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Novel minimal physiologically-based model for the prediction of passive tubular reabsorption and renal excretion clearance

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ABSTRACT

Purpose: Develop a minimal mechanistic model based on *in vitro–in vivo* extrapolation (IVIVE) principles to predict extent of passive tubular reabsorption. Assess the ability of the model developed to predict extent of passive tubular reabsorption (F_{reab}) and renal excretion clearance (CL_R) from *in vitro* permeability data and tubular physiological parameters.

Methods: Model system parameters were informed by physiological data collated following extensive literature analysis. A database of clinical CL_R was collated for 157 drugs. A subset of 45 drugs was selected for model validation; for those, Caco-2 permeability (P_{app}) data were measured under pH 6.5–7.4 gradient conditions and used to predict F_{reab} and subsequently CL_R. An empirical calibration approach was proposed to account for the effect of inter-assay/laboratory variation in P_{app} on the IVIVE of F_{reab}.

Results: The 5-compartmental model accounted for regional differences in tubular surface area and flow rates and successfully predicted the extent of tubular reabsorption of 45 drugs for which filtration and reabsorption were contributing to renal excretion. Subsequently, predicted CL_R was within 3-fold of the observed values for 87% of drugs in this dataset, with an overall gmfe of 1.96. Consideration of the empirical calibration method improved overall prediction of CL_R (gmfe = 1.73 for 34 drugs in the internal validation dataset), in particular for basic drugs and drugs with low extent of tubular reabsorption.

Conclusions: The novel 5-compartment model represents an important addition to the IVIVE toolbox for physiologically-based prediction of renal tubular reabsorption and CL_R. Physiological basis of the model proposed allows its application in future mechanistic kidney models in preclinical species and human.

1. Introduction

Renal excretion is considered a major route of elimination for many drugs (*e.g.*, metformin, acyclovir and digoxin) (Morrissey et al., 2013; Tucker, 1981; Varma et al., 2009). Prediction of human renal excretion clearance (CL_R) prior to commencing first-in-man clinical studies currently relies on *in silico* methods based on physico-chemical properties (Dave and Morris, 2015a; Ito et al., 2013; Paine et al., 2010; Varma et al., 2009) and/or allometric scaling (Huh et al., 2011; Paine et al., 2011). Despite wide use of these methods, they do not provide mechanistic insight into the underlying processes contributing to renal excretion and have limited ability to account for any changes in the renal physiology. Mechanistic understanding of various pharmacokinetic (PK) processes has become a necessary part of model-informed decision making for special populations (*e.g.*, obese or patients with renal impairment), as well as devising dosage regimens for use in such populations (Jadhav et al., 2015). The mechanistic approach becomes even more important when certain sub-groups ('complex' patients) exhibit various co-morbidities which make clinical studies

Abbreviations: AUC, area under the plasma concentration-time profile; BCRP, breast cancer resistance protein; CD, collecting duct; C_p, plasma concentration; CL_R, renal excretion clearance; $CL_{R,\ filt}$, renal filtration clearance; $CL_{R,\ int,reab}$, intrinsic permeability clearance in renal tubule; $CL_{R,sec}$, renal secretion clearance; DT, distal tubule; $f_{u,p}$, fraction of drug unbound in plasma; F_{reab}, fraction of the drug reabsorbed in the renal tubule; F_{reab}', intermediate model parameter, representing the fraction of the equilibrium reached between unbound drug concentration in the plasma and urine; GFR, glomerular filtration rate; gmfe, geometric mean fold error; IVIVE, *in vitro–in vivo* extrapolation; LogD, octanol-buffer distribution coefficient; LoH, loop of Henle; MATE, multidrug and toxin extrusion protein; MRP, multidrug resistance protein; OAT, organic anion transporter; OCT, organic cation transporter; OATP, organic anion-transporting peptides; OCTN, organic cation/L-carnitine transporter; P_{app}, apparent permeability; PBPK, physiologically-based pharmacokinetic; P-gp, P-glycoprotein; PT, proximal tubule; RMSE, root mean squared error; TFR, tubular flow rate; TSA, tubular surface area.

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very difficult, if not impossible (Rostami-Hodjegan, 2015). Thus, understanding various elements of renal excretion may offer advantages through prediction of potential differences in various patients under the framework of physiologically-based pharmacokinetic (PBPK) modelling (Zhao et al., 2011). In addition, many currently developed drugs undergo extensive active tubular secretion (Morrissey et al., 2013) for which prediction of CL_R by mechanistic PBPK models (Felmlee et al., 2013; Neuhoff et al., 2013; Posada et al., 2015) is considered more promising in comparison with *in silico* and allometric scaling.

While efforts have been made at predicting renal metabolic clearance from *in vitro* data (Gill et al., 2012, 2013), successful prediction of CL_R using *in vitro–in vivo* extrapolation (IVIVE) remains a challenge. In order to quantitatively and mechanistically predict CL_R using IVIVE, each of the contributing processes (glomerular filtration, active secretion and tubular reabsorption, Eq. (1)) must be considered independently.

$$CL_R = (CL_{R,filt} + CL_{R,sec}) \times (1 - F_{reab}) \quad (1)$$

Filtration clearance ($CL_{R,filt}$) is readily predicted from glomerular filtration rate (GFR) and fraction unbound in plasma ($f_{u,p}$). In cases where both secretion and reabsorption contribute to elimination, confidence in prediction of the fraction reabsorbed (F_{reab}) is equally important as the accurate prediction of renal secretion clearance ($CL_{R,sec}$). Whereas reabsorption is predominantly a passive process, secretion is actively mediated by a range of drug transporters expressed in the kidney such as OAT1, OAT3, OCT2 and MATE2-K (Morrissey et al., 2013).

A number of mathematical models concerning physiological functions of the kidney (*e.g.*, urine concentrating mechanism, solute transport regulation) exist (Layton, 2011; Weinstein, 2015), but may not be readily adaptable for use in renal PBPK models. Further, these models were developed based on physiological and experimental data in rat kidney (*e.g.*, from micropuncture studies) for which analogous data in human are lacking. Recently, a static model for the prediction of CL_R using *in vitro* permeability data from LLC-PK_1 cell monolayers was proposed and its performance was assessed against a relatively small and restricted dataset (Kunze et al., 2014). The model considered both active secretion and tubular reabsorption, and used the proximal tubule surface area as the IVIVE scaling factor for the apparent permeability (P_{app}) data. However, the remaining tubular regions (*e.g.*, collecting duct), which may contribute to passive tubular reabsorption, were not considered (Kunze et al., 2014).

A dynamic kidney model that facilitates IVIVE of renal transporter kinetics and passive permeability has recently been reported (Neuhoff et al., 2013). Although very promising, paucity of data on relevant physiological scaling factors and some of the system data (*e.g.*, transporter abundance) limit model application and validation. In addition, adequate consideration of the heterogeneity of the renal tubule, important for prediction of passive permeability clearance in each tubular segment, is lacking. Current reports on the use of physiologically-based kidney models for 'bottom-up' prediction of renal drug disposition often rely on clinical plasma and/or urine drug concentration data for derivation/optimisation of transporter kinetic parameters and their scaling factors (Dave and Morris, 2015b; Felmlee et al., 2013; Hsu et al., 2014; Watanabe et al., 2011), analogous to the trends seen with prediction of hepatic clearance (Galetin, 2014; Zamek-Gliszczynski et al., 2013). For example, IVIVE of human $CL_{R,sec}$ from *in vitro* uptake data obtained in precision cut kidney slices required an empirical scaling factor of 10 in order to obtain agreement between predicted and observed values (Watanabe et al., 2011). In an analogous manner OAT3 maximal uptake rate (V_{max}) was optimised using plasma concentration–time profiles to refine prediction of pemetrexed CL_R using a PBPK kidney model, and account for differences in transporter expression and activity between the *in vitro* transfected cell system and *in vivo* (Posada et al., 2015).

The aim of this study was to develop a mechanistic model to predict extent of passive tubular reabsorption from *in vitro* permeability data and tubular physiological parameters. The second aim was to assess the ability of the model developed to predict CL_R for a range of drugs for which filtration or reabsorption appeared to be the dominant mechanisms contributing to CL_R. The physiological aspects of the model were informed from the data collated following an extensive literature analysis. A database of *in vivo* CL_R and corresponding F_{reab} was collated for 157 drugs. For a subset of 45 selected drugs, *in vitro* permeability data were generated in Caco-2 cell monolayer under pH 6.5–7.4 gradient conditions. Subsequently, the tubular reabsorption model developed was applied to predict regional and overall passive tubular reabsorption for the selected drug subset ($n = 45$). An empirical calibration approach was proposed to account for the effect of inter-assay/laboratory variation in P_{app} on the IVIVE of F_{reab} using a set of reference drugs as calibrators ($n = 11$). The novel mechanistic 5-compartment model developed enables prediction of the contribution of passive tubular reabsorption to CL_R in a physiologically-based manner and is seen as an integral part of complex kidney models.

2. Materials and methods

2.1. Clinical data collation

CL_R data were collated from literature sources and, wherever possible, data were acquired from primary studies. Further data were gathered from review papers where sufficient details on the trial design had been reported. In addition, data from unpublished clinical studies available at https://www.clinicaltrials.gov were also included in the analysis. Where CL_R values were not reported in the study, Eqs. (2) and (3) were used to calculate CL_R from published urinary excretion and plasma concentration data. Reports of a drug not being detected unchanged in urine, or having "negligible" CL_R, were not considered for collation. Data available in graphical format were digitised using GetData Graph Digitizer v2.25 (http://getdata-graph-digitizer.com/).

$$CL_R = \frac{\text{Amount excreted in urine}_{0-t}}{AUC_{0-t}} \quad (2)$$

$$CL_R = \frac{\text{Urinary excretion rate}}{C_{p,midpoint}} \quad (3)$$

where $AUC_{0\text{-}t}$ represents the area under the plasma concentration–time profile, and $C_{p,midpoint}$ represents the plasma concentration at the midpoint of the urinary collection interval from which the urinary excretion rate was measured.

Only CL_R data acquired following administration of a drug to healthy adult subjects were included in the database. Data from diseased, obese, elderly or alcoholic subjects were excluded, but exclusion criteria based on sex or ethnicity were not applied. Data acquired after co-administration of multiple drugs (*e.g.*, from drug–drug interaction studies) were generally excluded. An exception was made for trimethoprim and sulfamethoxazole because these drugs are generally co-administered and there is a paucity of data following single drug administration. These studies were considered acceptable as there have been no reports in the literature of interactions at the level of renal excretion between sulfamethoxazole–trimethoprim. Aminoglycosides (amikacin, gentamicin, isepamicin, netilmicin, sisomicin and tobramycin) were excluded. These drugs are reported to accumulate in proximal tubule cells, possibly due to endocytotic luminal uptake mediated by the megalin receptor, causing nephrotoxicity (Moestrup et al., 1995; Nagai and Takano, 2004; Schmitz et al., 2002). Drugs with enantiomer specific renal excretion were excluded, an example being cetirizine (Strolin et al., 2008).

In contrast to previous databases (Varma et al., 2009), CL_R data in this database are reported as absolute values, *i.e.* without normalisation for body weight or body surface area. Normalisation was not considered as the majority of literature studies (>75%) reported absolute CL_R values and substantial portion of studies did not report either body weight or body surface area of subjects. In addition, recent