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ARTICLE

Mechanistic Models as Framework for Understanding Biomarker Disposition: Prediction of Creatinine-Drug Interactions

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Creatinine is widely used as a biomarker of glomerular filtration, and, hence, renal function. However, transporter-mediated secretion also contributes to its renal clearance, albeit to a lesser degree. Inhibition of these transporters causes transient serum creatinine elevation, which can be mistaken as impaired renal function. The current study developed mechanistic models of creatinine kinetics within physiologically based framework accounting for multiple transporters involved in creatinine renal elimination, assuming either unidirectional or bidirectional-OCT2 transport (driven by electrochemical gradient). Robustness of creatinine models was assessed by predicting creatinine-drug interactions with 10 perpetrators; performance evaluation accounted for 5% intra-individual variability in serum creatinine. Models showed comparable predictive performances of the maximum steady-state effect regardless of OCT2 directionality assumptions. However, only the bidirectional-OCT2 model successfully predicted the minimal effect of ranitidine. The dynamic nature of models provides clear advantage to static approaches and most advanced framework for evaluating interplay between multiple processes in creatinine renal disposition.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Serum creatinine can be elevated by drugs that inhibit renal transporters that can be incorrectly interpreted as kidney injury. *In vitro* data suggest that creatinine transport by OCT2 is driven by electrochemical gradient, supporting bidirectional mechanism of OCT2. Data on quantitative contribution of individual transporters to creatinine renal disposition are inconsistent.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Can mechanistic creatinine model enable prediction of creatinine-drug interactions? Is bidirectional transport of OCT2 an important consideration for creatinine renal disposition?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Physiologically based creatinine model for prediction of creatinine-drug interactions (steady-state and time course). The most comprehensive performance evaluation of static and dynamic creatinine models, with consideration of intra-individual variability in serum creatinine.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
☑ Recommendation to consider bidirectional transport mechanism of OCT2 when assessing OCT2/MATE-mediated interactions. Physiological structure of creatinine model allows extension to patient populations and investigation of the intra-individual variability in serum creatinine.

Serum creatinine is a widely used clinical biomarker of glomerular filtration rate and overall renal function. Current guidelines for chronic kidney disease and acute kidney injury define these conditions partly through serum creatinine measurements.[1,2] In the case of chronic kidney disease, serum creatinine is used to calculate estimated glomerular filtration rate (eGFR) with equations validated against exogenously administered filtration markers (e.g., iothalamate).[3,4] The eGFR often guides optimal drug dose adjustments for patients with impaired renal function.[5]

Creatinine is mostly unbound in plasma,[6] supporting the use of its plasma clearance as a glomerular filtration rate (GFR) marker. Despite the high correlation between GFR and creatinine renal excretion clearance, the latter exceeds the former at the population level, indicative of active secretion mediated by transporters expressed in the proximal tubule

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cells.[7,8] Net secretion represents on average 9% of creatinine renal excretion clearance (CL_R) based on inulin as a GFR marker in subjects with normal renal function, although this contribution varies widely (2–15%) between studies.[8] Data based on iothalamate and iohexol as alternative GFR markers suggest up to 24% and 38% contribution of active secretion to creatinine clearance, respectively.[9] Current *in vitro* data on creatinine specificity for renal transporters are inconsistent, with involvement of organic cation transporter 2 (OCT2) and organic anion transporter 2 (OAT2) indicated on the basolateral membrane, and multidrug and toxin extrusion (MATE)1 and MATE2-K transporters, on the apical (luminal facing) membrane of the proximal tubule cells (**Table S1**). In addition, involvement of OAT4 and OCT3 has been proposed.[10]

The use of creatinine clearance (or its estimate based on serum concentration) as biomarker of renal function assumes a parallel change in transporter-related secretion and glomerular filtration in the event of kidney injury. The above assumption can be violated when the transporter activities are modulated for reasons not related to kidney injury resulting in false impression (based on creatinine clearance) of renal injury. Several drugs have been associated with transient elevations in serum creatinine attributed to inhibition of transporters involved in creatinine secretion (**Figure 1**), with no serious adverse renal events. Although average increases in serum creatinine as a result of renal transporter inhibition typically fall below the clinical threshold for acute kidney injury,[1] this may not be the case for some individuals. Potential for misinterpretation of elevated serum creatinine as a loss of renal function in the patient highlights importance of in-depth understanding of transporter-mediated creatinine-drug interactions, and ability to predict these *a priori*.

The physiologically-based pharmacokinetic (PBPK) modeling approach is widely used for quantitative prediction of drug-drug interactions (DDIs).[11–13] PBPK models for DDI predictions rely upon quantitative translation of *in vitro* data through use of *in vitro-in vivo* extrapolation (IVIVE). Examples of creatinine models and their application to predict effects following administration of different drugs have been reported in the literature.[14,15] In contrast to those examples, the current study developed physiologically based creatinine models accounting for its synthesis and mechanistic description of processes occurring in the renal proximal tubule. These mechanistic models incorporated multiple transporters involved in creatinine renal elimination, assuming either unidirectional or bidirectional OCT2 transport (driven by electrochemical gradient). Accounting for bidirectional transport by OCT2 was previously demonstrated as an important consideration in PBPK simulation of cimetidine-metformin DDI.[16,17] In a companion paper,[18] technical details of the stepwise development

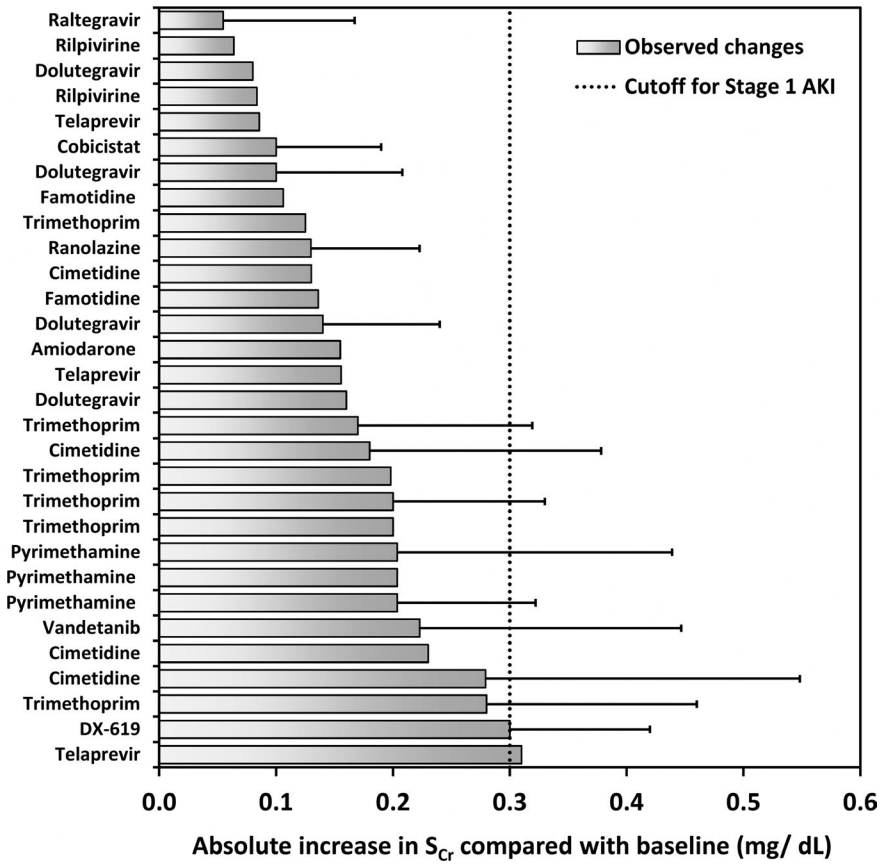

Figure 1 Transient increases in serum creatinine (S_{Cr}) observed during administration of specific drugs to subjects with normal renal function. Each grey bar represents the mean observed increase in serum creatinine from baseline (measured before administration of drug) during drug administration for a particular study; error bars represent SD for that study (see **Table S2** for references). Dotted line represents the clinical threshold for acute kidney injury (AKI; serum creatinine increase of 0.3 mg/dL compared with baseline).[1]

of mechanistic creatinine models and their refinement with clinical creatinine-trimethoprim interaction data are reported.

Following initial model optimization against trimethoprim clinical data, critical evaluation of the ability of different creatinine models to predict quantitatively creatinine-drug interactions with 10 perpetrator drugs was performed. This performance evaluation accounted for intra-individual variability in serum creatinine. To test the robustness of creatinine models, perpetrators selected showed different inhibitory effect on transporters involved in creatinine renal disposition. In addition, the creatinine models were compared with respect to their ability to describe the mechanisms of creatinine secretion and re-absorption.

METHODS

Initial evaluation of the creatinine-drug interaction risk via OAT1, OCT2, and MATEs

Serum creatinine profiles, drug plasma concentration-time data, and fraction unbound in plasma ($f_{u,p}$) for 11 inhibitors were collated and analyzed, as detailed in Scotcher *et al*.[18] There was no literature evidence on the direct effect of these inhibitors on creatinine synthesis. Literature reported half-maximal inhibitory concentration (IC_{50}) data for individual transporters of interest were collated; whenever possible *in vitro* inhibition data obtained with creatinine as transporter probe were used (**Table 1**).

Initial qualitative evaluation of clinical creatinine-drug interaction potential was performed using the basic DDI prediction model.[19] In brief, compounds' classification toward risk of clinical creatinine interaction were based on proposed unbound maximum concentration in plasma ($C_{max,u}$)/IC_{50} cutoffs of 0.1 and 0.02 for OCT/OAT and MATE transporters, respectively.[19,20] Changes in serum creatinine < 5%, associated with intra-individual variability in serum creatinine,[21] were classed as "negative."

Physiological structure of creatinine models

Detailed description of the stepwise development of mechanistic kidney models for creatinine and corresponding assumptions are reported in a companion paper.[18] Two models were selected for subsequent performance evaluation of their ability to predict creatinine-drug interactions. The workflow of model development and compartmental structure of the models are presented in **Figure S1** and **Figure 2**. The models differed in describing the mechanism of transport by OCT2, assuming either unidirectional uptake of creatinine via OCT2, or bidirectional transport (net membrane permeation) of creatinine via OCT2. The bidirectional-OCT2 model considered the role of membrane potential in the electrochemical gradient driven transport rate by OCT2, as described in Eqs. 1 and 2:

$$J_{o\to i,\mathrm{OCT2}} = \mathrm{CL}_{\mathrm{int,OCT2,preMP}} \cdot \frac{N}{\left(e^{N}-1\right)} \cdot \left(C_o \cdot f_{\mathrm{ion,o}} - e^{N} \cdot C_i \cdot f_{\mathrm{ion,i}}\right) \quad (1)$$

$$N = \frac{z \cdot \Phi \cdot F}{R \cdot T} \quad (2)$$

where $J_{o\to i,OCT2}$ is the net flux via OCT2 in direction outside to inside of cell; $CL_{int,OCT2,preMP}$ is intrinsic clearance of OCT2, before impact of membrane potential is applied; C_o and C_i are concentrations of creatinine outside and inside the proximal tubule cell, respectively; $f_{ion,o}$ and $f_{ion,i}$ are the ionized

Table 1 Summary of perpetrator drug properties[a]

Perpetrator drug	C_{max} in μM (Dose)	$f_{u,p}$[b]	IC_{50} (μM)[c,d]				Largest reported % change in S_{Cr} (Dose)
			OAT2	OCT2	MATE1	MATE2-K	
Cimetidine	12 (1,600 mg/day)	0.824	102.3	36.3	3.78	23.7	25.8 (1,600 mg/day)
DX-619	22.0 (800 mg/day)	0.320	1,000[e]	0.94	0.82	0.1	32.3 (800 mg/day)
Cobicistat	1.55 (150 mg/day)	0.063	24.1	10.7	4.1	22.5	10.6 (150 mg/day)
Dolutegravir	13.1 (100 mg/day)	0.006	1,000[e]	8.25	5.8	49.3	16.7 (100 mg/day)
Indomethacin	5.59 (50 mg)	0.060	2.1	1,000	1,000	1,000	0 (150 mg/day)
Pyrimethamine	2.3 (50 mg)	0.112	1,000[e]	0.93	0.17	0.35	26.1 (75 mg/week[f])
Famotidine (low dose)	0.39 (40 mg/day)	0.724	184	27.9	0.27	7.3	1 (40 mg/day)
Famotidine (high dose)	0.93 (200 mg)	0.724	184	27.9	0.27	7.3	17.9 (800 mg/day)
Ranolazine	6.01 (2,000 mg/day)	0.372	109	7.42	5.56	55.4	20.0[g] (2,000 mg/day)
Rilpivirine	0.6 (25 mg/day)	0.005	1,000[e]	0.38	0.25	0.28	10.6 (25 mg/day)
Trimethoprim	6.9 (20 mg/kg/day)	0.510	1,000[e]	25.8	1.62	0.58	31[h] (20 mg/kg/day)
Ranitidine	3.72 (300 mg/day)	0.728	1,000[e]	2.41	5.6	3.4	0 (300 mg/day)

C_{max}, maximal plasma concentration; $f_{u,p}$, fraction unbound in plasma; IC_{50}, half-maximal inhibitory concentration; S_{Cr}, serum creatinine.
[a]References listed in **Table S3**.
[b]$f_{u,p}$ is average value if reported in multiple studies.
[c]IC_{50} values obtained using creatinine as a probe, except for DX-619 (creatinine as probe for OCT2, tetraethylammonium as probe for MATE1 and MATE2-K), rilpivirine (metformin as probe for OCT2, MATE1 and MATE2-K), and indomethacin (creatinine as probe for OAT2, metformin as probe for OCT2, MATE1 and MATE2-K).
[d]No pre-incubation with inhibitor, although other studies suggest pre-incubation effects on IC_{50} (see **Supplemental Material, Section 3**).
[e]No inhibition observed, IC_{50} set to 1,000 μM for simulation of creatinine-drug interactions.
[f]Co-administered with 200 mg dapsone.
[g]Value falls to 12.0% after adjusting for placebo-control.
[h]Represents largest mean value, largest reported change in specific individual was 181%.

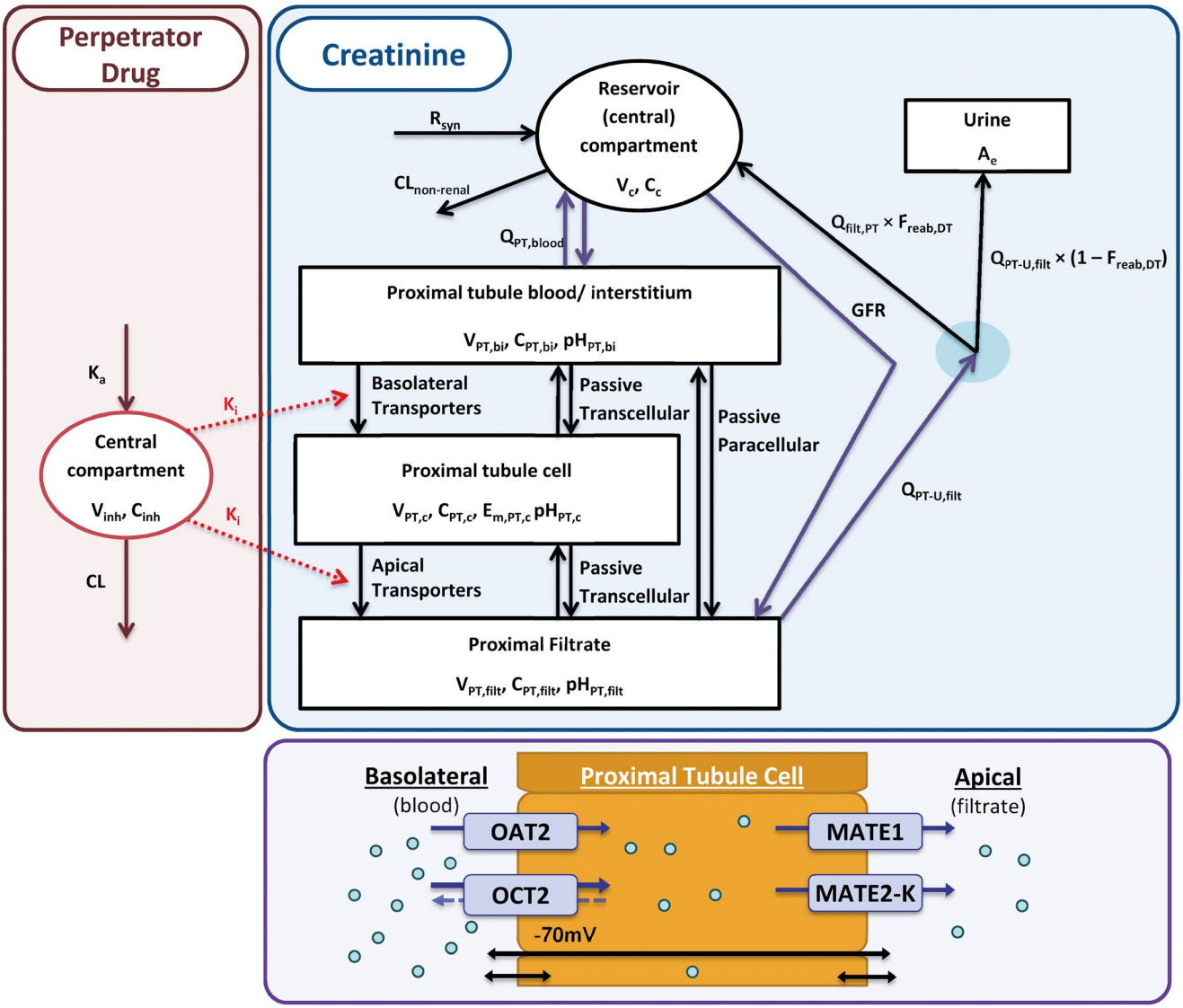

Figure 2 Compartmental structure of model used for simulation of creatinine-drug interactions.[18] Blue shaded area presents schematic of creatinine mechanistic kidney model. The concentration (C_x (mg/L)) in each x^{th} compartment is a model state, with the amount excreted in urine (A_e) also representing a state. The central (reservoir) compartment (subscript *c*), which represents the blood plasma receives, the input function representing creatinine synthesis rate (R_{syn} (mg/hour)). Nonrenal clearance ($CL_{non-renal}$) represents a minor elimination route from the central compartment. The central compartment is linked with the proximal tubule blood/interstitium compartment (subscript PT,bi) in a physiologically realistic manner through the proximal tubule blood flow ($Q_{PT,blood}$ (L/hour)), and to the proximal filtrate (subscript PT,filt) through glomerular filtration rate (GFR (L/hour)). Filtrate flow out of the proximal filtrate is described with a flow rate parameter ($Q_{PT-U,filt}$ (L/hour)). Passive permeability of creatinine in non-proximal nephron regions, the loop of Henle, distal tubule and collecting ducts are described under assumption of first-order re-absorption using fraction reabsorbed ($F_{reab,DT}$) parameter. In proximal tubule cells (subscript PT,c), the roles of passive permeability (transcellular and paracellular) permeability and transporters expressed on the basolateral (organic anion transporter 2 (OAT2) and organic cation transporter 2 (OCT2)) and apical (multidrug and toxin extrusion protein (MATE) 1 and 2-K) membranes are presented in the purple shaded area. OCT2 was modeled as either an uptake transporter or as a bidirectional transporter in variant creatinine models. As a bidirectional transporter, net flux by OCT2 is a function of the electrochemical gradient of creatinine and the membrane potential ($E_{m,PT,c}$ (70 mV); see Eqs. 1 and 2). The red shaded area shows a one-compartment model used to simulate the plasma concentration of the perpetrator (inhibitor) drug (subscript inh), with oral absorption rate constant (K_a) and elimination clearance (CL). The plasma concentration of perpetrator drug, along with its half maximal inhibitory concentration (IC_{50}) or inhibitory constant (K_i), is used to drive inhibition of transporter activity in the creatinine model (Eq. 5).

fraction of creatinine outside and inside the proximal tubule cell, respectively; *z*, Φ, *F*, *R*, and *T* are the valence of creatinine, the membrane potential, Faraday's constant, the Gas constant, and the absolute temperature, respectively.

Differential equations describing proximal tubule cell concentrations for the uptake-OCT2 (Eq. 3) and bidirectional-OCT2 (Eq. 4) models are shown below; the full set of model equations is listed in ref. 18

$$V_{PT,c}\frac{dC_{PT,c}}{dt} = C_{PT,bi} \cdot (CL_{PD,trans} + CL_{int,OAT2} + CL_{int,OCT2} \cdot f_{ion,PT,bi}) + C_{PT,filt} \cdot CL_{PD,trans} - C_{PT,c} \cdot (2 \cdot CL_{PD,trans} + CL_{int,MATE1} + CL_{int,MATE2-K}) \quad (3)$$

$$V_{PT,c}\frac{dC_{PT,c}}{dt} = C_{PT,bi} \cdot (CL_{PD,trans} + CL_{int,OAT2}) - CL_{int,OCT2,preMP} \cdot \frac{N_{PTC}}{(e^{N_{PTC}} - 1)} \cdot (e^{N_{PTC}} \cdot C_{PT,c} \cdot f_{ion,PT,c} - C_{PT,bi} \cdot f_{ion,PT,bi}) + C_{PT,filt} \cdot CL_{PD,trans} - C_{PT,c} \cdot (2 \cdot CL_{PD,trans} + CL_{int,MATE1} + CL_{int,MATE2-K}) \quad (4)$$

where $V_{PT,c}$, $C_{PT,c}$, $C_{PT,bi}$, $C_{PT,filt}$, $CL_{PD,trans}$, $CL_{int,OAT2}$, $CL_{int,OCT2}$, $CL_{int,OCT2,preMP}$, $CL_{int,MATE1}$, $CL_{int,MATE2-K}$, $f_{ion,PT,bi}$, and $f_{ion,PT,c}$ are the volume of the proximal tubule cells, the concentration of creatinine in the proximal tubule cells, blood and filtrate, the transmembrane passive permeability, and the intrinsic clearances for OAT2, OCT2 (uptake), OCT2 (bidirectional, before impact of membrane potential is accounted for), MATE1 and MATE2-K, and the fraction ionized of creatinine in the blood/ interstitium and cell, respectively.

MATE transport is proton gradient driven and was assumed to mediate efflux under *in vivo* physiological conditions.[22] Passive permeability in proximal tubule and re-absorption in remaining tubular regions of nephron were initially implemented by IVIVE of *in vitro* apparent permeability data scaled by corresponding tubular surface areas; subsequently, this parameter was also optimized by clinical data.[18]

Nakada *et al*. (2018) reported a static model for describing trimethoprim-creatinine interaction and related changes in serum creatinine concentrations and creatinine CL_R.[14] This one-compartment model accounted for processes of filtration, secretion, and re-absorption, but lacked physiological complexity of the models in the current study. In order to benchmark models and their predictive performance,[23] the Nakada model was also considered in comparative performance evaluation in the current study (implementation details in **Supplementary Material, Section 5**).

The plasma concentration-time profiles of all perpetrator drugs were described using one-compartment or two-compartment pharmacokinetic models, as described in ref. 18 (**Figure S2** for dolutegravir, as an example). For famotidine, different pharmacokinetic models were required for the high and low doses. The inhibitory effect of perpetrator drugs on OAT2, OCT2, MATE1, and MATE2-K transporters in uptake-OCT2 model is described by Eq. 5, adapted from ref. 24 and using *in vitro* data listed in **Table 1**. Analogous equation was applied by replacing transporter intrinsic clearance (CL_{int}) in Eq. 5 with net flux rate (J; see Eqs. 1 and 2) for OCT2 in the OCT2-bidirectional model, but using Eq. 5 for OAT2, MATE1, and MATE2-K. Equivalent equation was applied for simulation of creatinine-drug interactions with the Nakada model (see **Eq. S3**).

$$CL_{int,i,inh}(t) = \frac{CL_{int,i}}{1 + \frac{C_{p,j}(t) \cdot f_{u,p,j}}{IC_{50,i,j}}} \quad (5)$$

where $CL_{int,i,inh}(t)$ is the intrinsic clearance of the i^{th} transporter at time t after accounting for inhibition, $CL_{int,i}$ is the intrinsic clearance of i^{th} transporter (see Eqs. 3 and 4), $C_{p,j}(t)$ is the plasma concentration of perpetrator j at time t, $f_{u,p,j}$ is the fraction unbound in plasma for perpetrator j, and $IC_{50,i,j}$ is the unbound concentration of perpetrator j causing 50% inhibition of transporter i.

Creatinine-drug interactions were simulated following the study designs described in respective clinical studies. Simulations were performed for a nominal duration of 96 hours to ensure steady-state serum creatinine concentrations in the simulation before initiating administration of perpetrators.

Evaluation of predictive performance of creatinine mechanistic models

Creatinine physiologically based models developed in the current study were optimized by creatinine-trimethoprim clinical interaction data to recover creatinine CL_R; use of CL_R data alone (without perturbation by interaction) were insufficient.[18] The creatinine-trimethoprim interaction data were excluded from the evaluation of the model performance, consistent with PBPK modeling best practices.[24–28] Predictive performance of unidirectional or bidirectional OCT2 creatinine models was evaluated by assessing the number of data points within prediction limits **Eqs. S4–S7**. Where individual serum creatinine concentration data were reported, mean values were also calculated to evaluate prediction success. Predictive performance was evaluated using either (a) maximum change (single perpetrator administration) or steady-state changes (repeated administration) in serum creatinine, or (b) complete profiles of creatinine-drug interactions, where these were reported. The latter case included data following withdrawal of drug (i.e., serum creatinine returning to baseline).

Commonly applied twofold limits were modified to account for intra-individual variability in baseline serum creatinine concentration (limits **Eqs. S4–S7**). Consideration of stricter prediction limits was adapted from Guest *et al*.[29] to ensure appropriate distinction of true-negative and false-negative interaction prediction (i.e., when observed percentage change in serum creatinine concentration is close to 0%). The limits coalesce when the observed change is 0% and approach the traditional twofold limits with more pronounced interaction. These new prediction limits considered intra-individual percentage coefficient of variation in baseline serum creatinine concentration (CV_I). The EuBIVAS Project recently reported CV_I of 4.4% and 4.7% based on enzymatic and alkaline picrate methods, respectively,[21] and CV_I of 4.7% was used in the current study.

Models of creatinine and perpetrator drugs, described as systems of ordinary differential equations, were implemented in Simulink version 8.9 (R2017a), The MathsWorks (Natick, MA), using a variable-step numerical solver. The exact solver was automatically selected by the Simulink software, but was typically ode15s (numerical differentiation formulas). Solver settings were not changed from the default values, with the exception that relative tolerance was set to 1/100,000. Simulation data were exported to Matlab R2017a, The MathsWorks for statistical analyses and generation of figures.

Table 2 Summary of perpetrator drug pharmacokinetic models[a]

	Pharmacokinetic model parameters[b]						
Perpetrator drug	**CL (L/hour)**	**V_2[c] (L)**	**k_a (1/hour)**	**Q (L/hour)**	**V_3[c] (L)**	**K_m (μM)**	**V_{max} (μmol/hour)**
Cimetidine	39.20	115.80	1.57	–	–	–	–
DX-619	9.00	114.80	–	–	–	–	–
Cobicistat	10.00	67.40	0.49	–	–	–	–
Dolutegravir	0.65	9.09	4.73	0.43	3.08	–	–
Indomethacin	3.77	7.71	1.86	2.24	19.30	–	–
Pyrimethamine	1.14	137.18	2.04	–	–	–	–
Famotidine (low dose)	60.74	89.46	0.27	–	–	–	–
Famotidine (high dose)	116.00	715.00	1.14	–	–	–	–
Ranolazine	22.40	110.00	0.06	–	–	4.80	128.6
Rilpivirine	8.84	142.83	0.19	11.96	311.62	–	–
Trimethoprim	3.78	97.82	4.37	–	–	–	–
Ranitidine	33.40	135.00	0.72	–	–	–	–

CL, clearance, C_p, plasma concentration; k_a, absorption rate constant, K_m, Michaelis constant, Q, inter-compartment clearance, V_{max}, maximum rate of elimination, V_x, volume of compartment indicated by subscript x.
[a]References listed in **Table S3**.
[b]See ref. 18 for full description and equations of the models, in brief, these are (i) a one-compartment model with linear elimination, (ii) a two-compartment model with linear elimination, and (iii) a one-compartment model with linear and nonlinear (Michaelis-Menten kinetics) elimination.
[c]V_2 and V_3 represent the volumes of the central (plasma) and peripheral compartments, respectively.

RESULTS

Data collation

Clinical and *in vitro* inhibition data (**Tables 1** and **2**) were collated for trimethoprim, cimetidine, DX-619, cobicistat, dolutegravir, pyrimethamine, famotidine, ranolazine, and rilpivirine; these drugs were associated with renal transporter inhibition leading to changes in serum creatinine concentration. In addition, the data set included indomethacin and ranitidine, which exhibited minimal effect on serum creatinine (**Table 1**). In total, 193 measurements of changes in serum creatinine from 28 studies were collated, with the percentage change in serum creatinine ranging from –3.5% to 181%.

Qualitative evaluation of the creatinine-drug interaction risk via OAT2, OCT2, and MATEs

A qualitative approach for evaluation of renal transporter DDI risk was initially applied. *In vitro* transporter IC_{50} data obtained with creatinine as a transporter probe were used where available to overcome any potential substrate-dependent inhibition issues associated with OCT2/MATEs (**Table 1**). In this preliminary analysis, most drugs that caused transient elevated serum creatinine were correctly identified for subsequent follow-up clinical evaluation, with $C_{max,u}/IC_{50}$ exceeding cutoff of 0.02 for MATE1 (**Figure 3**). However, false-positive (e.g., ranitidine and lower dose famotidine) and false-negative (e.g., cobicistat) outcomes were evident, consistent with previous analysis performed with metformin IC_{50} data.[30] Similar trend was noted for MATE2-K, with weaker relationship for OCT2 and no apparent trend for OAT2 (**Figure 3**).

Quantitative model-based prediction of creatinine-drug interactions

Physiologically based kidney models for creatinine were developed and accounted for the roles of transporters and passive permeability via transcellular and paracellular routes in the proximal tubule.[18] In contrast to previous modeling attempts,[14,15,31] mechanistic creatinine models developed here explicitly defined the proximal tubule cell compartment, including membrane localization of OAT2 and OCT2 (basolateral) and MATE1 and MATE2-K (apical) transporters (**Figure 2**). In addition, the role of the resting membrane potential on transport rate and direction was considered for OCT2, as described previously for metformin.[17,32]

Stepwise approach in creatinine model development is illustrated in **Figure S1**. Initial proteomic-informed IVIVE of CL_{int} underestimated creatinine CL_R by up to 14%. Therefore, clinical trimethoprim-creatinine data (76 measurements from six studies) were used to refine some of the key model parameters, while retaining relative contributions of each transporter to overall proximal tubule uptake and efflux as in the IVIVE approach. In the uptake-OCT2 model, OAT2 and OCT2 had similar contributions to creatinine transporter-mediated uptake in the proximal tubule (46% and 54%, respectively), whereas MATE2-K (76%) had larger contribution than MATE1 (24%) to transporter-mediated efflux (**Table S6**). The total transporter uptake $CL_{int,u}$ was 67-fold greater than total transporter efflux $CL_{int,u}$. Relative transporter activities for the bidirectional-OCT2 model were concentration-dependent, with maximum contribution of OCT2 of 54% to creatinine uptake under basolateral-cellular sink conditions (i.e., concentration inside cell << concentration outside cell); at the other extreme, under cellular-basolateral sink conditions, OCT2-mediated efflux dominated over uptake by OAT2.

Following successful recovery of the observed creatinine CL_R, creatinine models were applied to predict creatinine-drug interactions with 10 perpetrator drugs. The inhibitory effects on OAT2, OCT2, MATE1, and MATE2-K were considered using corresponding *in vitro* IC_{50} data. Impact of complete transporter inhibition on the simulated steady-state creatinine concentrations in plasma and proximal tubule cell and

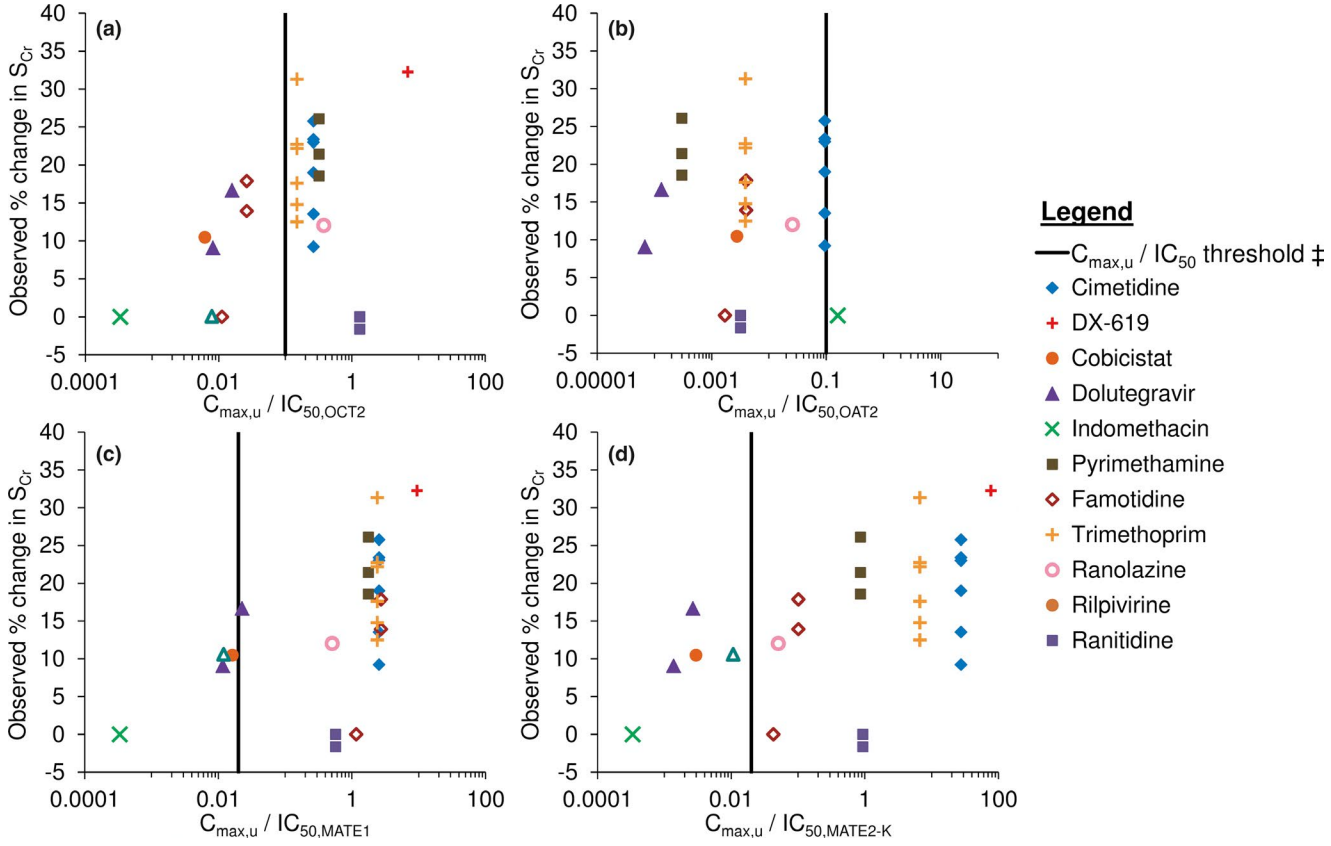

Figure 3 Comparison of $C_{max,u}/IC_{50}$ with observed % change in serum creatinine (S_{Cr}) for 11 perpetrators. IC_{50} values are for OCT2 (**a**), OAT2 (**b**), MATE1 (**c**), and MATE2-K (**d**). Clinically observed percentage change in serum creatinine, C_{max}, $f_{u,p}$, and *in vitro* IC_{50} (creatinine as substrate where available) data were obtained from literature (**Table 1** and **Table S4**). C_{max}, maximum concentration of drug in plasma; $C_{max,u}$, maximum unbound concentration of in plasma; $f_{u,p}$, fraction unbound in plasma; $IC_{50,MATE1}$, half maximal inhibitory concentration for MATE1 transporter; MATE1, multidrug and toxin extrusion 1. ‡$C_{max,u}/IC_{50}$ threshold correspond to those proposed for potential drug-drug interaction risk evaluation of 0.1 (OCT2 and OAT2) or 0.02 (MATE1 and MATE2-K).[19,20]

proximal tubule cell-to-plasma partition coefficient was investigated; summary of different scenarios and their effect on rate-determining step in creatinine disposition is in **Table S7**. The data for trimethoprim were not included in model performance evaluation to separate the "model development" and "verification" data sets and ensure robust evaluation. Three models were considered in this analysis, namely the uptake-OCT2 and bidirectional-OCT2 mechanistic creatinine models as described above, and a mechanistic static model reported in the literature ("Nakada model").[14]

For the first time, the evaluation of prediction success of creatinine-drug interactions accounted also for the 5% intra-individual variability in serum creatinine.[21] The newly developed prediction limits allowed apparently negative changes in creatinine concentrations observed in some instances (likely arising from intra-individual variability) to be considered in the evaluation of model predictive performance (**Supplementary Material, Section 7**). The need for more restrictive limits than those used generally for DDI prediction arises from smaller observed changes in serum creatinine (typically not exceeding ~ 30% increase) than considered clinically relevant for metabolism or transporter DDIs. The ability of the mechanistic creatinine models to predict changes in creatinine CL_R could not be assessed for all creatinine-drug interactions investigated due to limited availability of clinical data.

Based upon maximum or steady-state change in serum creatinine data, the uptake-OCT2 model (59%) and Nakada model (61%) had similar overall predictive performance when considering the percentage of serum creatinine data (n = 117 measurements) within prediction error limits, with slightly worse performance of the bidirectional-OCT2 model (51%; **Table 3**). Overall trends remained when all creatinine data were included in the analysis (i.e., consideration of profiles of serum creatinine changes including potential return to baseline; **Figure S3** and **Table S8**). The uptake-OCT2 model had slightly better predictive performance for drugs that caused > 15% elevation in serum creatinine, for example, DX-619 and cimetidine. Uptake-OCT2 and mechanistic static model by Nakada predicted a false-positive interaction for ranitidine (up to 35% increase in serum creatinine at the highest dose), in contrast to no changes in serum creatinine reported clinically. Such false-positive prediction for ranitidine was not apparent with the bidirectional-OCT2 model. Regardless of the model used, an overall underprediction of the magnitude of interaction was evident (**Figure 4** and **Figure S4**), in particular for cobicistat, dolutegravir, ranolazine, and rilpivirine.

Table 3 Performance of creatinine models in predicting creatinine-drug interactions, considering only maximum change or changes at perpetrator steady-state for each study

		% within prediction limits[a]			% Underpredicted[a]			% Overpredicted[a]		
Perpetrator drug	**Total number of S_{Cr} measurements (*n* studies)**	**Uptake-OCT2 model**	**Bidirectional-OCT2 model**	**Nakada model**	**Uptake-OCT2 model**	**Bidirectional-OCT2 model**	**Nakada model**	**Uptake-OCT2 model**	**Bidirectional-OCT2 model**	**Nakada model**
Cimetidine	8 (6)	50	38	50	50	63	50	0	0	0
DX-619	77[b] (1)	74[c]	60[d]	75[c]	18[e]	39[f]	12[e]	8[g]	1[g]	13[g]
Cobicistat	1 (1)	0	0	0	100	100	100	0	0	0
Dolutegravir	2 (2)	0	0	0	100	100	100	0	0	0
Indomethacin	2 (1)	100	100	100	0	0	0	0	0	0
Pyrimethamine	4 (2)	75	75	100	25	25	0	0	0	0
Famotidine (low dose)	1 (1)	100	100	100	0	0	0	0	0	0
Famotidine (high dose)	2 (1)	0	0	0	100	100	100	0	0	0
Ranolazine	5 (1)	40	0	0	60	100	100	0	0	0
Rilpivirine	9 (1)	0	0	0	100	100	100	0	0	0
Ranitidine	6 (5)	0	83	33	0	0	0	100	17	67
All[b]	117 (22)	59	51	61	31	47	27	10	2	12
All[h]	47 (22)	38	40	40	47	53	49	15	6	11

S_{Cr}, serum creatinine.
[a]See **Eqs. S4–S7** for prediction limits.
[b]Includes data from *n* = 11 individual subjects for DX-619, remaining data represent mean values reported in literature.
[c]Value becomes 86% if mean data are considered.
[d]Value becomes 71% if mean data are considered;
[e]Value becomes 14% if mean data are considered.
[f]Value becomes 29% if mean data are considered.
[g]Value becomes 0% if mean data are considered.
[h]Mean data for DX-619 considered.

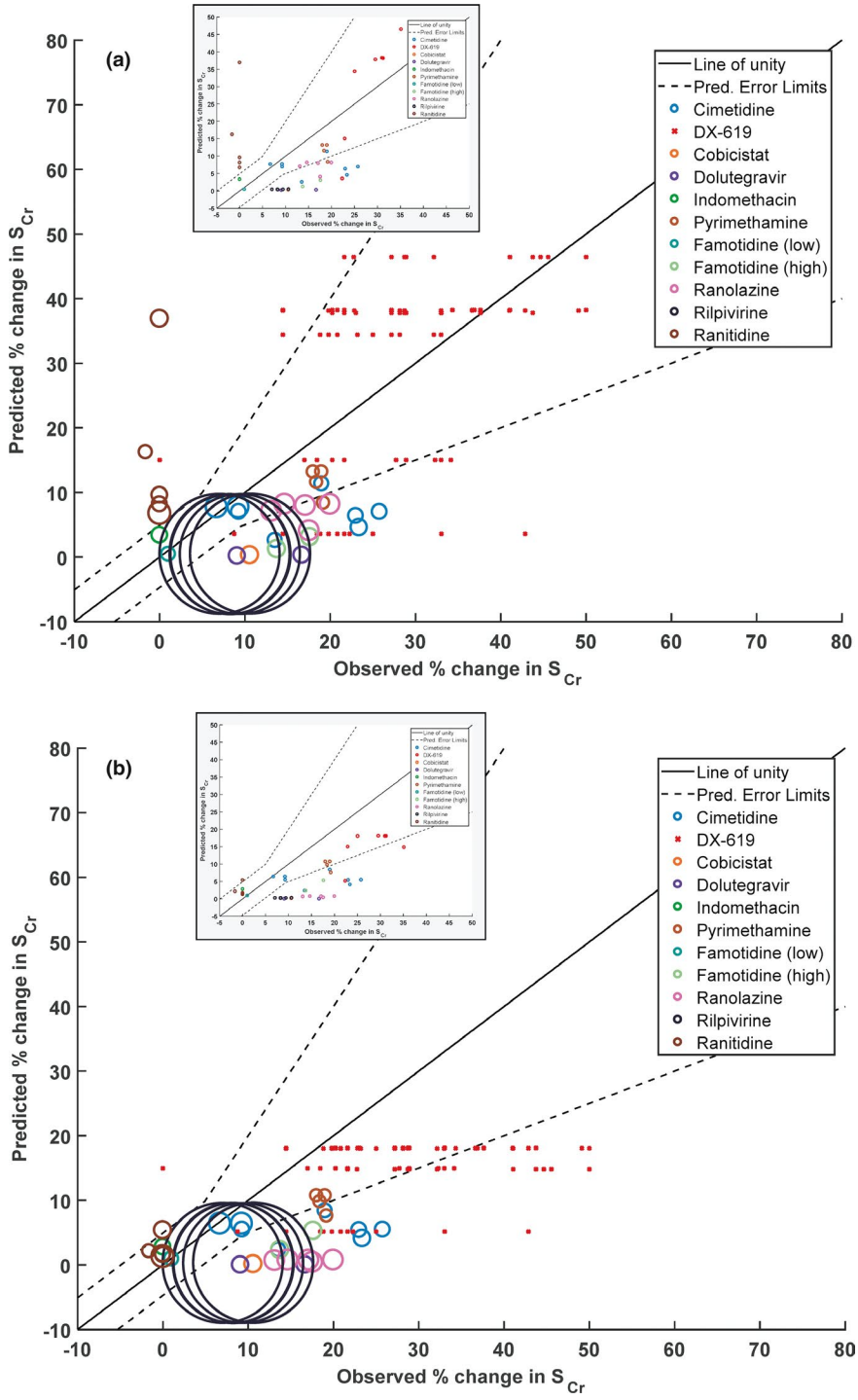

Figure 4 Predictions of creatinine-drug interactions for steady-state or maximal percentage change in serum creatinine (S_{Cr}) data using the uptake-OCT2 model (**a**), and bidirectional-OCT2 model (**b**). For studies with repeated administration of perpetrator, percentage change in serum creatinine data at steady-state were included; for studies with single administration of the perpetrator drug, or for which steady-state was not reached, the maximal percentage change value was included. Prediction error limits (dashed lines) are described by **Eqs. S4–S7**. Open circles indicate observed data reported as mean, with the area of the circle proportional to the number of subjects in the relevant clinical study, crosses indicate individual data. Inset figures show the central tendency for the observed data.

DISCUSSION

Transient increase in serum creatinine is commonly observed as a result of transporter-mediated interactions, in particular, via OCT2/MATE inhibition. Despite this, creatinine is not deemed as the most sensitive endogenous biomarker for these transporters.[33] However, considering its wide use as a biomarker of renal function, ability to

correctly predict and differentiate renal transporter interaction from reduced renal function due to drug-induced kidney injury is important. The current study developed a physiologically based creatinine model incorporating multiple processes in the proximal tubule in a mechanistic manner. The primary goal was the assessment of the creatinine models' ability to predict creatinine-drug interactions via different renal transporters (individual or combined).

Predictive performance of creatinine models

The current analysis represents the most systematic evaluation of creatinine models to-date; models investigated differed in their complexity and physiological description of proximal tubule. This evaluation showed overall comparable predictive performance of static and dynamic models when assessing maximum steady-state effect of perpetrators on serum creatinine. Ability of current models to predict correctly minor (< 10%) changes in serum creatinine is seen as an advantage to less mechanistic approaches that may result in false-positive outcomes. A mechanistic creatinine model that featured bidirectional OCT2 transport was the only model that correctly predicted negligible interaction between ranitidine and creatinine (**Table 3**). Consideration of the electrochemical gradient driving force for OCT2 transport is consistent with previously reported metformin PBPK model,[16,17] *in vitro* data demonstrating an effect of membrane potential on creatinine accumulation (**Table S1**), and *in vitro* data reporting efflux transport of tetraethylammonium and acetylcholine by OCT2.[34,35] Despite current limited number of perpetrators to test this model, OCT2 transport driving force is seen as an important consideration for complex interactions with dual OCT2/MATE inhibitors, in particular in cases when inhibitor's intracellular concentration may be higher than in plasma.

Although the overall predictive ability of the mechanistic static and dynamic models was comparable when predicting steady-state effect of perpetrators, additional criteria should be considered in model evaluation. The mechanistic dynamic models allowed quantitative integration of creatinine renal disposition, interrogation of mechanistic assumptions, and identification of knowledge gaps and uncertainties (in fraction transported, permeability data, and tubular re-absorption). All above is consistent with quantitative systems pharmacology approach (i.e., a useful model is one that permits new mechanistic insight to be gained).[36] In addition, dynamic models allowed simulation of time course of changes in serum creatinine, together with newly proposed prediction limits that accounted for intra-individual variability in serum creatinine for the evaluation of prediction success of biomarker interactions.

Rate-determining step and contribution of specific transporters in creatinine renal disposition

Current modeling efforts identify uptake via OCT2 and OAT2 as rate-determining processes driving creatinine disposition in the proximal tubule cells. Despite underprediction of creatinine active secretion by bottom-up proteomics-informed IVIVE (limitations discussed in ref. 18), current study provides the most robust to-date estimate of the relative creatinine transport clearances for OCT2, OAT2, MATE1, and MATE2-K and passive permeability to support model development (**Table S6**). Consequences of complete inhibition of individual transporters on proximal tubule cell-to-plasma partition coefficient and rate-determining step in creatinine disposition are summarized in **Table S7**. Many clinical creatinine-drug interactions can be rationalized by inhibition of MATEs alone (**Figure 3**), and role of this transporter is supported by clinical genotyping studies (**Table S9**). Likewise, OCT2 genotype data support its role in creatinine renal disposition (**Table S9**), together with *in silico* structural modeling of creatinine interactions with variants of OCT2.[37] Conversely, confidence toward the *in vivo* role of OAT2 is limited by inconsistent *in vitro* uptake data from OAT2-transfected cell lines,[38] limited genetic variability, and lack of drugs known or expected to inhibit OAT2 *in vivo*.

Accurate estimation of fraction transported is a general challenge associated with development of PBPK models for transporter substrates. Overlapping substrate specificity[19,33] and inconsistencies in *in vitro* data between laboratories (**Table S1**) contribute to this. The latter may be due to differences in transporter expression in specific transfected cell lines, proportion of transporter protein located in plasma membrane (vs. intracellular pool), and/or proteomic methods used. Use of different cell culture/assay conditions may also affect transporter functional activity; for example, *in vitro* creatinine transport studies use varying levels of glutamate in culture medium despite its proposed relevance for OAT2 uptake transport *in vitro*. Furthermore, net observed transmembrane effects may be a poor reflection of the activity of individual transporter(s), as several transporters may be acting in opposing and/or parallel directions. As such, quantifying transport rates, and fraction transported by a particular transporter from observed "net effect" data (e.g., *in vivo* CL_R) may be challenging without clear understanding of the rate-determining step or availability of selective transporter inhibitors.[12] *In silico* cell models may assist with delineation of relevant kinetic parameters/processes, but typically rely upon availability of rich data.[39]

Does creatinine undergo tubular re-absorption?

Optimized apparent permeability in the uptake-OCT2 ($29\ cm/s \times 10^{-6}$) and bidirectional-OCT2 ($14\ cm/s \times 10^{-6}$) creatinine models were order of magnitude greater than average reported *in vitro* apparent permeability ($1.15\ cm/s \times 10^{-6}$). This trend was evident also in the static Nakada model, which required a fraction re-absorbed of 34% to recover creatinine-trimethoprim interaction data; value that was much higher than < 10% predicted by available mechanistic re-absorption models.[40] So far, limited evidence supports possibility of saturable tubular reabsorption of creatinine,[41] whereas reports of urine flow dependent CL_R or creatinine-to-inulin clearance ratio have inconsistent findings.[42–44] One potential candidate for creatinine tubular re-absorption is OAT4 expressed on the apical membrane of the proximal tubule,[45] although relevant *in vitro* data to support this are equivocal.[10,15] OCT2 may also mediate creatinine re-absorption at the basolateral membrane of proximal tubule cells, particularly under

conditions of MATE inhibition. Potential role of OCT2 in creatinine re-absorption is supported also by finding that the bidirectional-OCT2 model improved the prediction of negligible clinical creatinine-ranitidine interaction compared with other models (**Figures 3 and 4**). Consideration of active tubular re-absorption may refine existing models and high estimates of passive re-absorption currently required in all models to recapitulate the observations.

Although creatinine is commonly considered as completely unbound in plasma, scarce primary studies are available,[6] whereas a creatinine binding site of albumin has been reported.[46] An additional consideration is that the blood-to-plasma concentration ratio of 1 was assumed in the model due to lack of supporting data. Creatinine is OAT2 substrate (expressed in red blood cells), and showed saturable uptake into human red blood cells.[47] Given the scarcity of measured $f_{u,p}$ and blood-to-plasma ratio data, and their importance in the models, measurements using modern techniques would be beneficial.

Relevance of renal disposition of MATE inhibitors

The underprediction trend seen for certain interactions in the current data set may be a reflection of the pragmatic approach that relied on simulated plasma concentrations of inhibitors to drive transporter inhibition. This assumption may not be correct for inhibitors of MATE transporters, which face the intracellular space and tubular filtrate rather than plasma.[48] Mechanistic modeling of cellular perpetrator concentrations was beyond the scope of the current study, and challenged by information gaps on whether the perpetrators themselves are substrates for renal transporters (**Table S10**). A preliminary approach was applied to estimate the unbound proximal tubule luminal (i.e., filtrate) to plasma concentration ratio ($K_{p,uu,filtrate}$) of perpetrators (**Table S10**). Although improvement of underprediction was seen in the case of famotidine ($K_{p,uu,filtrate}$ = 9.5), it also led to overprediction of interaction for ranitidine ($K_{p,uu,filtrate}$ = 15.8), highlighting a need for further refinement and consideration of mechanistic models of perpetrator renal disposition for evaluation of such interactions.

In conclusion, the current study evaluated physiologically based creatinine models for prediction of transporter-mediated creatinine-drug interaction. Increasing evidence of OCT2 bidirectional transport mechanism supports its consideration in the model. The physiological structure of the models has added advantage over static methods; it allows simulations of dynamics of creatinine-drug interactions over time, investigation of the interplay of transporter processes and provides an excellent platform for investigation of the intra-individual and interindividual variability in serum creatinine. Considering knowledge gaps in creatinine renal disposition highlighted here, it is important to refine and re-evaluate the mechanistic model when new data become available. In contrast to static models, the physiological basis of the mechanistic creatinine model allows its future application to specific populations (e.g., impaired renal function) and corresponding evaluation of transporter-mediated interaction risk in this patient cohort.

Supporting Information. Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (www.psp-journal.com).

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