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Authors
Hsueh, C-H
Hsu, V
Zhao, P
et al.

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PBPK Modeling of the Effect of Reduced Kidney Function on the Pharmacokinetics of Drugs Excreted Renally by Organic Anion Transporters

C-H Hsueh1,2,3, V Hsu2, P Zhao2, L Zhang2, KM Giacomini1 and S-M Huang2

Altered pharmacokinetics (PK) in subjects with chronic kidney disease (CKD) may lead to dosing adjustment of certain drugs in subjects with CKD. It can be valuable to quantitatively predict PK in CKD for the management of drug dosing in these subjects. We developedphysiologically based pharmacokinetic (PBPK) models of seven renally eliminated drugs: adefovir, avibactam, entecavir, famotidine, ganciclovir, oseltamivir carboxylate, and sitagliptin. These drugs are all substrates of renal organic anion transporters (OATs). Drug models verified using PK data from healthy subjects (HS) were coupled with physiological models representing CKD that incorporated prior knowledge of effects of CKD on hepatic and renal elimination. The models reasonably described clinically observed PK changes in subjects with CKD (compared to subjects with normal renal function), with predicted AUC changes within 50% of the observed changes. PBPK models can be used to prospectively predict PK of renally eliminated OAT substrates in subjects with CKD.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
- PBPK modeling is a useful tool but could be challenging when predicting drug pharmacokinetics (PK) in a specific population such as the CKD population.

WHAT QUESTION DID THIS STUDY ADDRESS?
- To determine if PBPK models informed by current knowledge can be used to predict PK in CKD subjects for drugs that are excreted renally by organic anion transporters (OATs).

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
- Predictive performance appears to be established for predicting PK of renally cleared OAT substrates in CKD subjects by PBPK models.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
- For a renally cleared OAT substrate, considerations proposed in this work can be taken to quantitatively predict its PK and to support dosing recommendations in CKD.

The kidneys play a critical role in drug disposition. Of the top prescribed drugs in the US, more than 90% of the renally eliminated drugs undergo (net) tubular secretion, which may significantly contribute to the renal clearance (CLr).1 As a substantial portion of people in the US suffer from chronic kidney diseases (CKD) and many of them require multiple medications, dosing drugs properly based on the altered pharmacokinetic (PK) in these subjects is important.2 In CKD subjects, reduction in both glomerular filtration and tubular secretion can lead to a significant decrease in CLr for renally eliminated drugs. Consequently, for those subjects doses may need to be adjusted.

An effective dosage adjustment of a therapeutic drug in CKD subjects requires an understanding of its clearance mechanism and quantitative effects of CKD on various pathways relevant to the drug’s disposition. For renally cleared drugs, changes in glomerular filtration can be quantified in CKD. However, there seems to be a lack of consensus on the effect of CKD on tubular secretion. Bricker et al. introduced the intact nephron hypothesis (INH), which proposed that damaged nephrons stop working completely, whereas undamaged nephrons are functionally normal in subjects with reduced kidney function.3,4 The INH, which predicts that glomerular filtration and tubular secretion decline in parallel, is consistent with CLr for some drugs in subjects with various degrees of CKD, e.g., memantine.5 Our recent study of drugs that are transported by organic anion transporters (OATs) might challenge this hypothesis.6 We showed that reductions of glomerular filtration rate (GFR) and the secretory clearance (CLsec via tubular secretion) for these drugs were disproportional, particularly in severe CKD subjects. The reduction in CLsec appears to be greater than that of GFR. We also showed that the activity of OATs can be inhibited by uremic solutes at clinically relevant concentrations. These solutes are metabolites synthesized in the body, and can accumulate to very high concentrations in advanced CKD subjects.6 This may explain the disproportional deteriorations of glomerular filtration and tubular secretion observed in subjects with CKD for these OAT substrates. The

1Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, California, USA; 2Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland, USA; 3Oak Ridge Institute for Science and Education (ORISE) Fellow, Oak Ridge, Tennessee, USA. Correspondence: Ping Zhao (Ping.zhao@gatesfoundation.org)

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inhibition of other renal transporters by uremic solutes remains to be elucidated. In addition to renal elimination pathways, CKD might affect hepatic drug metabolism.6,7

The process-specific changes in CKD make prospective prediction of drug PK a challenging task. Because CKD involves a complex/dynamic alteration of physiology, the physiologically based pharmacokinetic (PBPK) model, which incorporates both drug-specific parameters and physiological parameters, can be used to predict the effect of CKD on drug exposure.8 In this study, we developed PBPK models for seven renally cleared drugs that are OAT substrates. The need to consider inhibition of OAT activities in addition to INH was evaluated and confirmed through our analysis. These findings allowed us to propose a model-based framework for prospectively predicting PK profiles of renally cleared OAT substrates in CKD subjects. The key hypotheses are depicted in Figure 1.

RESULTS
PBPK models in healthy subjects (HS)
The drug-specific parameters of the seven drugs are shown in Table 1. All drugs are predominantly excreted by the kidney (fraction excreted unchanged (fe) >0.65) and are known substrates of OATs. Of note, the tubular secretion of these drugs accounts for between 27% for avibactam (AVI) and 78% for entecavir (ETV) of the corresponding CLr, suggesting the importance of the renal tubular secretion in their elimination, presumably by OATs. We assumed OATs contribute to most of the CLsec for these drugs but the potential involvement of other transporters could not be ruled out. The models were verified with datasets that were not used during model development. Examples of the simulated data vs. observed data in the verification dataset can be found in Supplementary Figure 1 and Supplementary Table 1. These base models also described well the PK (area under the curve (AUC) and CLr) of HS in the CKD trials (Supplementary Table 2).

Prediction of the effect of CKD on drug PK using PBPK modeling
Drug PK in CKD populations was prospectively predicted using models developed and verified in HS in virtual populations representing specific CKD groups (Figure 2, Supplementary Table 2). The models appear to reasonably describe observed changes in AUC and CLr in CKDs, as measured by AUC ratio (AUCR, CKD/HS) and CLr ratio (CLrR, CKD/HS) across seven drugs for various CKD stages. All predicted AUCRs (Figure 2, middle panel) and most of the predicted CLrRs were within 1.5-fold of the observed data (Figure 2, dashed lines). Predicted CLrRs for adevofivr (ADV) was not within the 1.5-fold of the observed data in severe CKD (0.66 of observed value). In addition, 14 and 15 of the 21 predicted AUCR (67%) and CLrR (71%), respectively, were within a more stringent 1.25-fold of the observed data (Figure 2, dotted lines).

DISCUSSION
We demonstrated that PBPK models considering effects of CKD on tubular secretion (assuming INH), hepatic elimination, and inhibition of OATs by uremic solutes reasonably described PK of seven renally cleared OAT substrates in patients of different CKD stages.9–13 INH reasonably predicted CLrR in the early stages of CKD
Anatomic evidence shows that the renal injury in CKD could occur in glomeruli but not in renal tubules (aglomerular tubules) and vice versa (atubular glomerulus). The INH hypothesizes that, despite this anatomical observation showing heterogeneous sources of renal damage, once a nephron is injured, it does not belong to the functional nephron population.3,4 The persisting nephrons will compensate for the nephron loss. In a way, the nephrons remain functionally homogenous. This has been supported by animal studies.1 By physically injuring a single kidney and surgically separating the urinary bladder into two, researchers were able to compare diseased and healthy kidney in the same animal.
The ratio of effective renal plasma flow over GFR and the ratio of maximum tubular excretion of para-aminohippuric acid (PAH) over GFR were comparable for diseased and healthy kidneys. This endorsed the concept of the INH. In our models, we reduce CLsec in accordance with GFR by reducing the number of proximal tubular cells per gram kidney (PTCPGK). This is based on the pilot study by Hsu et al. Adjustment of this number represents INH better, as it describes the loss of tubular cells. PBPK models with this adjustment appear to describe observed changes in CLr in mild and/or moderate CKD.

**Insufficiency of the INH to explain PK change in severe CKD**

Interestingly, the INH alone underpredicted the reduction in CLr (higher predicted CLrR) in severe CKD in our PBPK models (**Supplemental Figure 2**). In the animal model described above, uremic solutes would not accumulate, since at least one kidney remains intact. In contrast, in an animal model of acute kidney injury induced by chemicals, both kidneys would be damaged and uremic solutes are expected to accumulate. Notably, PAH clearance showed a greater reduction than GFR in the acute kidney injury, consistent with a suppression in tubular

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**Table 1  Drug-specific parameters in PBPK models**

<table>
<thead>
<tr>
<th>Drug</th>
<th>ADV</th>
<th>AVI</th>
<th>ETV</th>
<th>FAM</th>
<th>GCV</th>
<th>OC</th>
<th>SITA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (g/mol)</td>
<td>273</td>
<td>265</td>
<td>277</td>
<td>338</td>
<td>255</td>
<td>284</td>
<td>407</td>
</tr>
<tr>
<td>logP</td>
<td>-2.8</td>
<td>-1.8</td>
<td>-1.4</td>
<td>-0.98</td>
<td>1.66</td>
<td>-2.4</td>
<td>1.95</td>
</tr>
<tr>
<td>Compound type</td>
<td>amph</td>
<td>acid</td>
<td>amph</td>
<td>base</td>
<td>amph</td>
<td>amph</td>
<td>base</td>
</tr>
<tr>
<td>pKa</td>
<td>2/6.8</td>
<td>0</td>
<td>2.77/8</td>
<td>6.83</td>
<td>2.2/9.4</td>
<td>3.6/8.2</td>
<td>8.8</td>
</tr>
<tr>
<td>B/P</td>
<td>0.59-28</td>
<td>0.7642</td>
<td>1.41</td>
<td>0.932</td>
<td>1.36</td>
<td>0.6</td>
<td>1.49</td>
</tr>
<tr>
<td>fu</td>
<td>0.96</td>
<td>0.93</td>
<td>0.8741</td>
<td>0.83</td>
<td>0.9836</td>
<td>0.97</td>
<td>0.65</td>
</tr>
</tbody>
</table>

**Absorption**

<table>
<thead>
<tr>
<th>Model</th>
<th>ADAM</th>
<th>N/A</th>
<th>1st order</th>
<th>N/A</th>
<th>1st order</th>
<th>1st order</th>
<th>1st order</th>
</tr>
</thead>
<tbody>
<tr>
<td>fa</td>
<td>N/A</td>
<td>0.9441</td>
<td>N/A</td>
<td>0.6135</td>
<td>0.8</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Ka (1/h)</td>
<td>N/A</td>
<td>1.2541</td>
<td>N/A</td>
<td>2.5636</td>
<td>0.11</td>
<td>0.3330</td>
<td></td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>N/A</td>
<td>031</td>
<td>N/A</td>
<td>0.3136</td>
<td>1.38</td>
<td>030</td>
<td></td>
</tr>
<tr>
<td>Peff, man (10^-4 cm/s)</td>
<td>0.25</td>
<td>427</td>
<td>N/A</td>
<td>0.91</td>
<td>5.38</td>
<td>9.56</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**Distribution**

| Vss (L/kg) | 0.426 | 0.237 | 0.91 | 1.234 | 0.836 | 0.97 | 0.65 |
| Kp scalar | 1.3 | 0.7 | 2.5 | 2.5 | 0.5 | 0.8 | 0.9 |

**Elimination**

| CLr (L/h) | 15.626 | 9.2337 | 23.142 | 18.633 | 12.236 | 19 | 17.430 |
| CLur (L/h) | 1.326 | 0.937 | 13.342 | 9.133 | 036 | 0 | 7.630 |
| %TS of CLr | 56 | 27 | 78 | 68 | 42 | 63 | 73 |
| fe (%) | 83.728 | 9237 | 7042 | 6733 | 10036 | 10039 | 6530 |

**Transport**

| CLint,T by Effd (μl/min/106 cells) | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| CLPD (ml/min/106 proximal tubular cells) | 3.28 | 0 | 0 | 8.45 | 0 | 1.86 | 0 |

ADV, adefovir; amph, ampholyte; AVI, avibactam; B/P, blood to plasma ratio; CLint,T by Eff, in vitro efflux transporter-mediated clearance for the apical transporter; CLint,T by OATs, in vitro OATs-mediated clearance; CLr, renal clearance; CLur, non-renal clearance; ETV, entecavir; fa, fraction absorbed; FAM, famotidine; fe, fraction excreted unchanged; fu, Fraction unbound in plasma; GCV, ganciclovir; Ka, First order absorption rate constant; OC, oseltamivir carboxylate; %TS of CLr, percentage of tubular secretion contributed to renal clearance, calculated as (CLr – fu*GFR) / CLr, where CLr is the renal clearance, fu is the fraction unbound and GFR is the glomerular filtration rate; Peff, man, effective permeability in man; SITA, sitagliptin; Vss, volume of distribution at steady state.

*Unless otherwise specified, values were collected from online resources such as drugbank (https://www.drugbank.ca/) and pubchem (https://pubchem.ncbi.nlm.nih.gov/); 1predicted44,45 and Kp scalar was adjusted to match clinical observations; 2fitted from plasma concentration-time profiles by parameter estimation while keeping relative activity factor/relative expression factor (RAF/REF) = 1 in HS; 3CLint,T by Eff was unidentifiable and was obtained by sensitivity analysis to match drug accumulation in the urine (fe) as reported by Hsu et al14 The default value of 1 was then used for all drugs since it was sufficient to reasonably describe observed CLr and fe; 4passive diffusion clearance, estimated from the passive permeability measured in HEK293 cells using metformin as the standard47; 5predicted by software’s mechanistic permeability model; 6modified from Hsu et al.14; 7percentage of dose recovered in urine unchanged ml/min/106 cells to tubule.

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The ratio of effective renal plasma flow over GFR and the ratio of maximum tubular excretion of para-aminohippuric acid (PAH) over GFR were comparable for diseased and healthy kidneys. This endorsed the concept of the INH. In our models, we reduce CLsec in accordance with GFR by reducing the number of proximal tubular cells per gram kidney (PTCPGK). This is based on the pilot study by Hsu et al. Adjustment of this number represents INH better, as it describes the loss of tubular cells. PBPK models with this adjustment appear to describe observed changes in CLr, in mild and/or moderate CKD.
activity by the solutes.\textsuperscript{15,16} PAH is a well-known substrate of OATs. In the analysis by Hsueh \textit{et al}., drugs that were excreted by OATs showed a greater reduction of $CL_{sec}$ compared to GFR, particularly in severe CKD.\textsuperscript{6} The study also demonstrated that several uremic solutes were clinically relevant inhibitors of OATs. Overall, the evidence suggests that on top of the INH, reduction of intrinsic clearance mediated by OATs ($CL_{OATs}$) is observed clinically, and is presumably a result of an accumulation of uremic solutes. Indeed, an additional reduction in the $CL_{OATs}$ is needed to restore the observed clinically $CL_{rR}$ in our models. Overall, the simulations support our proposal of incorporating inhibition of $CL_{OATs}$ in severe CKD (Figure 3).

**Degree of the $CL_{OATs}$ reduction in CKD**

It might not be necessary to adjust $CL_{OATs}$ for mild and moderate CKD (Supplementary Figure 2, right panel). The INH appears sufficient to capture the $CL_{rRs}$ observed clinically, even though statistical analyses favor a 0–10% reduction in $CL_{OATs}$ in moderate CKD (Supplementary Table 3). In contrast, it is clear that INH alone is not sufficient to describe the $CL_{rRs}$ in severe CKD. A reduction in $CL_{OATs}$ is necessary. The best values to reduce $CL_{OATs}$ in severe CKD fall within 40–55%. Based on the analysis from 18 CKD trials, the median value of $CL_{OATs}$ reductions is around 27% and 59% for moderate and severe CKD, respectively (values used in prospective predictions in Figure 2).\textsuperscript{6} The values are different from the best descriptors here (27% vs. 0–10% for moderate CKD and 59% vs. 40–55% for severe CKD). The concentrations of uremic solutes are susceptible to the impacts of environment, GFR, food, etc. If uremic solutes are indeed the key factor that leads to the reduction in the $CL_{OATs}$, differential accumulations of uremic solutes among CKD trials might potentially explain the difference in the best descriptors. In addition, we assumed that OATs are responsible for $CL_{sec}$ of all test drugs. A significant contribution by other transporters to
CL_{sec} may affect prediction and calculation of best descriptors. Nevertheless, both support the need to reduce CL_{OATs} in CKD, particularly in severe CKD. The assumption that OATs are responsible for all CL_{sec} also ensures the conservativeness of prospective prediction of an investigational OAT substrate in severe CKD.

The understanding of uremic solutes in CKD is increasing but still requires much work. For example, the reported concentrations are usually from subjects with end stage renal disease (ESRD) during the off-dialysis period. Concentrations of many solutes in other stages of CKD remain unavailable. Furthermore, it is unclear if there are other unidentified solutes that potentially inhibit OATs. As such, although adjusting the CL_{OATs} based on the inhibitory potencies ([I]/IC_{50}) of uremic solutes is more mechanistic, it is not deployed here.

Degree of the nonrenal clearance (CL_{nr}) reduction in CKD
Increasing data suggest that CKD impairs CL_{nr} as well as CL_{fr}.9,10,13,17 Thus, it is now recommended that changes in CL_{nr} be included in the development of PBPK models to predict PK of drugs in CKD.18,19 Our data partially confirmed these findings. The confirmation of CL_{fr} followed by AUCR allowed us to examine the need of CL_{nr} reduction. Even when the predicted CL_{fr} matched the observed data, the AUCR could still be underpredicted unless a reduction in the CL_{nr} was applied. This is most obvious for severe CKD and for drugs with relatively high CL_{nr}, such as ETV, sitagliptin (SITA), and famotidine (FAM) (Supplementary Figure 2, middle panel, and Supplementary Figure 3). Intuitively for adeovir (ADV), AVI, ganciclovir (GCV), and oseltamivir carboxylate (OC), whose CL_{nr} contributes little to the total CL (fe >0.8), the effect of reduced CL_{nr} on the predicted AUCR is negligible. Different nonrenal elimination pathways are responsible for the seven compounds studied here, and contributed to the total CL from 0% (OC) to 37% (ETV). The best scaling factor for the CL_{nr} in CKD was not evaluated in our study because of its relatively insignificant contribution compared to the renal pathway. Due to the lack of knowledge of the quantitative impact of CKD on different nonrenal elimination pathways, the same scaling factor was considered to account for the reduction of CL_{nr} in CKD. That was 31% and 36% reduction in the CL_{nr} for moderate and severe CKD, respectively, which represented the average values from the ranges reported by Sayama et al.19 It appears that the use of these factors worked for drugs predominately renally excreted. However, for those drugs whose CL_{nr} contribute significantly to the total CL, it should be evaluated carefully.

Other variables that might be considered
In this study, we assumed that CKD does not affect oral absorption. However, increased bioavailability in the subjects with CKD has been reported.13 This could be due to the reduced CL_{nr} (reduced first pass effect) or reduced intestinal P-glycoprotein (P-gp).20 ETV and SITA are substrates of P-gp. Since the fraction absorbed (Fa) for these two drugs are relatively high (0.94 for ETV and 1 for SITA), the effect of CKD on intestinal P-gp (e.g., a reduction of its activity) may be negligible. Active uptake, if any, into the liver or other elimination organs except for the kidneys was not considered in our model either. The expressions/activities of those transporters might be subject to change in CKD. Although the procedure of reducing CL_{nr} could in part account for the effect of transporters in other organs, the incorporation of other transporter information may improve model performance.

In this study, fraction unbound in plasma (fu) was assumed to be constant regardless of the degree of CKD. It is appropriate here, because six out of the seven drugs had fu values greater than 0.8. SITA is the most protein-bound drug in this study (fu = 0.65) and was reported to show no change of fu in subjects with severe CKD.21 However, for those highly protein-bound drugs, significant changes in protein binding by CKD are expected. It might be necessary to consider altered fu.

As OATs are highly abundant in the kidney and interact with structurally diverse organic anions, our assumption that the seven drugs studied here were eliminated via OATs may be valid. When coadministered with probenecid, CL_{sec} of FAM reduced by ~89%,22 suggesting little involvement of other transporters. The contribution of transporters other than OATs cannot be excluded. Further studies are needed to understand if an additional reduction is required for CL_{fr} mediated by other transporters, such as OCT2.23

It is also unclear whether our approach can be generalized to drugs with a smaller contribution of secretion, whose renal elimination is sensitive to urine pH and flow, and which undergo significant reabsorption.

Renal blood flow reduces in CKD. The effect of renal blood flow change on CL_{fr} of test drugs remains unknown. However, there is a report showing that the ratio of renal blood flow and kidney size remains consistent in CKD.24 The software considers changes in kidney size by CKD.

Prediction of drug disposition in subjects with CKD
Based on our analyses and current understanding of the impact of CKD on drug elimination, we summarize the use of PBPK models to predict the PK of renally cleared drugs (fe >0.65) secreted by renal OATs in CKD populations (Figure 3). The procedure begins with development and verification of a drug PK model in HS. For mild CKD, the use of INH, which assumes proportional reduction of GFR and CL_{sec}, appears sufficient. For moderate CKD, reduction in CL_{nr} should be considered besides the INH. In addition to these two adjustments (INH and reduced CL_{nr}), a further reduction in CL_{OATs} is required for predicting PK in severe CKD.

CONCLUSION
Application of PBPK models has been employed in drug development to evaluate drug PK, and to support dosing recommendations.8 Clinical PK studies in CKD are often needed in the early phase of drug development and the design and conduct of such studies can be challenging. Here we have demonstrated the reasonable performance of PBPK models to predict the PK of renally cleared OAT substrates in CKD. Our analysis indicates that for mild or moderate CKD the INH is sufficient to predict
reductions in CLr; however, for severe CKD an adjustment for reduced CLsec needs to be made, potentially as a result of the accumulation of uremic solutes that inhibit OATs. Although there are limitations and further verification/refinement is needed, considerations proposed in this work can be taken to quantitatively predict PK in CKD and to support dosing recommendations, specifically for a renally cleared drug that is an OAT substrate.

**METHODS**

Figure 1 summarizes the key hypotheses: 1) A nephron is the smallest functional unit of the kidney and is composed of the glomerulus and renal tubules. 2) In the early stages of CKD, GFR reduces in subjects with CKD with a proportional reduction of the tubular secretion, as suggested by INH. 3) OATs are critical to the elimination of many drugs excreted by tubular secretion. 4) As the renal function further declines, circulating uremic solutes accumulate to concentrations that can inhibit OATs in subjects with CKD, further reducing CLOATs in the functional nephrons. 5) In addition to CLr, CLnr reduces in subjects with CKD.

**Drug selection and development of drug PBPK models in HS**

Seven drugs with fe from 0.65 to 1 were selected. The PBPK models were constructed using a population-based PBPK software SimCYP (a Certara Company, Sheffield, UK, v. 15.1). Drug-dependent parameters were collected from the literature and are shown in Table 1. The PBPK models in HS were developed by incorporating physicochemical properties, in vitro data on drug disposition, and observed PK in HS. Full PBPK distribution models were selected for all drugs to enable the use of the mechanistic kidney model (Mech KiM) (see below). Tissue distributions were predicted by Rodgers et al. and the “Kp Scalar” was adjusted to match the observed volume of distribution at steady state (Vss).

CLnr was back-calculated from systemic clearance (CLsys) and CLr, and was assigned to an undefined hepatic clearance. Values of CLsys after intravenous (i.v.) drug administration were available for all drugs except for ETV. For ETV, an fa of 0.94 was obtained from a human mass balance study and fraction escaping intestinal metabolism of 1 was assumed because the drug has not been shown to be metabolized by metabolizing enzymes that are significantly expressed in enterocytes. These assumptions allowed the use of retrograde analysis to derive CLnr from apparent clearance after oral drug administration. CLr was described by Mech KiM. Tubular secretion was parameterized by a net tubular uptake transporter to represent the OAT-mediated process as described previously. Passive permeability of the renal tubular cell was included in the models of ADV, FAM, and OC (passive diffusion clearance, CLpass, Table 1), as in vitro data were available. This was calculated from the measured passive permeability in HEK293 cells using metformin as the standard. Passive diffusion clearance was assumed zero for AVI, ETV, GCV, and SITA due to the lack of passive permeability information. Then CLOATs, were obtained using the parameter estimation function against plasma concentration–time profiles after i.v. administration (Table 1), except for ETV (see below).

Parameters describing oral absorption were acquired after the estimation of CLOATs. Fraction absorbed (fa) was estimated from the absolute bioavailability studies (GCV, OC, and SITA) or a mass balance study (ETV). First-order absorption rate constant (ka) and lag time (Tlag) were optimized to match the observed concentration–time profiles after oral administration using the parameter estimation function. As i.v. PK data were not available for ETV, CLOATs was estimated along with ka and Tlag. It is of note that adefovir dipivoxil, valganciclovir, and oseltamivir are the prodrugs of ADV, GCV, and OC, respectively. Approximation of the conversion of prodrugs to active metabolites was accomplished by using a first-order (for GCV and OC) or an Advanced Dissolution, Absorption, and Metabolism model (for ADV) oral absorption function as the drug input, assuming that the administered prodrugs are instantaneously converted to the respective metabolites. The ADAM model was used for ADV because it best described its biotransformation process based on the plasma concentration–time profile of ADV after oral administration of adefovir dipivoxil. The doses were calculated based on the amounts of ADV, GCV, and OC in their produg forms. The values of coefficient of variation (CV) for all drug specific parameters were set to 30%.

**Physiological models in CKD populations**

Once a drug model was generated and verified in HS, it was coupled with physiological models representing CKD populations (or virtual populations, described below) to simulate drug PK in CKD. Stages of CKD were generally defined as follows: HS (GFR ≥90 ml/min), mild CKD (60–89 ml/min), moderate CKD (30–59 ml/min), and severe CKD (15–29 ml/min). Software’s built-in “Sim-Healthy volunteer,” “Sim-RenalGFR_30-60,” and “Sim-RenalGFR_less30” virtual populations were used to further define the different physiological models. In addition to physiology changes already implemented in these virtual populations, the following modifications were made before simulation of drug PK in CKD was conducted.

First, PTCPGK, a system parameter defined in the Mech KiM model, was adjusted to represent INH by keeping a proportional reduction of GFR and tubular secretion for a given CKD group (Supplementary Table 4) according to the equation below:

\[
\frac{CL_{r,\text{HS}}-fu \times GFR_{\text{HS}}}{GFR_{\text{HS}}} = \frac{CL_{r,\text{CKD}}-fu \times GFR_{\text{CKD}}}{GFR_{\text{CKD}}}
\]

where fe is the fraction unbound in plasma.

Next, the inhibitory effect of uremic solutes on the OATs and the effect of CKD on hepatic elimination were considered by applying the relative activity factor/relative expression factor (RAF/REF) values of 0.73 and 0.41 to CLint,OATs in moderate and severe CKDs, respectively, for each test drug. These RAF/REF values represent a 27% and 59% reduction in secretory clearance in addition to INH, as derived from an analysis of 18 drugs by Hsueh et al. according to a static equation. Of note, all drugs from this study were part of that analysis. Then a generic degree of reduction was applied to CLnr. We applied an average reduction of 31% and 36% for moderate and severe CKD, respectively, as reported by Sayama et al.

**Simulation design for CKD trials**

Observed PK profiles and information on study design from CKD trials were collected from the literature. In our simulations, age, GFR, sex, and dosing regimen were matched with the reported clinical trials, as summarized in Supplementary Table 5. Each simulation was conducted using 50 subjects (5 subjects × 10 trials). Modification was made to match the observed GFR for a specific study. This was accomplished by adjusting the plasma creatinine concentration in a given virtual population of the software to achieve the target GFR. For example, for mild CKD, the “Sim-Healthy volunteer” population was used as the template and an adjustment of creatinine concentration was made. As sex and age were not reported in CKD trials of ADV, a 50% female population was assumed. Due to the fact that CKD subjects are generally older, the age of subjects in the CKD group was also assumed to be older (30 ± 7 years for HV, 38 ± 6 years for mild CKD, and 50 ± 8 years for moderate and severe CKD).

**Model performance evaluation**

The predicted AUCRs and CLrRs were compared to evaluate the model predictability. Less than 50% deviation from the observed ratio was arbitrarily regarded as a successful prediction. Root mean square deviation (RMSD) and geometric mean fold error (GMFE) were used to compare the accuracy of model prediction. They are:
Additional Supporting Information may be found in the online version of this article.

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CONFLICT OF INTEREST/DISCLOSURE

The authors declare no conflict of interest. The views expressed in this article do not necessarily reflect the official policies nor endorsements of the Department of Health and Human Services.

AUTHOR CONTRIBUTIONS

C.H.H., V.H., P.Z., L.Z., K.M.G., and S-M.H. wrote the article; C.H.H., P.Z., L.Z., K.M.G., and S.M.H. designed the research; C.H.H. and V.H. performed the research; C.H.H. and V.H. analyzed the data.

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