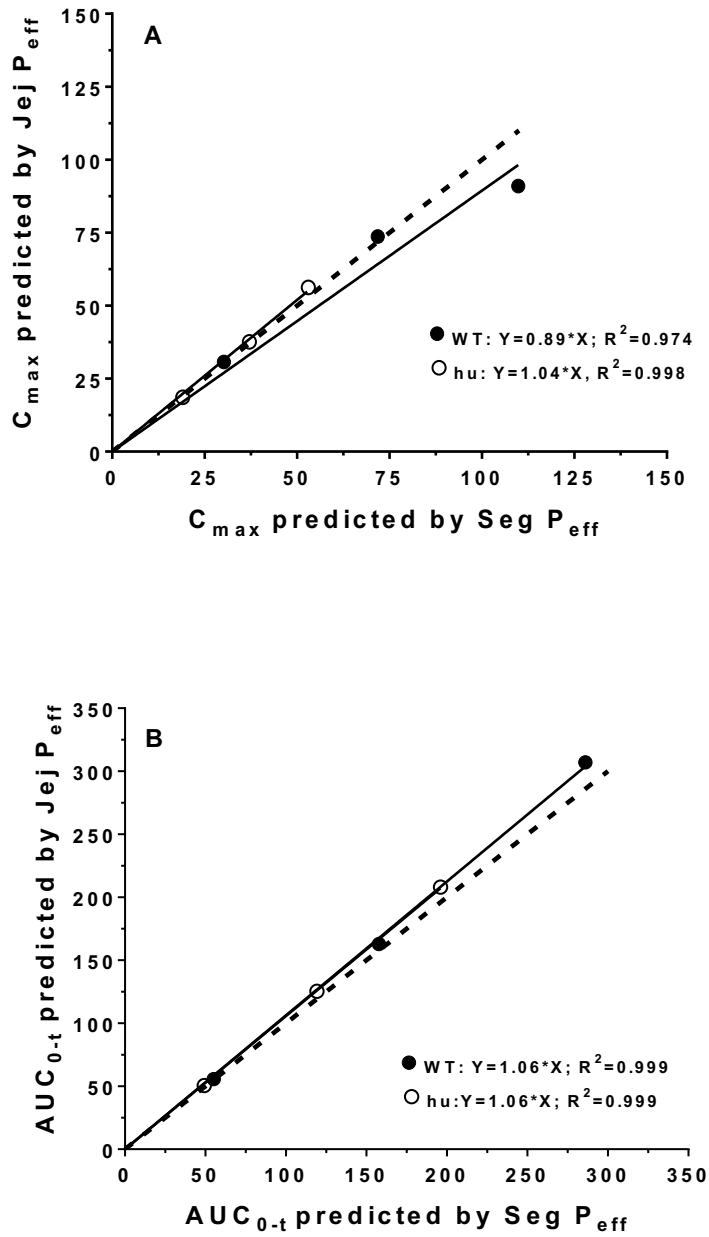


Figure 6

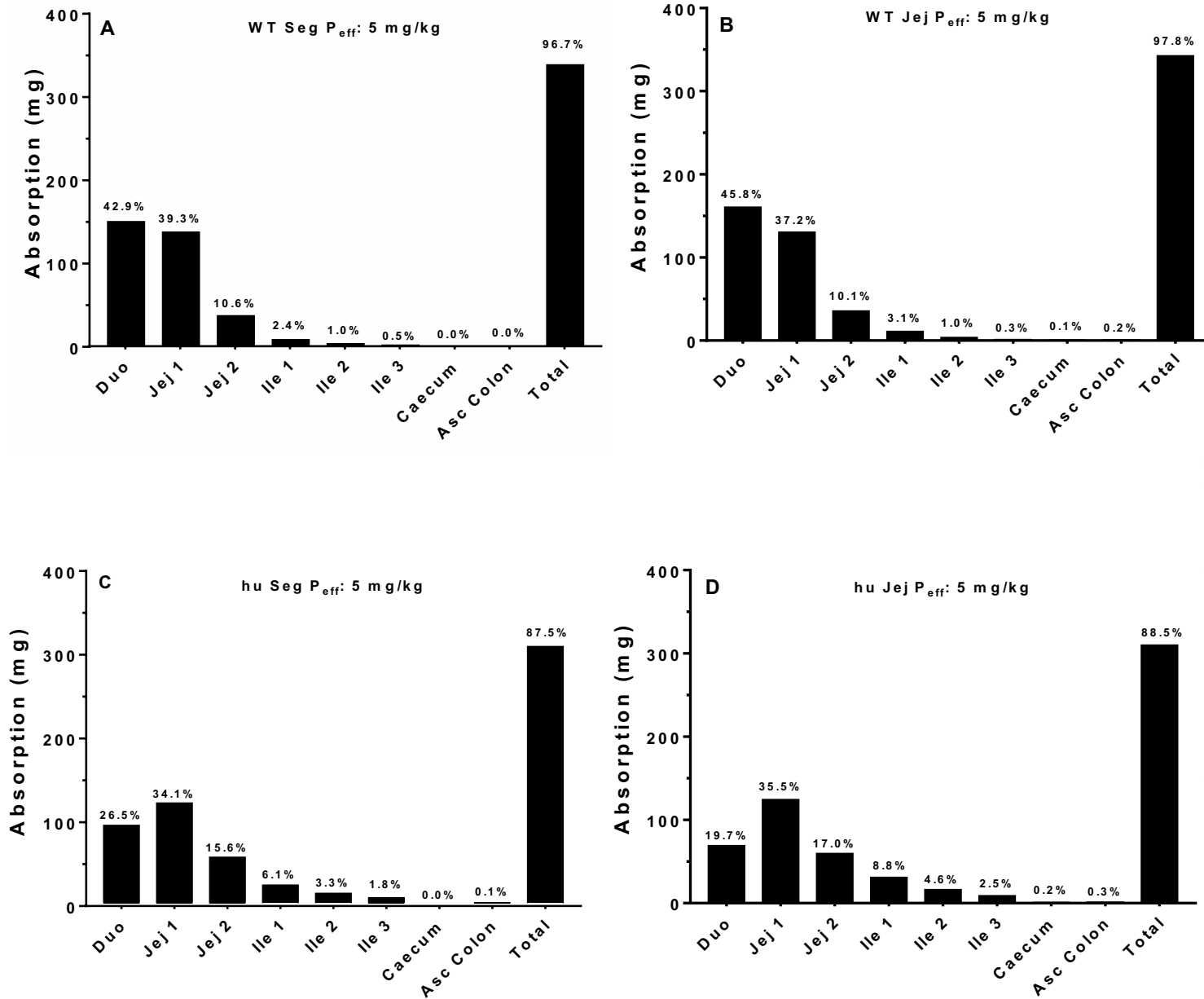


Figure 7

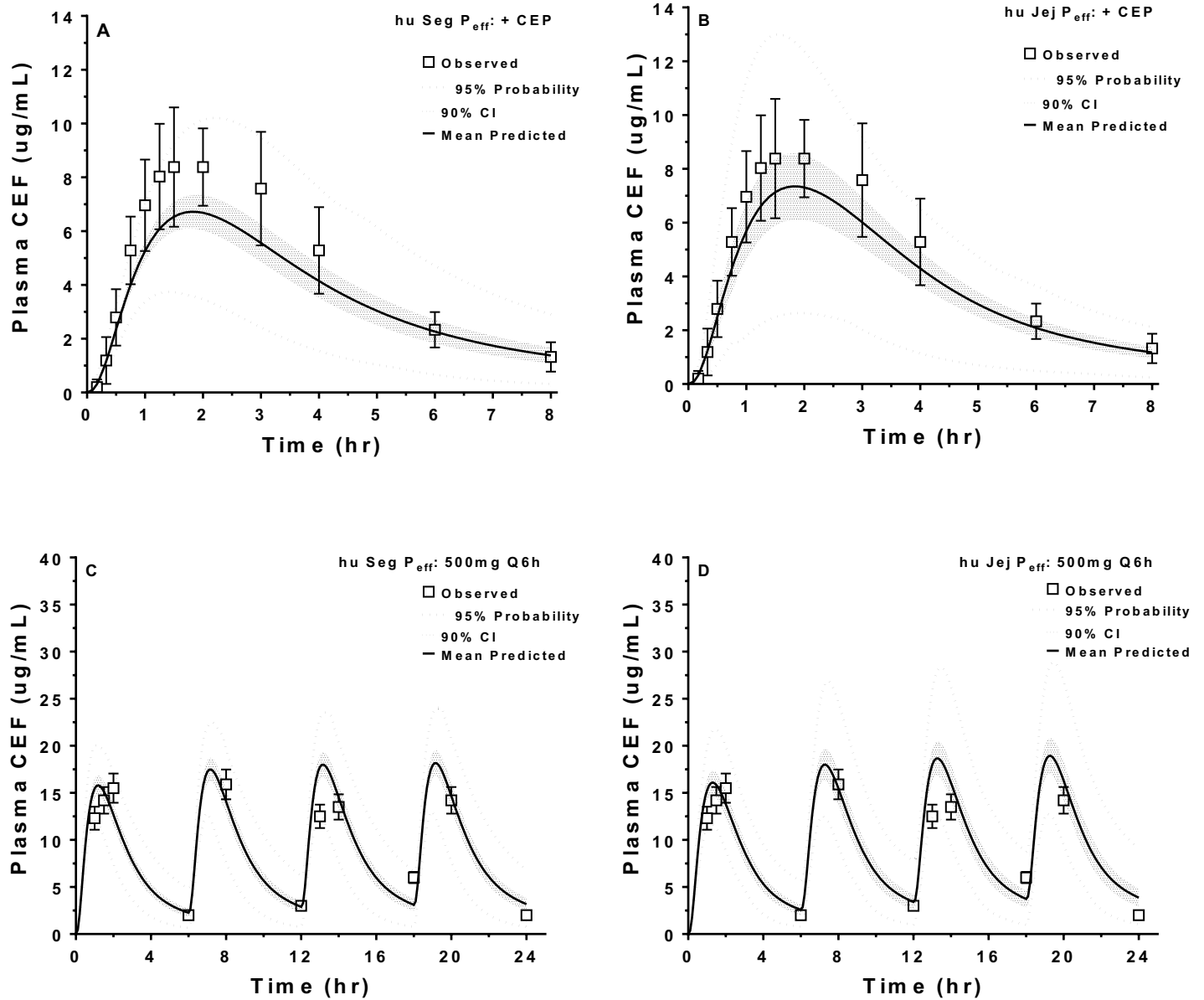


Figure 8

**Supplemental Data*****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

**Method to Estimate the Concentration-Dependent Permeability of Cefadroxil in Mouse Intestinal Segments during Single Escalating Doses and during Multiple Dosing.** The jejunal permeability values of cefadroxil were obtained from wildtype and *huPepT1* mice during *in situ* perfusions of the drug over a concentration range of 0.01-25 mM (Hu and Smith, 2016). The concentration-dependent permeability of cefadroxil (shown in Figure S1) was then fit to the equation:

$$P_{eff} = P_{eff,max} \left( 1 - \frac{I}{IC_{50} + I} \right)$$

where  $P_{eff,max}$  is the jejunal permeability of cefadroxil at 10  $\mu$ M, a concentration that is at least 270x lower than the drug's  $K_m$  value in both genotypes,  $I$  is the inhibitor concentration of cefadroxil in this self-inhibition study, and  $IC_{50}$  is the concentration of inhibitor at which the  $P_{eff}$  of cefadroxil is one-half its maximum value. The parameter estimates are shown in Table S1.

The observed jejunal  $P_{\text{eff}}$  values of 10  $\mu\text{M}$  cefadroxil in wildtype and *huPepT1* mice are presented in Table S2, along with  $P_{\text{eff}}$  estimates made in mouse jejunum from human single escalating doses (i.e., 4.1 mM at 5 mg/kg, 12.4 mM at 15 mg/kg, 24.8 mM at 30 mg/kg) and during multiple dosing [i.e., 5.5 mM at 500 mg (6.7 mg/kg) every 6 hours].

The  $P_{\text{eff}}$  values of 10  $\mu\text{M}$  cefadroxil in other intestinal segments were obtained from wildtype and *huPepT1* mice during *in situ* perfusions of the drug (Hu and Smith, 2016).  $P_{\text{eff}}$  estimates at other dose levels were then determined by proportionality (Table S2). For example, the duodenal  $P_{\text{eff}}$  of cefadroxil at 5 mg/kg was estimated in wildtype mice as:  $0.40/0.62 \times 0.55 = 0.36 \times 10^{-4}$  cm/sec. These values in mice were then scaled for human subjects by:  $P_{\text{eff, human}} = P_{\text{eff, mouse}} \cdot (R_{\text{human}}/R_{\text{mouse}})$  where R is radius of intestine (Tables 3 and S2).

**Method to Estimate the Permeability of Cefadroxil in Intestinal Segments of *huPepT1* Mice during the 5 mg/kg Cefadroxil  $\pm$  45 mg/kg Cephalexin Drug-Drug Interaction Study.**

Since the *in situ* intestinal permeability of cephalexin was unknown, we assumed that cefadroxil and cephalexin had the same  $IC_{50}$  values. Thus, cephalexin equivalents (i.e., 38.9 mM at 45 mg/kg) were added to cefadroxil (i.e., 4.1 mM at 5 mg/kg) and, at a total concentration of 43.0 mM, the jejunal permeability of cefadroxil in *huPepT1* mice was estimated according to the above equation as  $0.023 \times 10^{-4}$  cm/sec.  $P_{\text{eff}}$  estimates in the other intestinal segments were then determined by proportionality (Table S2). For example, the duodenal  $P_{\text{eff}}$  in *huPepT1* mice was estimated as:  $0.023/0.111 \times 0.154 = 0.032 \times 10^{-4}$  cm/sec. As before, these values in mice were then scaled for human subjects by:  $P_{\text{eff, human}} = P_{\text{eff, mouse}} \cdot (R_{\text{human}}/R_{\text{mouse}})$  where R is radius of intestine. (Tables 3 and S2).

TABLE S1

Model fitting of permeability parameters in wildtype (WT) and humanized (hu) PepT1 mice

Parameter	WT	hu
$P_{eff,max}$ (cm/sec x $10^{-4}$ )	$0.71 \pm 0.07$	$0.18 \pm 0.04$
$IC_{50}$ (mM)	$4.85 \pm 1.73$	$6.52 \pm 4.26$

TABLE S2

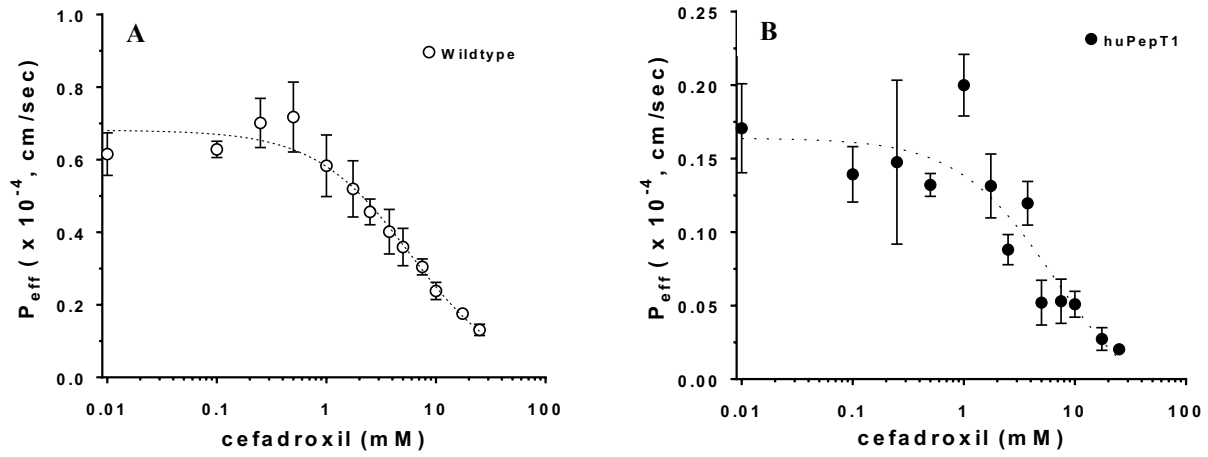
Observed and predicted intestinal  $P_{\text{eff}}$  of cefadroxil in wildtype (WT) and humanized (hu) *PepT1* mice when estimated by the *in situ* permeability of small and large intestines

	Mouse	Human	WT	hu	WT	hu	WT	hu	WT	hu	WT	hu	hu
	Radius (cm)		<b>10 <math>\mu\text{M}</math></b>		5 mg/kg		15 mg/kg		30 mg/kg		500 mg (6.7 mg/kg)		CEF + CEP
Stomach	0.40	9.67	<b>0.00</b>	<b>0.00</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Duodenum	0.14	1.53	<b>0.55</b>	<b>0.24</b>	0.361	0.154	0.201	0.088	0.128	0.053	0.317	0.136	0.032
Jejunum 1	0.13	1.45	<b>0.62</b>	<b>0.17</b>	0.402	0.111	0.223	0.063	0.142	0.038	0.352	0.098	0.023
Jejunum 2	0.12	1.29	<b>0.62</b>	<b>0.17</b>	0.402	0.111	0.223	0.063	0.142	0.038	0.352	0.098	0.023
Ileum 1	0.11	1.13	<b>0.29</b>	<b>0.12</b>	0.190	0.078	0.106	0.044	0.067	0.027	0.167	0.069	0.016
Ileum 2	0.10	0.98	<b>0.29</b>	<b>0.12</b>	0.190	0.078	0.106	0.044	0.067	0.027	0.167	0.069	0.016
Ileum 3	0.09	0.82	<b>0.29</b>	<b>0.12</b>	0.190	0.078	0.106	0.044	0.067	0.027	0.167	0.069	0.016
Caecum	0.62	3.39	<b>0.00</b>	<b>0.06</b>	0.003	0.036	0.001	0.020	0.001	0.012	0.002	0.032	0.007
Ascending Colon	0.33	2.41	<b>0.00</b>	<b>0.06</b>	0.003	0.032	0.001	0.018	0.001	0.011	0.002	0.029	0.007

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

Observed  $P_{\text{eff}}$  values of 10  $\mu\text{M}$  cefadroxil in the small and large intestines of WT and *huPepT1* mice were obtained previously by our laboratory (Hu and Smith, 2016), and shown in bold text.

Predicted  $P_{\text{eff}}$  values of cefadroxil in the small and large intestines of WT and *huPepT1* mice are shown after human single oral doses of 5, 15 and 30 mg/kg, after 500 mg (6.7 mg/kg) oral doses every six hours, and after 5 mg/kg cefadroxil plus 45 mg/kg cephalexin (CEF + CEP).



**Figure S1.** The concentration-dependent jejunal permeability of cefadroxil in wildtype (panel A) and humanized mice (panel B).