Research Article

Theme: Kidney Transporters: Importance in Clearance, Disease, and Drug-Drug Interactions
Guest Editors: Marilyn Morris and Colin Brown

Mechanistic Models Describing Active Renal Reabsorption and Secretion: A Simulation-Based Study

Melanie A. Felmlee,¹ Rutwij A. Dave,¹ and Marilyn E. Morris^{1,2}

Received 7 September 2012; accepted 31 October 2012; published online 30 November 2012

Abstract. The objective of the present study was to evaluate mechanistic pharmacokinetic models describing active renal secretion and reabsorption over a range of Michaelis–Menten parameter estimates and doses. Plasma concentration and urinary excretion profiles were simulated and renal clearance (CL_r) was calculated for two pharmacokinetic models describing active renal reabsorption (R1/R2), two models describing active secretion (S1/S2), and a model containing both processes. A range of doses (1–1,000 mg/kg) was evaluated, and V_{max} and K_m parameter estimates were varied over a 100-fold range. Similar CL_r values were predicted for reabsorption models (R1/R2) with variations in V_{max} and K_m . Tubular secretion models (S1/S2) yielded similar relationships between Michaelis–Menten parameter perturbations and CL_r , but the predicted CL_r values were threefold higher for model S1. For both reabsorption and secretion models, the greatest changes in CL_r were observed with perturbations in V_{max} , suggesting the need for an accurate estimate of this parameter. When intrinsic clearance was substituted for Michaelis–Menten parameters, it failed to predict similar CL_r values even within the linear range. For models S1 and S2, renal secretion was predominant at low doses, whereas renal clearance was driven by fraction unbound in plasma at high doses. Simulations demonstrated the importance of Michaelis–Menten parameter estimates (especially V_{max}) for determining CL_r . K_m estimates can easily be obtained directly from *in vitro* studies. However, additional scaling of *in vitro* V_{max} estimates using *in vitro/in vivo* extrapolation methods are required to incorporate these parameters into pharmacokinetic models.

KEY WORDS: kidney transport parameters; models; reabsorption; renal clearance; secretion.

INTRODUCTION

The kidney represents a major clearance organ within the body and is involved in the elimination of therapeutic agents, toxins, and endogenous metabolites. Renal excretion involves four distinct processes: (1) glomerular filtration; (2) active tubular secretion; (3) active reabsorption; and (4) passive reabsorption. The extent to which a compound is filtered at the glomerulus is dependent on the molecular size and protein binding. Active tubular secretion and reabsorption are transporter-mediated processes typically occurring within the proximal tubule of the kidney. The involvement of active transport processes will be dependent on the compound's affinity for the

wide range of transporters expressed in the kidney and, as such, has the potential to lead to drug–drug interactions. Passive reabsorption is a nonsaturable process that occurs along a concentration gradient and is dependent on the lipid solubility and pK_a of the compound and the pH and flow rate of urine. While not all of these processes are involved in the renal clearance of every compound, it is important to understand the interaction between these processes to allow for predictions of renal clearance.

Active renal reabsorption and tubular secretion occur in the proximal tubules of the kidney and are mediated by a number of specific anion and cation transporters expressed at the apical and basolateral membranes of proximal tubular cells. Nutrient and peptide transporters (e.g., MCTs, SMCTs, PEPT1, SGLT2) (1–4) are involved in the active reabsorption of endogenous compounds; however, these transporters may also be involved in the transport of therapeutic agents, such as γ -hydroxybutyric acid and β -lactam antibiotics (3,5). Transporters involved in tubular secretion (e.g., OAT1/3, OCT2, OCTNs, MATE1) eliminate endogenous waste products and therapeutic agents (6–11). These transporters exhibit broad substrate specificity and may have a high capacity for transport of therapeutic agents.

Various approaches have been used in the literature to predict pharmacokinetic profiles from *in vitro* data, including

Guest Editors: Marilyn Morris and Colin Brown

Electronic supplementary material The online version of this article (doi:10.1208/s12248-012-9437-3) contains supplementary material, which is available to authorized users.

¹ Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, The State University of New York, Buffalo, New York 14214, USA.

² To whom correspondence should be addressed. (e-mail: memorris@buffalo.edu)

allometry and *in vitro/in vivo* extrapolation (IVIVE). While both simple and modified allometric methods can accurately predict renal clearance involving glomerular filtration and passive reabsorption, these methods fail to accurately predict renal clearance when active transport processes are involved (12,13). Physiologically based IVIVE predictions have been successfully applied to the prediction of metabolic and biliary clearance and active tubular secretion (14,15); however, they have not been applied for the prediction of active renal reabsorption. *In vitro* studies in kidney cell lines or in cell lines overexpressing a specific transporter can be used to generate kinetic data and Michaelis–Menten parameter estimates. The transport kinetic parameters (K_m and V_{max}) can be combined with transporter expression data and incorporated into a mechanistic pharmacokinetic model of renal clearance for both tubular secretion and reabsorption to provide *a priori* predictions of the renal clearance of a compound and the potential for drug–drug interactions and toxicity.

In our evaluation, we used four published mechanistic models with varying degrees of complexity describing active renal reabsorption (16,17) and active renal secretion (18,19). These mechanistic models include specific transport kinetic parameters that are driven by a physiologically relevant drug concentration (plasma/blood or tubular lumen) as opposed to evaluating renal clearance as a singular parameter. Both compartmental and more physiologically based models of active tubular secretion and reabsorption have been utilized in the literature. Two additional models of renal reabsorption have been described in the literature (20,21); however, one model has limited applicability as it treats urine as a distribution compartment and the other utilizes plasma drug concentrations to drive renal reabsorption. Therefore, these models were excluded from the present analysis. In contrast to the compartmental models, the models developed for γ -hydroxybutyric acid and phenolsulfonphthalein (PSP) incorporate system-specific parameters, including renal blood flow, glomerular filtration rate (GFR), and urine flow which can be fixed (17). A mechanistic model for passive renal reabsorption has been described in the literature (22); however, it was not evaluated in the present study. All of these models were developed for single chemical entities and their broader applicability in assessing the contribution of active renal secretion and reabsorption to the pharmacokinetics of additional compounds has yet to be evaluated over a range of parameter estimates. The development of a universally applicable mechanistic model is desirable to evaluate renal clearance across a range of compounds that are substrates for secretory or reabsorption transporters. In such a model, physiological system-specific parameters can be fixed, while drug-specific parameters can be incorporated, and therefore, the model could be utilized to make *a priori* predictions of renal clearance based on *in vitro* transporter kinetic parameters through IVIVE methodologies. The objective of the present study was to evaluate and compare previously published mechanistic pharmacokinetic models of active tubular secretion and reabsorption and to combine the models to determine a universal model to describe these processes. The models were evaluated over a range of Michaelis–Menten parameter estimates and doses to determine their impact on model outputs (plasma concentration and urinary excretion) and calculated pharmacokinetic

parameters (renal clearance). Furthermore, effects of changes in fraction unbound in plasma ($f_{u,p}$) on renal clearance for secretion models were also evaluated over the range of doses and Michaelis–Menten parameter estimates.

METHODS

Structural Models

Four structural models of active renal reabsorption and tubular secretion (Fig. 1) were selected for analysis based on the available models within the literature (16–19). The differential equations for the structural models are listed in the supplemental data. Model R1 includes renal tubule and urine compartments separated by urine flow with active reabsorption from the renal tubules (16). Model R2 represents a more physiological model of the kidney with renal blood flow, glomerular filtration from the kidney compartment, proximal and distal tubule compartments, and a urine compartment (17). Model S1 is a one-compartment model with linear and saturable clearance processes described by glomerular filtration and active tubular secretion, as well as linear nonrenal clearance (18). Model S2 is a mechanistic semiphysiological model, incorporating renal blood flow from the central compartment to the kidneys. Drug enters the tubular lumen by glomerular filtration and the remaining drug is available for active secretion into the tubular cells with subsequent excretion into the lumen (19). Model S2 was extended to include active renal reabsorption from the tubular lumen to evaluate whether both process could be evaluated using the semiphysiological structural model. The secretion and reabsorption components of this model can be utilized depending on the specific compound being assessed. One basic assumption of all models evaluated is the compartmental drug concentration, the concentration that is present at the transporter and is available for transport. Although any input model could be employed, only intravenous bolus administration will be considered in the present study. In addition, distributional models were not considered to simplify the analysis. Compartmental or physiologically based distributional models could be combined with the renal clearance models.

Simulation Studies

Simulations of plasma concentrations and urinary excretion were conducted in Phoenix WinNonLin version 6.1 (Pharsight Corp., Mountain View, CA, USA) using the structural models detailed in the previous section. Profiles for active reabsorption models were simulated for 24 h and secretion models were simulated for 8 h to ensure the total amount of drug excreted in urine ($A_{e,inf}$) was obtained. Drug was administered as an intravenous bolus of 1–1,000 mg/kg (R1/R2, combined model) or 1–500 mg/kg (S1/S2). Nonrenal clearance (CL_{nr}) was fixed at 1 ml/min/kg for all models, so renal clearance was the primary elimination pathway. V_{max} and K_m values were varied over a 100-fold range to determine their impact on model outputs. Reabsorption model parameters are listed in Table I and are based on previous assessments of the active renal reabsorption of γ -hydroxybutyric acid in rats which is known to exhibit

Fig. 1. Structural models (**a** R1, **b** R2, **c** S1, and **d** S2) describing active renal reabsorption and secretion. Detailed descriptions for the parameters are listed in Table I

nonlinear renal clearance caused by saturation of active reabsorption. For the reabsorption models: K_m was fixed at 0.5 mg/ml and V_{max} was varied from 1 to 100 mg/min/kg; V_{max} was fixed at 10 mg/min/kg and K_m was varied from 0.05 to 5 mg/ml. Secretion model parameters (Table I) are based on PSP in rats (19,23). The physiological parameters were scaled to rat from dog values listed in Russel *et al.* (19) and the first-order transfer rate of the drug from tubular cells to lumen was fixed to the dog value. The effect of changes in $f_{u,p}$ (from 0.01 to 1) of PSP on renal clearance (CL_r) was evaluated for models S1 and S2. For the secretion models: K_m was fixed at 0.01 mg/ml and V_{max} was varied from 0.025 to 2.5 mg/min/kg; V_{max} was fixed at

0.5 mg/min/kg and K_m was varied from 0.001 to 0.1 mg/kg. In addition, simulations were conducted using intrinsic transport clearance (CL_{int}) for all models. For reabsorption, CL_{int} was varied from 2 to 200 ml/min/kg, and for secretion, CL_{int} was varied from 5 to 500 ml/min/kg. These values correspond to the ranges of CL_{int} obtained from the V_{max} and K_m values used.

Data Analysis

Area under the plasma concentration time curve (AUC) was calculated in Phoenix WinNonLin using the linear trapezoidal rule. Renal clearance (CL_r) was calculated by

Table I. Nominal Pharmacokinetic Parameter Values for Renal Reabsorption and Tubular Secretion Models in Rats

Parameter (units)	Description	Value	Applicable model(s)
GFR (ml/min/kg)	Glomerular filtration rate	10	R1, R2, S1, S2
Q_R (ml/min/kg)	Renal blood flow	41	R1, R2, S2
UF (ml/min/kg)	Urine flow	0.3	R1, R2, S2
V_c (ml/kg) ^a	Volume of the central compartment	312	R1
V_c (ml/kg)	Volume of the central compartment	298.7	R2
V_t (ml/kg) ^b	Volume of the renal tubules	13.3	R1
V_{pt} (ml/kg) ^c	Volume of the proximal tubule	10	R2
V_{dt} (ml/kg) ^c	Volume of the distal tubule	3.3	R2
V_k (ml/kg)	Volume of kidney blood	13.3	R2
V_c (ml/kg)	Volume of central compartment	300	S1, S2
V_{rp} (ml/kg)	Volume of renal plasma	6.5	S2
V_{tu} (ml/kg)	Volume of tubular urine	0.45	S2
V_{tc} (ml/kg) ^d	Volume of tubular cells	19.6	S2
K_t (/min)	First-order rate of transfer	0.17	S2

Parameter estimates are based on previous assessments of the renal reabsorption of γ -hydroxybutyric acid (17) (for models R1 and R2) and of the active secretion of PSP and physiological parameters were scaled to rat from dog (19) (for models S1 and S2)

^a Volume of the central compartment for model R1 was obtained by adding the V_c and V_k for model R2

^b Volume of the renal tubules for model R1 was obtained by adding the proximal and distal tubule volumes for model R2

^c Luminal volume of the proximal and distal tubules

^d Intracellular volume of renal tubular cells

$A_{e,inf}$ divided by AUC. Data were plotted in Sigma Plot version 11 (Systat Software Inc, San Jose, CA, USA).

RESULTS

Active Tubular Reabsorption Models

Model R1 (Fig. 1a) represents a compartmental model and model R2 (Fig. 1b) represents a semiphysiological model for evaluating the impact of transporter-mediated renal reabsorption on overall renal clearance. The impact of dose and Michaelis–Menten parameter estimates for both models are presented in Fig. 2. The expectation for these

reabsorption models is that renal clearance will increase with increasing dose as reabsorption becomes saturated and a greater amount of drug is excreted in urine. In addition, a high capacity for transport should result in no renal clearance as the drug is completely reabsorbed. Renal clearance demonstrated similar predicted values with variation in dose and K_m or V_{max} for both reabsorption models (Fig. 2a, b). Renal clearance increased with increasing dose consistent with saturation of renal reabsorption. The greatest change in renal clearance was observed with perturbations in V_{max} , suggesting that this parameter is critically important for determining renal excretion. Increases in V_{max} resulted in decreases in renal clearance, with high V_{max} values leading to

Fig. 2. Mesh plots evaluating the relationship between renal clearance (CL_r), dose, and Michaelis–Menten parameters estimates (V_{max} and K_m) or intrinsic reabsorption clearance (Cl_{int}) for active renal reabsorption models (R1 and R2). **a** V_{max} was varied from 1 to 100 mg/min/kg, while K_m was fixed at 0.5 mg/ml. **b** K_m was varied from 0.05 to 5 mg/ml, while V_{max} was fixed at 10 mg/min/kg. **c** Cl_{int} was varied from 2 to 200 ml/min/kg which covers that same range as the Cl_{int} calculated from V_{max} and K_m estimates. The color gradient ranges from blue (CL_r equal to 0) to red (maximal CL_r indicated by the y-axis)

complete reabsorption of the drug (Fig. 2a). In addition, decreased affinity (high K_m value) led to increased renal clearance as drug concentrations in the tubular lumen fell below the K_m value (Fig. 2b). For all parameter perturbations, changes in renal clearance resulted in changes in the total amount of drug excreted in the urine with increased renal clearance resulting in an increased amount of drug excreted in urine (data not shown). Due to the observed nonlinear renal reabsorption and, therefore, renal clearance, there is a shift in the fraction eliminated by renal and nonrenal pathways. When $V_{\max}$ is high, all drug is reabsorbed from the lumen; therefore, nonrenal clearance becomes the predominant elimination pathway. These results are consistent with observed data for changes in γ -hydroxybutyric acid renal clearance and total amount excreted in the urine when transporter-mediated reabsorption is inhibited in a competitive manner (increase in apparent K_m) (17); additionally, the estimated *in vivo* K_m for γ -hydroxybutyric acid (0.46 mg/ml) (17) is close to the determined *in vitro* K_m (0.48 mg/ml) (24).

In contrast, when Cl_{int} was substituted for K_m and $V_{\max}$ in both models, the observed changes in renal clearance did not correspond to those obtained when K_m and $V_{\max}$ were used (Fig. 2c), even with low doses in the expected linear range. When Cl_{int} was perturbed, no dose-dependent changes in renal clearance were observed. When Cl_{int} was high, similar predictions were obtained (compared to $V_{\max}$ simulations) with complete reabsorption and CL_r of 0. Cl_{int} cannot be utilized to describe nonlinear processes and, therefore, should not be utilized in structural models of active renal reabsorption. Based on the simulation results, either model could be utilized for the prediction of renal reabsorption from *in vitro* transporter kinetic data; however, it is more advantageous to use the physiologically based model (R2) as system-specific parameters can be fixed and *in vitro* estimates of drug-specific properties (such as transport kinetic parameters) can be integrated for a range of species.

Active Tubular Secretion Models

Model S1 (Fig. 1c) represents the most simplistic mechanistic model of active secretion with glomerular filtration and active secretion from the central compartment (18). Model S2 (Fig. 1d) (19) is a semiphysiological mechanistic model similar in structure to model R2, with compartments representing the tubular lumen and proximal tubule cells connected by blood and urine flows. The impact of dose and Michaelis–Menten parameter estimates for both models are presented in Fig. 3. The basic assumption for these models is that renal clearance will always be greater than $GFR \times f_{u,p}$ in the absence of passive reabsorption and that saturation of secretion will result in decreasing renal clearance. While the shape of the mesh plots when dose and Michaelis–Menten parameter estimates were perturbed is similar, the predicted renal clearance values are threefold higher for model S1 (Fig. 3). These higher predictions are consistent with the structure of the model, with a higher concentration of drug (central compartment concentration) available for secretion, while in model S2, only drug that is not filtered at the glomerulus is available for secretion. As such, K_m estimates from model S1 likely would not correlate with K_m estimates obtained *in vitro*. In contrast to the

reabsorption models, only minor dose-dependent effects on renal clearance were observed under these simulation conditions. However, alterations in filtration clearance due to protein binding may cause this relationship to be altered and the minimum renal clearance value will be reduced. At higher doses, renal clearance decreased to approximately GFR as a result of saturation of active secretion. Increasing $V_{\max}$ had the greatest impact on renal clearance when a low dose was administered (Fig. 3a). K_m only influenced predicted renal clearance at low doses and low K_m (Fig. 3b) values. As K_m or dose increased, renal clearance decreased to approximately GFR. For all Michaelis–Menten parameter perturbations, no changes were observed in the total amount of drug excreted in the urine. The predicted total amount of drug excreted in the urine was dependent on dose, which is consistent with basic pharmacokinetic concepts. When Cl_{int} was substituted for the Michaelis–Menten parameter estimates, the predicted renal clearance was not dose-dependent and increased to the same maximal renal clearance for all doses (Fig. 3c). Utilizing this single parameter does not allow for the evaluation of nonlinear renal clearance resulting from transporter saturation and should not be incorporated into structural models of active secretion.

When protein binding was considered, CL_r increased as the value of fraction unbound of PSP in plasma was increased for models S1 and S2 (Figs. 4 and 5). For all doses, perturbations in $V_{\max}$ estimates (Fig. 4) had a greater impact on predicted values of CL_r compared with perturbation in K_m estimates (Fig. 5), as described previously. At the lowest dose (1 mg/kg) evaluated (Figs. 4a and 5a), the contribution of renal secretion to total renal clearance was greater than that of $GFR \times f_{u,p}$; however, as the dose was increased to 500 mg/kg (Figs. 4c and 5c), $GFR \times f_{u,p}$ became the predominant determinant of CL_r . Changes in the values of the Michaelis–Menten parameter estimates ($V_{\max}$ and K_m) lead to substantial increases in the predicted CL_r values at low doses, while at high doses, perturbations did not impact overall renal clearance. At high doses, renal secretion is saturated (renal concentrations $\gg K_m$) and CL_r is determined by $GFR \times f_{u,p}$. At the low dose, model S1 predicted greater values of renal clearance compared with model S2, which was expected due to the structure of the two models. However, at the high dose, both models performed similarly, which indicated saturation of renal secretion. This observation can be attributed to ongoing glomerular filtration, which contributes predominantly to the renal clearance as renal secretion is saturated. Based on these results, model S2 is the more appropriate model for the prediction of renal secretion using physiological parameters and scaled *in vitro* transport kinetic data.

Combined Model

Model S2 was extended to include active renal reabsorption with saturable absorption from the tubular lumen into tubular cells, followed by secretion into the renal plasma compartment. A rapid first-order rate constant was utilized for secretion into the renal plasma compartment, so reabsorption from the lumen was the rate-limiting step. To evaluate a compound only undergoing reabsorption, the

Fig. 3. Mesh plots evaluating the relationship between renal clearance (CL_r), dose, and Michaelis-Menten parameters estimates (V_{max} and K_m) or intrinsic secretion clearance (CL_{int}) for active tubular secretion models (S1 and S2). **a** V_{max} was varied from 0.025 to 2.5 mg/min/kg, while K_m was fixed at 0.01 mg/ml. **b** K_m was varied from 0.001 to 0.1 mg/ml, while V_{max} was fixed at 0.5 mg/min/kg. **c** CL_{int} was varied from 5 to 500 ml/min/kg which covers that same range as the CL_{int} calculated from V_{max} and K_m estimates. The color gradient ranges from blue (CL_r equal to GFR) to red (maximal CL_r indicated by the y-axis)

secretion parameters in the model were turned off. The model predictions following perturbations in dose and Michaelis-Menten parameter estimates are detailed in Fig. 6. Consistent with the results obtained using models R1 and R2, renal clearance increased with increasing dose at low V_{max} values and high V_{max} values resulted in renal clearance decreasing to 0 (Fig. 6a). A similar relationship was also observed for changes in K_m between the models R1/R2 and the combined model (Fig. 6b). These results suggest that the combined model could be used to predict renal clearance for compounds undergoing both active secretion, reabsorption, and both processes.

DISCUSSION

Renal excretion is a major elimination pathway involved in the removal of a number of therapeutic agents from the body. Active renal reabsorption and tubular secretion are major processes determining the overall renal clearance of a compound; however, few mechanistic pharmacokinetic models have been developed to quantitatively describe these processes. Development of mechanistic models to describe active transport processes will allow for *a priori* predictions of renal clearance based on IVIVE of transport kinetic

Fig. 4. Mesh plots evaluating the relationship between renal clearance (CL_r), $f_{u,p}$, and V_{max} for models S1 and S2, where V_{max} was varied from 0.025 to 2.5 mg/min/kg, $f_{u,p}$ was varied from 0.01 to 1, and K_m was fixed at 0.01 mg/ml. **a** 1 mg/kg dose, **b** 100 mg/kg dose, and **c** 500 mg/kg dose. The color gradient ranges from blue (CL_r equal to $GFR \times f_{u,p}$) to red (maximal CL_r indicated by the y-axis)

parameters, as well as predictions of the potential for clinically significant drug–drug interactions. The present study is the first comparative analysis of published models of active renal reabsorption and tubular secretion aimed at determining the drug-specific parameters required to accurately predict renal clearance. Based on the results of the present study, physiologically based models incorporating both active reabsorption and secretion well described renal clearance. In addition, these models require only a few drug-specific kinetic parameters that can be obtained from *in vitro* studies, while the remaining system-specific parameters can be fixed to

literature values. The present study evaluated a wide range of doses and transporter kinetic parameters; however, future studies are required to validate the combined model using pharmacokinetic data from a number of compounds with different extents of active renal reabsorption and tubular secretion, as well as *in vitro* transport kinetic parameter estimates.

In the present study, perturbations in both K_m and V_{max} resulted in changes in renal clearance determined in both secretion and reabsorption models; however, the greatest changes were observed with alterations in V_{max} (Figs. 2a, 3a,

Fig. 5. Mesh plots evaluating the relationship between renal clearance (CL_r), $f_{u,p}$, and K_m for models S1 and S2, where K_m was varied from 0.001 to 0.1 mg/ml, $f_{u,p}$ was varied from 0.01 to 1, and V_{max} was fixed at 0.5 mg/min/kg. **a** 1 mg/kg dose, **b** 100 mg/kg dose, and **c** 500 mg/kg dose. The color gradient ranges from blue (CL_r equal to $GFR \times f_{u,p}$) to red (maximal CL_r indicated by the y-axis)

4, and 6a). Changes in renal clearance were also observed when an intrinsic secretion or reabsorption clearance term was used, but they did not correspond to the predictions obtained with the individual Michaelis–Menten parameters even within the linear range of transport. This suggests that use of intrinsic clearance to predict active renal reabsorption and tubular secretion may result in mispredictions as the extent of reabsorption/secretion is highly dependent on the capacity for drug transport. Furthermore, these findings suggest that good initial estimates of the Michaelis–Menten parameters are needed to accurately predict a drug's renal

clearance. The Michaelis–Menten parameters for renal transport can be estimated based on *in vitro* or *in vivo* data. Both K_m and V_{max} parameters may be estimated from *in vitro* cell uptake studies. While K_m values can be translated directly to an *in vivo* model, V_{max} estimates may not be accurate in renal cell lines or transfected cells lines as they are dependent on the relationship between protein expression of the transporter in the cell line and kidney tissue. As such, IVIVE scalars relating protein or mRNA expression between *in vitro* and *in vivo* systems are necessary to determine accurate scalars for V_{max} (25). Ideally, the generation of IVIVE scalars can be

Fig. 6. Mesh plots evaluating the relationship between renal clearance (CL_r), dose, and Michaelis-Menten parameter estimates (V_{max} and K_m) for model S2 with reabsorption parameters added. For these scenarios, the secretion parameters were turned off. **a** Reabsorption V_{max} was varied from 1 to 100 mg/min/kg, while K_m was fixed at 0.5 mg/ml. **b** Reabsorption K_m was varied from 0.05 to 5 mg/ml, while V_{max} was fixed at 10 mg/min/kg. The color gradient ranges from blue (CL_r equal to 0) to red (maximal CL_r indicated by the y-axis)

achieved by determining the absolute or relative transporter expression through quantification of protein expression in cell lines and kidney samples; however, information regarding protein expression in rat and human kidney samples is lacking (26,27). In order to improve predictions, an understanding of the population variability in expression is necessary, requiring the quantitative analysis of a large pool of human kidney samples, with concurrent analysis of cell culture samples from which *in vitro* kinetic parameters have been generated. Analysis of mRNA expression could also be used to determine scalars that are specific to the laboratory in which they were generated. Currently, mRNA transporter expression data is only available in rodent kidney; there is not sufficient literature data to obtain these scalars for renal drug

transport. An additional hurdle is the interlaboratory variability in cell lines and tissues utilized necessitating the generation of laboratory-specific scalars. V_{max} estimates may also be influenced by the existence of polymorphic variants of individual transporters (28,29) and, therefore, will be important to characterize the variants present in tissue and cell lines.

The effects of changes in fraction unbound in plasma on renal clearance for secretion models over the range of doses and Michaelis-Menten parameter estimates were also evaluated. At low doses (Figs. 4a and 5a), renal clearance was determined by changes in V_{max} and K_m , and at high doses, renal clearance was dependent on $GFR \times f_{u,p}$. These relationships confirm the need to incorporate *in vitro* or *in vivo* estimates of $f_{u,p}$ especially when doses and, therefore, drug concentrations greatly exceed the K_m for renal secretion.

One limitation of the present models is that they exclusively consider glomerular filtration and active reabsorption and tubular secretion. The combined model incorporating active reabsorption and secretion can be further expanded to include additional factors influencing renal clearance including passive reabsorption. System-specific parameters, such as GFR and urine and blood flow, may be altered to reflect changes that occur in various disease states or with drug treatment, therefore determining their impact on renal clearance and other model outputs. Additional drug-specific parameters can also be incorporated. A large number of drugs undergo passive reabsorption or have a combination of many of these processes contributing to their observed urinary excretion profiles and total renal clearance. Passive reabsorption may be predicted based on the physicochemical properties of a compound (30). Additionally, physiologically based pharmacokinetic models of passive reabsorption have been proposed in the literature (22,31). The influence of a compounds' ionization state within the tubular lumen could also be incorporated with total drug available for active reabsorption and unionized drug available for passive reabsorption. These factors could be added to the combined physiological model presented here to establish a universal mechanistic model of renal clearance that would be widely applicable for the prediction of total renal clearance and the potential for drug-drug interactions. These models should be further evaluated with respect to the dependence on physiological factors, such as renal plasma flow, pH dependence, and the expression of specific drug transporters in the kidney.

CONCLUSIONS

In the present study, we evaluated four mechanistic pharmacokinetic models describing active renal reabsorption or tubular secretion and a model incorporating both processes. Simulations demonstrated the importance of Michaelis-Menten parameters, especially V_{max} , for the prediction of renal clearance, while the incorporation of CL_{int} resulted in underestimation of renal clearance under the conditions evaluated. The applicability of the combined model should be validated using a range of compounds with variable transport. *In vitro* studies should be conducted with compounds which undergo only secretion or reabsorption to enable estimation of Michaelis-Menten parameters for active reabsorption and tubular secretion. Furthermore, *in vitro*

transport studies and evaluations of transporter expression should be undertaken to allow for *a priori* predictions of renal clearance for a range of compounds.

ACKNOWLEDGMENTS

This study was supported in part by NIH grant DA-023223 and Roche Inc.

REFERENCES

- Ganapathy V, Thangaraju M, Gopal E, Martin PM, Itagaki S, Miyauchi S, *et al.* Sodium-coupled monocarboxylate transporters in normal tissues and in cancer. *AAPS J.* 2008;10(1):193–9. Epub 2008/05/01.
- Leibach FH, Ganapathy V. Peptide transporters in the intestine and the kidney. *Annu Rev Nutr.* 1996;16:99–119. Epub 1996/01/01.
- Morris ME, Felmler MA. Overview of the proton-coupled MCT (SLC16A) family of transporters: characterization, function and role in the transport of the drug of abuse gamma-hydroxybutyric acid. *AAPS J.* 2008;10(2):311–21. Epub 2008/06/05.
- Launay-Vacher V, Izzedine H, Karie S, Hulot JS, Baumelou A, Deray G. Renal tubular drug transporters. *Nephron Physiol.* 2006;103(3):97–106. Epub 2006/03/24.
- Inui KI, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. *Kidney Int.* 2000;58(3):944–58. Epub 2000/09/06.
- Muller F, Fromm MF. Transporter-mediated drug–drug interactions. *Pharmacogenomics.* 2011;12(7):1017–37. Epub 2011/07/27.
- Ohashi R, Tamai I, Yabuuchi H, Nezu JJ, Oku A, Sai Y, *et al.* Na (+)-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. *J Pharmacol Exp Ther.* 1999;291(2):778–84. Epub 1999/10/19.
- Tamai I, Nakanishi T, Kobayashi D, China K, Kosugi Y, Nezu J, *et al.* Involvement of OCTN1 (SLC22A4) in pH-dependent transport of organic cations. *Mol Pharm.* 2004;1(1):57–66. Epub 2005/04/19.
- Hasegawa M, Kusuhara H, Endou H, Sugiyama Y. Contribution of organic anion transporters to the renal uptake of anionic compounds and nucleoside derivatives in rat. *J Pharmacol Exp Ther.* 2003;305(3):1087–97. Epub 2003/03/28.
- Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res.* 2007;24(7):1227–51. Epub 2007/05/03.
- Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. *Biochem Pharmacol.* 2007;74(2):359–71. Epub 2007/05/19.
- Fagerholm U. Prediction of human pharmacokinetics—renal metabolic and excretion clearance. *J Pharm Pharmacol.* 2007;59(11):1463–71. Epub 2007/11/03.
- Paine SW, Menochet K, Denton R, McGinnity DF, Riley RJ. Prediction of human renal clearance from preclinical species for a diverse set of drugs that exhibit both active secretion and net reabsorption. *Drug Metab Dispos Biol Fate Chem.* 2011;39(6):1008–13. Epub 2011/03/02.
- Watanabe T, Kusuhara H, Debori Y, Maeda K, Kondo T, Nakayama H, *et al.* Prediction of the overall renal tubular secretion and hepatic clearance of anionic drugs and a renal drug–drug interaction involving organic anion transporter 3 in humans by *in vitro* uptake experiments. *Drug Metab Dispos Biol Fate Chem.* 2011;39(6):1031–8. Epub 2011/03/09.
- Watanabe T, Kusuhara H, Sugiyama Y. Application of physiologically based pharmacokinetic modeling and clearance concept to drugs showing transporter-mediated distribution and clearance in humans. *J Pharmacokinet Pharmacodyn.* 2010;37(6):575–90. Epub 2010/11/11.
- Graumlich JF, Ludden TM, Conry-Cantilena C, Cantilena Jr LR, Wang Y, Levine M. Pharmacokinetic model of ascorbic acid in healthy male volunteers during depletion and repletion. *Pharm Res.* 1997;14(9):1133–9. Epub 1997/11/05.
- Felmler MA, Wang Q, Cui D, Roiko SA, Morris ME. Mechanistic toxicokinetic model for gamma-hydroxybutyric acid: inhibition of active renal reabsorption as a potential therapeutic strategy. *AAPS J.* 2010;12(3):407–16. Epub 2010/05/13.
- Landersdorfer CB, Kirkpatrick CM, Kinzig M, Bulitta JB, Holzgrabe U, Sorgel F. Inhibition of flucloxacillin tubular renal secretion by piperacillin. *Br J Clin Pharmacol.* 2008;66(5):648–59. Epub 2008/11/27.
- Russel FG, Wouterse AC, van Ginneken CA. Physiologically based pharmacokinetic model for the renal clearance of phenol-sulphonphthalein and the interaction with probenecid and salicylic acid in the dog. *J Pharmacokinet Biopharm.* 1987;15(4):349–68. Epub 1987/08/01.
- Jusko WJ, Levy G. Pharmacokinetic evidence for saturable renal tubular reabsorption of riboflavin. *J Pharm Sci.* 1970;59(6):765–72. Epub 1970/06/01.
- Wang X, Wang Q, Morris ME. Pharmacokinetic interaction between the flavonoid luteolin and gamma-hydroxybutyrate in rats: potential involvement of monocarboxylate transporters. *AAPS J.* 2008;10(1):47–55. Epub 2008/05/01.
- Komiya I. Urine flow dependence of renal clearance and interrelation of renal reabsorption and physicochemical properties of drugs. *Drug Metab Dispos Biol Fate Chem.* 1986;14(2):239–45. Epub 1986/03/01.
- Itagaki S, Shimamoto S, Hirano T, Iseki K, Sugawara M, Nishimura S, *et al.* Comparison of urinary excretion of phenol-sulphonphthalein in an animal model for Wilson's disease (Long-Evans Cinnamon rats) with that in normal Wistar rats: involvement of primary active organic anion transporter. *J Pharm Pharm Sci Publ Can Soc Pharm Sci, Soc Can Sci Pharm.* 2004;7(2):227–34. Epub 2004/09/16.
- Wang Q, Darling IM, Morris ME. Transport of gamma-hydroxybutyrate in rat kidney membrane vesicles: role of monocarboxylate transporters. *J Pharmacol Exp Ther.* 2006;318(2):751–61. Epub 2006/05/19.
- Proctor NJ, Tucker GT, Rostami-Hodjegan A. Predicting drug clearance from recombinantly expressed CYPs: intersystem extrapolation factors. *Xenobiotica.* 2004;34(2):151–78. Epub 2004/02/27.
- Brown CD, Sayer R, Windass AS, Haslam IS, De Broe ME, D'Haese PC, *et al.* Characterisation of human tubular cell monolayers as a model of proximal tubular xenobiotic handling. *Toxicol Appl Pharmacol.* 2008;233(3):428–38.
- Tahara H, Kusuhara H, Chida M, Fuse E, Sugiyama Y. Is the monkey an appropriate animal model to examine drug–drug interactions involving renal clearance? Effect of probenecid on the renal elimination of H2 receptor antagonists. *J Pharmacol Exp Ther.* 2006;316(3):1187–94. Epub 2005/11/18.
- Shima JE, Komori T, Taylor TR, Stryke D, Kawamoto M, Johns SJ, *et al.* Genetic variants of human organic anion transporter 4 demonstrate altered transport of endogenous substrates. *Am J Physiol Renal Physiol.* 2010;299:F767–75. Epub 2010/07/30.
- Chen L, Takizawa M, Chen E, Schlessinger A, Choi JH, Segenthar J, *et al.* Genetic polymorphisms in the organic cation transporter 1, OCT1, in Chinese and Japanese populations, exhibit altered function. *J Pharmacol Exp Ther.* 2010;335:42–50. Epub 2010/07/20.
- Varma MV, Feng B, Obach RS, Troutman MD, Chupka J, Miller HR, *et al.* Physicochemical determinants of human renal clearance. *J Med Chem.* 2009;52(15):4844–52. Epub 2009/05/19.
- Hall S, Rowland M. Relationship between renal clearance, protein binding and urine flow for digitoxin, a compound of low clearance in the isolated perfused rat kidney. *J Pharmacol Exp Ther.* 1984;228(1):174–9. Epub 1984/01/01.