Prediction of pharmacokinetic behaviour by combining *in vivo* and *in vitro* data in physiologically based pharmacokinetic (PBPK) model: Parameter estimation and sensitivity analysis

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Abstract | A large number of failures of potential drug molecules in the late stages of the drug discovery process have been ascribed to poor *in vivo* pharmacokinetic properties. Physiologically based pharmacokinetic (PBPK) models can potentially be used to predict the *in vivo* drug disposition from the *in vitro* characterization of the drug molecules. One potential drawback of the existing models is the large number of parameters and the uncertainty associated with the parameter values. We present a framework for the estimation of kinetic parameters and sensitivity analysis for PBPK model. The existing model was enhanced by incorporating the mechanistic knowledge of drug disposition involving transporter proteins and metabolic enzymes. Some of the drug-specific model parameters have been estimated from the *in vitro* and *in vivo* data available in literature and rest are estimated by fitting the *in vivo* data and *in vitro* data to the model to minimize the error between model predictions and experimental values. The model predictions for tissue specific concentration profiles have been shown to agree with experimental data for rats. Further, the model predictions for plasma drug disposition in humans or animals for the five exemplary drugs agree well with the *in vivo* clinical trial data from literature. The selected drugs are of two different categories; cardiovascular system and diabetes. By using global sensitivity analysis we find that the parameters associated with liver, kidney, and renal excretion have the highest effect on the *in vivo* drug disposition. These analyses and predictions will help in making an early selection of compounds for development based on pharmacokinetic properties as well as for advancing personalized medicine which, in turn will improve therapy for specific subpopulations.

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1. Introduction

Drug discovery and development is a lengthy and costly process, requires an average of 15 years and US$ 880 M to generate a successful medicine[1,2]. The number of potential drug molecules synthesized in recent years has increased markedly. However, a majority of these candidate molecules fail at late stages of the discovery and development process. The realization that the cost of failure in the late stages of clinical trial is very high had motivated pharmaceutical and biotechnology companies to investigate better computer modelling and simulation tools. This allows resources to be concentrated on those compound that are most likely to succeeded and to do so at the early stages of development[3–5]. Models that accurately predict some aspect of Absorption, Distribution, Metabolism and Excretion, and Toxicity (ADME/Tox) can be of utility in early identification of drug candidates that may potentially fail.

There have been several attempts by different commercial firms and universities towards the development of physiologically based pharmacokinetic and pharmacodynamic models. These models have different capability and coverage (Table 1). For example, Gastroplus contains a detailed representation of drug transport and absorption in the gastrointestinal system[6,7], but does not account for the mechanistic details of metabolism. SimCYP deals with the detailed action of hepatic cytochrome P450 enzymes in drug metabolism[8–10]. Distribution of drugs to various organs and tissues is well addressed by PK-Sim and Cloe-PK. One of the major tasks in a pharmacokinetic model development is estimation of parameter values which is difficult to obtain without doing *in vivo* experiments. The majority of the current software does not provide any parameter values or provide any information on how to estimate the parameters. The other area of differentiation is the ability and method used to represent different individuals or species. pkExpress uses an empirical fit with human data for its ADME predictions, whereas, PK-Sim uses different sets of parameters to represent different species. But, the key drawback in the existing models is none of these softwares address vascular differences nor take into account disease state.

In recent years, impelled by exciting technologic advances, an advanced efficiency in gathering biological knowledge on transporter proteins, metabolic enzyme or drug–drug interactions has been realized. These specific metabolic enzymes or transporter proteins play an important role in the distribution and metabolism of all xenobiotics. Further, their expression varies with age, disease state, co-administered drug, which in turn significantly affect the pharmacokinetic behavior of drug molecules. Drug interaction with the metabolic enzymes (Cytochrome p450) or transporter proteins (OATP or MDR) results in inhibition or induction of the enzymes. Inhibition or induction of the metabolic enzymes could result in potentially toxic concentration of parent compound or sub therapeutic effect by reducing drug level below that required for efficacy. Beside these, another major source of variability in pharmacokinetics across ethnic groups is Single Nucleotide Polymorphisms (SNPs) and other genotypic differences within transport proteins and metabolic enzymes[11]. With the emerging biological knowledge it is now possible to acquire *in vitro* data for a potential drug in the screens involving these enzymes. However, none of the existing models use this data for quantitative prediction of the *in vivo* behaviour of drug molecules. Further, the large number of parameters and the uncertainty associated with them is a major drawback of the current model. There have been few initiatives towards quantification of uncertainty in parameter estimates and sensitivity of outcomes to particular parameters and combinations of parameters involved in PBPK models[12]. However, estimation of the PBPK parameter values and understanding their relative importance in prediction of *in vivo* pharmacokinetic behaviour is an aspect that needs more attention. Sensitivity analysis technique can be employed to uncover the critical parameters having significant effect on drug disposition in various organs.

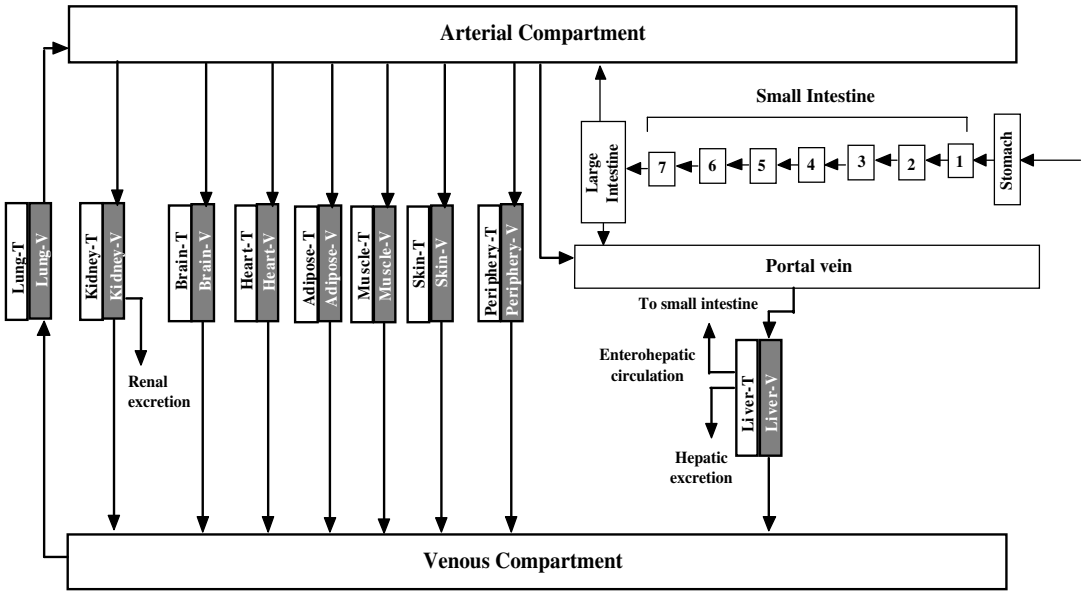
In the present study, PBPK model developed by Willmann S, *et al.*[3] is used as a basis for parameter estimation and sensitivity analysis. More structure has been added to the model by adding not only the knowledge of transporter proteins and metabolic enzymes, but also vascular and tissue-level differences due to patient variations to allow representation of the effect of differences in subject species, genotype, and history. In the present study a total of six drugs from two classes were chosen for model validation: (a) Cardio vascular system and (b) Diabetes. Further, a sensitivity analysis was carried out to determine the relatively important drug dependent parameters and their desired values for a chosen drug. This analysis will help us to investigate how a projected performance of the model varies along with the change in model parameters on which projections are based.

Table 1: List of available PBPK softwares

PBPK softwares	Key Results obtained	Key Drug specific information	Key Patient Data	Comments	References
GastroPlus™ (Simulations Plus)	Drug Absorption in Humans	Physicochemical, Permeability	Species	Main focus is on absorption	6
SimCYP	Drug Metabolism & Clearance	Microsomal data	Population PK	University Research Consortium	8–10
PK-Sim (Bayer)	Absorption & Distribution	Fat solubility, protein binding, MW and clearance	Organ blood flow, volume, fat & protein content	Lacks detailed metabolism	3
Cloe-PK (Cyprotex)	ADME	Physical drug properties, permeability.	None apparent	Collaborative/ Service model	46

A survey of the available commercial software and university initiatives towards pharmacokinetic modeling.

Figure 1: The proposed Physiologically Based Pharmacokinetic (PBPK) model structure for disposition of drugs in humans and animals. T: tissues; V: vascular; Arrows indicate bulk flow; Compartment contacts indicate membrane transport. The numbers in the boxes represent corresponding sub-compartments of small intestine. The model structure was taken from literature[3].



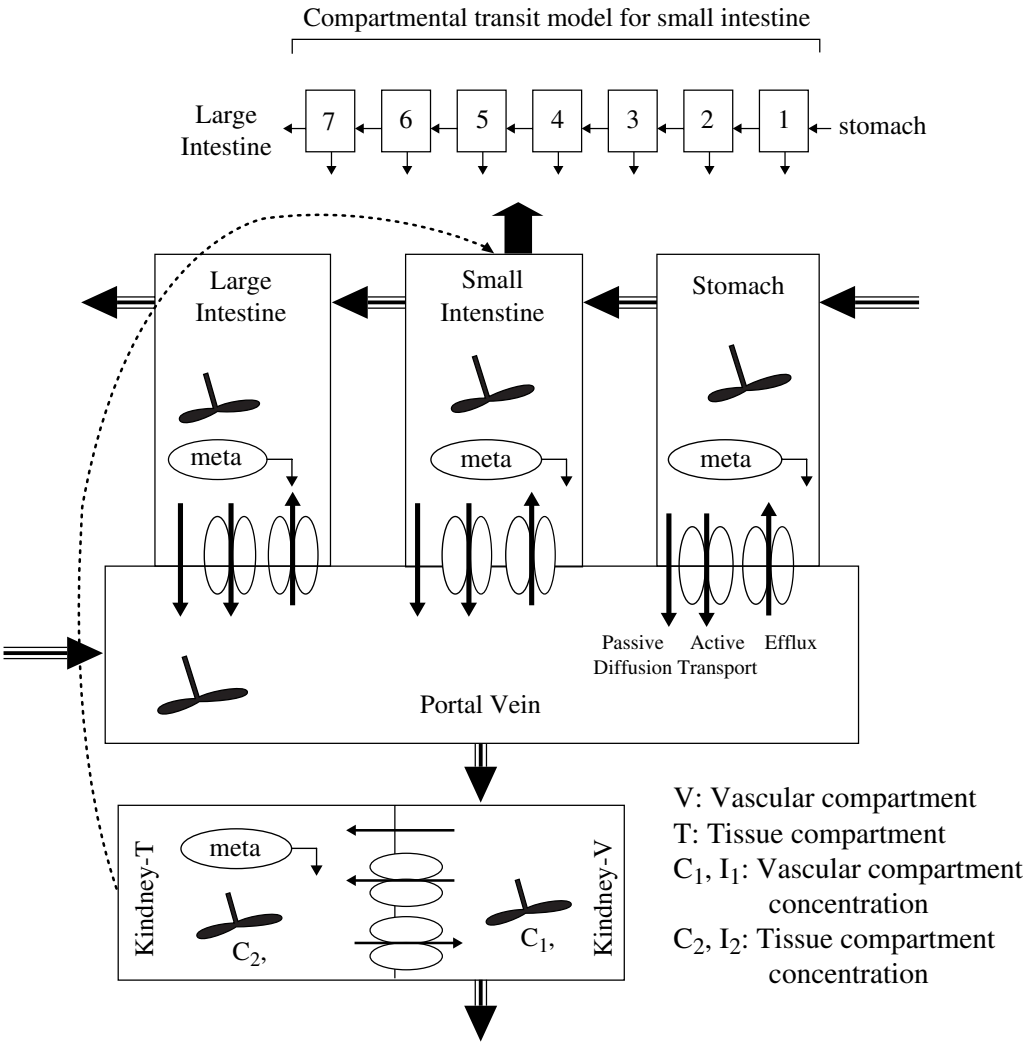
T: tissue; V:vascular; Arrows indicate bulk flow; compartment contact indicates membrane transport

2. Methods

2.1. *Model structure*

The PBPK model used in the present study was developed by Willmann S, *et al.*,[3]. The detail representation of the model structure is described as follows (Fig. 1). It is assumed that the body is made up of finite number of tissue types. Further, each tissue type is considered as made up of two compartments; a vascular compartment and an extra vascular compartment or tissue compartment with permeation barrier (e.g membrane) separating them. Connection between vascular and tissue compartment is via bulk flow (blood capillaries) and or transport. Each compartment (including arterial and venous compartment) is considered to be well mixed. Modelling of gastrointestinal absorption and first pass elimination together with representation of modelling of drug transport processes across a

Figure 2: Modelling of gastrointestinal absorption, drug transport and metabolism. Schematic representation of modelling of gastrointestinal absorption, drug transport process across a membrane and metabolism for a typical organ. A compartmental transit model has been incorporated in order to represent the transit flow of drugs through small intestine tract. Transit flow of drugs in the small intestine is described by seven compartments. Further, three mode of transport process between vascular and tissue compartment has been taken into account: passive diffusion, active transport by transporter proteins and efflux proteins. The rate of transport and metabolism is described by Michaelis-Menten type of kinetic equation. Drug–drug interaction was also taken into the account. The model structure was taken from literature[3].



membrane and metabolism for a typical organ is shown in Figure 2. A compartmental transit model has been incorporated to anatomize the transit flow of oral dosage forms through the human small intestine tract[7]. Transit flow of drugs in the small intestine can be described by seven compartments (Fig. 2). Transport between vascular and tissue compartments is assumed to take place via three mechanisms: passive diffusion, active transport by transporter proteins (inward) and efflux proteins (outward). Drug–drug interaction was accounted in terms of the inhibition of the respective enzyme or transport process by the presence of a second drug. The transport protein and efflux mediated transport and CYP mediated metabolism is described by Michaelis-Menten type of kinetic equation and hence each enzyme has its own set of Michaelis-Menten parameters. There may be enterohepatic recirculation of drugs mediated by coordinated action of several transport proteins. The mass balance equation for change in concentration of a drug in vascular and tissue compartment of an organ *i* are shown in equations (1) and (2), respectively. Equation for rate of diffusion (RD),

rate of transport (RT), rate of metabolism (RM) and rate of efflux (RE) are shown by equations (3)–(6), respectively. The commercial software package MATLAB (Mathworks Inc., Nattic, MA USA), was used for model development and simulation. The MATLAB ODE solver "ode15s" (gear type stiff solver) was chosen for numerical integration because of its variable-order, multistep integration algorithm works well enough with the stiff systems, such as a PBPK model that has both very fast and very slow dynamics.

$$\frac{\mathrm{d}}{\mathrm{d}t}(C_{1_i}) = \frac{F_{\mathrm{in},i}\cdot C_{\mathrm{artery}}}{V_{\mathrm{vascular},i}} - \frac{F_{\mathrm{out},i}\cdot C_{1_i}}{V_{\mathrm{vascular},i}} - \frac{RD_i}{V_{\mathrm{vascular},i}} - RT_i + \frac{RE_i\cdot V_{\mathrm{tissue},i}}{V_{\mathrm{vascular},i}} \quad (1)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}(C_{2_i}) = \frac{RD_i}{V_{\mathrm{tissue},i}} + \frac{RT_i\cdot V_{\mathrm{vascular},i}}{V_{\mathrm{tissue},i}} - RE_i - RM_i \quad (2)$$

$$RD_i = k_i\cdot SA_i\cdot\left(C_{1_i} - C_{2_i}\right) \quad (3)$$

$$RT_i = \frac{V_{\mathrm{max},TR}\cdot C_{1_i}}{K_{m,TR} + C_{1_i} + \frac{K_{i,TR}}{K_{m,TR}}\cdot I_{\mathrm{vascular},i}} \quad (4)$$

$$RM_i = \frac{V_{\mathrm{max,Meta}}\cdot C_{2_i}}{K_{m,\mathrm{Meta}} + C_{2_i} + \frac{K_{i,\mathrm{Meta}}}{K_{m,\mathrm{Meta}}}\cdot I_{\mathrm{tissue},i}} \quad (5)$$

$$RE_i = \frac{V_{\mathrm{max}}\cdot C_{2_i}}{K_{m,\mathrm{Efflux}} + C_{2_i} + \frac{K_{i,\mathrm{Efflux}}}{K_{m,\mathrm{Efflux}}}\cdot I_{\mathrm{tissue},i}} \quad (6)$$

2.2. *Parameter estimation*

PBPK models involve two categories of parameters: (a) Drug independent physiological parameters such as body weight, organ volume, blood flow rates etc.; (b) Drug specific parameters such as the kinetic parameters for transport and metabolism. For all major tissues considered in the model, the volumes and percent blood flow rates were from Brown *et al.*,[13] with the exception of arterial and venous blood volumes, which were from Bonate *et al.*[14] A pseudo-organ named 'periphery', accounts for rest of the body, both the highly perfused and less perfused organs. Tables 2 and 3 show a representative set of physiological parameter values for rat and human respectively.

Drug specific parameters were estimated from *in vitro* studies reported in the literature. A literature search was conducted to identify the *in vitro* data, e.g. kinetic parameters ($V_{\max}$ and K_m) for active transport (influx and efflux) and metabolism. To exemplify, the $V_{\max}$ and K_m for active transport of pravastatin was found to be 0.31 ± 0.13 nmol/min-mg.protein and 16.5 ± 9.6 micro-mol/lt respectively[15]. Similarly, *in vitro* $V_{\max}$ and K_m values for pravastatin metabolism in liver were estimated to be 128.4±149.5 pmol/min-mg.protein and 4887±2185 micro-mol/lt respectively[16]. $V_{\max}$ and K_m values for drug transport and metabolism for the same drug in stomach, kidney, and small intestine were calculated by using relative gene expression level of transporter proteins and metabolic enzyme in those organs. However, the *in vitro* drug specific parameters were transformed into the *in vivo* value in order to use in pharmacokinetic model. One of the major problems with the PBPK model based software is the lack of information/database on drug-specific parameters for the model. Some of these parameters are reported in the literature, but many are still unknown. In this study, we estimated several of the parameters by fitting the *in vivo* data and *in vitro* data to the model. We used the built-in function "fmincon" from the MATLAB optimization tool-box to estimate the kinetic parameters for transport, metabolism and efflux in all major tissues. The routine is utilized to find a constrained minimum of objective function of several variables. The objective function was defined as the deviation between experimental and model predicted values of variable such as plasma concentration profile of drugs in human and animals. In the present study optimization was achieved via *medium scale optimization* which uses a sequential quadratic programming (SQP) method to solve quadratic programming sub problem at each iteration. The maximum and minimum *in vivo* values were used as upper and lower bounds, respectively. Further, it is important to note that because of the underdetermined nature of the system, the kinetic parameters estimated in the present study is one of the feasible solution that exists in the feasible region of the solution space and do not provide an unique solution. A representative set of estimated drug specific parameter values for pravastatin in rats and digoxin in human is shown in Tables 4 and 5, respectively.

2.3. *Sensitivity analysis*

In recent years, computational models have been emerging as important tool in many type of scientific and engineering investigations. Over parameterization is a well known problem for such mathematical models. Therefore, in computer simulation, the important question which may arise is—which factor is most significant when the system involve a large number of factors. This question seems more relevant for biological systems. Biological parameters in particular often have a large uncertainty in their estimate and quite an often it is infeasible (if not impossible) to get a precise estimate of the parameters used in the

Table 2: A representative set of drug independent physiological parameter values for rats.

Panel A Tissue	**% CO***	**Value (L.min^{-1})**
Lung	CO	0.1104
Kidney	14.1	0.0155
Heart	5.1	0.0056
Brain	2.0	0.0022
Adipose	7	0.0077
Muscle	27.8	0.0306
Skin	5.8	0.0064
Bone	12.2	0.0135
Portal Vein	15.3	0.0169
Hepatic artery (into the liver)	3	3.312 x 10^{-3}
Liver-out	18.3	0.0200
Periphery	8.01	0.0088
Panel B		
Tissue	**% BW+**	**Value (gm)**
Stomach	0.46	1.15
Small Intestine	1.4	3.50
Large Intestine	0.84	2.10
Venous Compartment[a]	5.36	13.4
Arterial Compartment[a]	2.72	6.80
Lung	0.5	1.25
Kidney	0.73	1.82
Heart	0.3	0.75
Brain	0.6	1.50
Adipose	7	17.5
Muscle	40	100
Skin	19	47.5
Bone	7.3	18.2
Portal Vein	0.5	1.25
Liver	3.4	8.50
Periphery	4.88	12.2

*Mean Cardiac Output (CO) was taken as 0.1104 L.min^{-1}; +Body Weight (BW) was taken as 250 gm; [a]Values were taken from literature[13,14]; **Panel A** represents cardiac output and regional blood flow rate to all major organs. **Panel B** represents relative organ weight or volume of the organs.

model (some of them might actually be based on intelligent guesses). There have been several approaches reported in the literature for sensitivity analysis e.g. Morris method[17,18], Fourier Amplitude Sensitivity Testing (FAST)[19], Sobol method[20] etc. The Morris method is a one-factor-at-a-time (OAT) method using randomized sampling matrices. This allows direct observation of elementary effects. FAST method is the variance based sensitivity analysis methods, which compute the Total Sensitivity Indices (TSI) of the input parameter. Of the many available methods FAST method has been found to be computationally most efficient and independent of any assumptions about model structure. Further, FAST method not only study the effect of only one parameter, but also effect of all parameters varying together can be assessed by FAST. However, FAST method suffers from computational complexity for a large number of inputs. Sobol' method is similar to FAST method, but computationally less efficient than FAST method. However, in the present study our objective was to identify the critical factors which have significant effect on pharmacokinetic behaviour. To that end, we found Morris randomized OAT design[17,18] is most suitable for parameter screening. The main advantage of this method is that it dose not require any explicit assumption about the system. Further, the number of different simulation configuration required is linear in the number of factors and the results can be interpreted in a lucid graphical way. However, the main limitation of this method is that it does not provide the estimations for factors interactions. The Morris method is known as a global sensitivity analysis method, as it covers the entire space Ω, over which the parameters may vary. In this method, the main effect of a factor may be estimated by computing a number r, a measure of local sensitivity, at different points $x_1, x_2, \ldots, x_r$ in input space and then an average of all r measures can be taken to reduce the dependence on a specific point that a local sensitivity analysis has. The number of runs needed by this method is proportional to the number of parameter K.

The outline of the algorithm and parametric study used in the present work is described in

Table 3: A representative set of drug independent physiological parameter values for human

Panel A Tissue	**% CO***	**Value (L.min^{-1})**
Lung	CO	5.200
Kidney	17.5	0.910
Heart	4.0	0.208
Brain	11.4	0.593
Adipose	5.2	0.270
Muscle	19.1	0.993
Skin	5.8	0.301
Bone	4.2	0.218
Portal Vein	18.1	0.941
Hepatic artery (into the liver)	4.6	0.239
Liver-out	22.7	1.180
Periphery	10.1	0.525
Panel B		
Tissue	**% BW+**	**Value (kg)**
Stomach	0.21	0.147
Small Intestine	0.91	0.637
Large Intestine	0.53	0.371
Venous Compartment[a]	5.36	3.752
Arterial Compartment[a]	2.72	1.904
Lung	0.76	0.532
Kidney	0.44	0.308
Heart	0.5	0.350
Brain	2.0	1.400
Adipose	21.4	14.98
Muscle	40	28.00
Skin	3.7	2.590
Bone	14.3	10.01
Portal Vein	0.5	0.350
Liver	2.57	1.800
Periphery	3.2	2.240

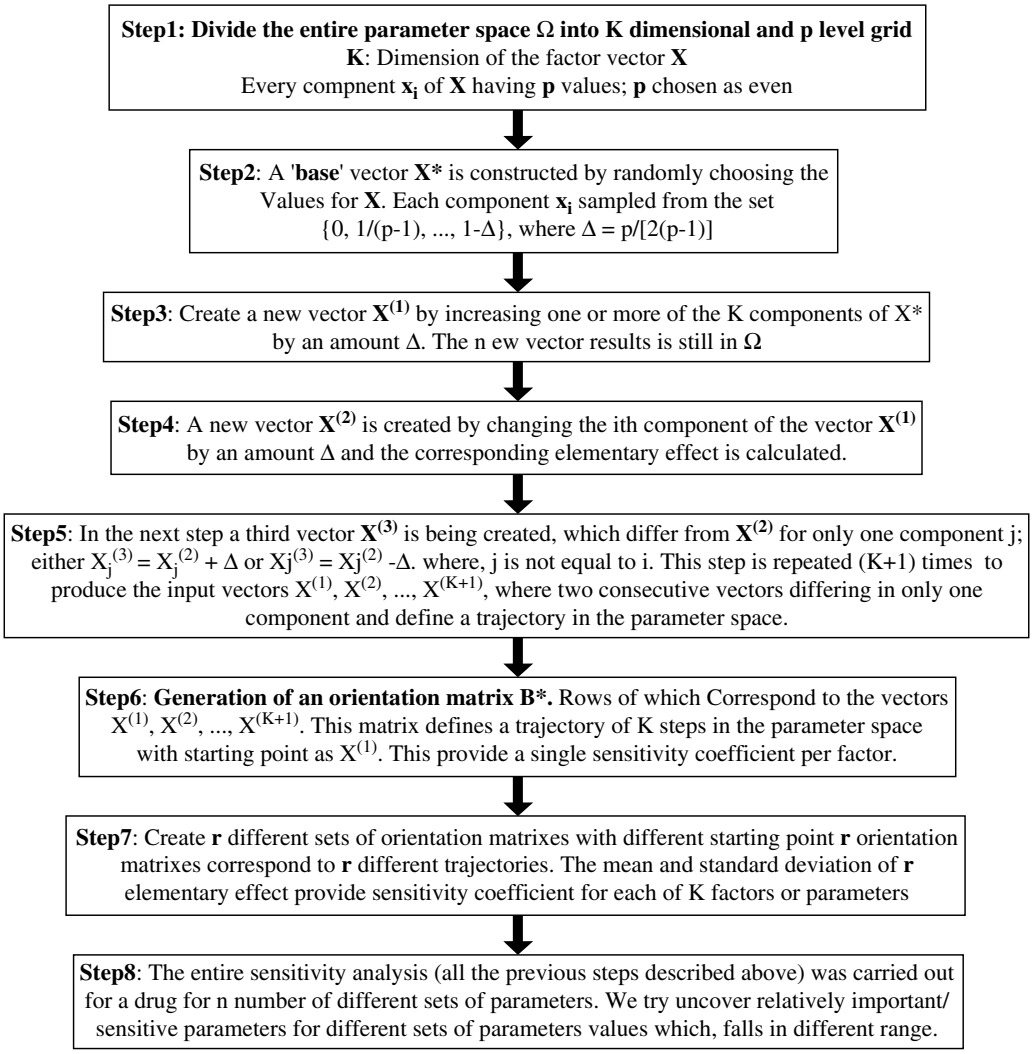
***** Mean Cardiac Output (CO) was taken as 5.2 L.min^{-1}; +Body Weight (BW) was taken as 70 kg; [a]Values were taken from literature[13,14]; **Panel A** represents cardiac output and regional blood flow rate to all major organs. **Panel B** represents relative organ weight or volume of the organs.

steps 1–8 of Figure 3. The entire global sensitivity analysis (steps 1–7) was performed on each of 24 sets of drug dependent parameters for each of the six drugs within the range of 0.2–3.0, 3.5–6.3, 6.5–9.3, 9.5–12.3, 12.5–15.3, 15.5–18.3, 18.5–21.3, 21.5–24.3, 24.5–27.3, 27.5–30.3, 30.5–33.3, 33.5–36.3, 36.5–39.3, 39.5–42.3, 42.5–45.3, 45.5–48.3, 48.5–51.3, 60–70, 75–85, 90–100, 110–120, 125–135, 140–150 and 0.01–1000 times the estimated parameter values respectively. The analysis was carried out to find out the important or sensitive drug specific parameters for different possible ranges of drug specific parameters of a drug. The responses considered for the present analysis were of two types: maximum plasma concentration of a drug, C_{max} and the area under the curve (AUC) of time-concentration profile of a drug in plasma.

3. Results

Pharmacokinetic model[3] used in the present study accounts for the role of transporter proteins present at the endothelial and epithelial barriers and the metabolic enzymes present in the tissues. Further, the model also incorporates vascular and tissue-level differences due to patient variations to allow representation of the effect of differences in individuals, genotype, and history. Therefore, the current model requires a number of parameters involving both drug independent physiological parameters and drug specific parameters. The drug independent physiological parameters were used as reported in the literatures for both human and rat (Table 2 and Table 3). We have estimated some of the drug specific parameters from the *in vitro* and *in vivo* data reported in the literatures, which were further fine-tuned by using an optimization tool *fmincon* in MATLAB. To exemplify, the parameters for pravastatin were estimated from the reported values of the rate constants of transport and metabolism under *in vitro* conditions[11,15,16]. The parameter values thus obtained were further fine-tuned by using the fmincon program in MATLAB by allowing a variance of 60% around the initial guess value. Further, a large number of unavailable drug specific

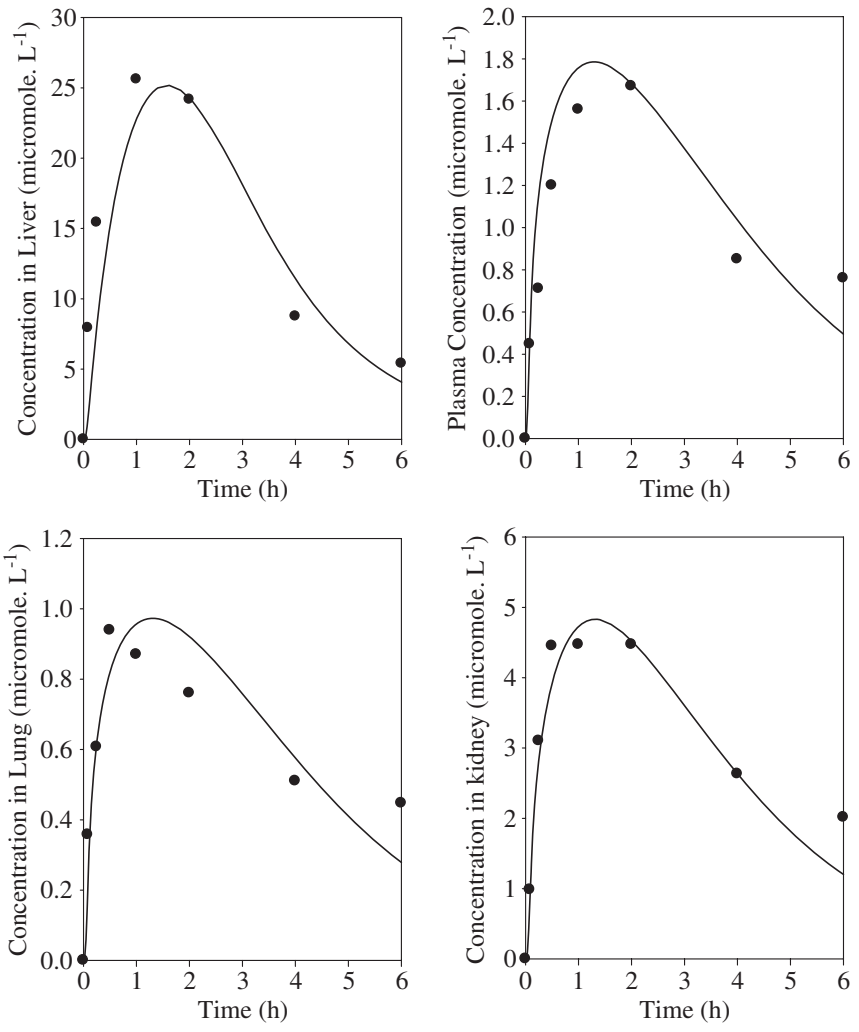
Figure 3: Morris global sensitivity analysis algorithm. Flow diagram for the global sensitivity analysis algorithm (Morris method) to find out the sensitive or important drug specific parameters which contribute most to model variability[17,18].



parameters were estimated through optimization algorithm as described in parameter estimation section. The estimated values of the drug specific parameters have been listed for pravastatin in rat (Table 4) and digoxin in human (Table 5). For both, rat and human liver is found to be the major site for metabolism as supported by the highest value of V_{max} followed by lung and kidney. However, activity of the transporter proteins towards active transport of the drug is found to be highest in kidney as observed from the values of kinetic parameters. A significant amount of drug is found to be excreted from kidney, termed as renal excretion. To validate the predictive ability of the model, tissue specific concentration profiles of pravastatin in rat were compared with the corresponding experimental values. Further, the model was validated for plasma drug disposition in humans for five representative drugs. It is observed from Figure 4 that the model predictions for tissue specific concentration profile of pravastatin in rat are in good agreement with the experimental results. Further, the model fit of the simulated plasma concentrations with the experimental values for a single dose administration was satisfactory for the five drugs with the R^2 values ranging between 0.9 and 0.95 (Figure 5).

A sensitivity analysis was carried out for all of the six drugs to investigate how a projected performance of the model varies with the change in model parameters. In the present study, by applying a global sensitivity analysis technique we uncover the critical drug dependent parameters

Figure 4: Simulation results for pravastatin in different organs of rats is compared with experimental data. Model validation for disposition of pravastatin in rats. Pravastatin was administered orally to Wistar-Imamichi rats at a dose of 20 mg/kg. The experimental data was taken from Komai *et al*.,[21].

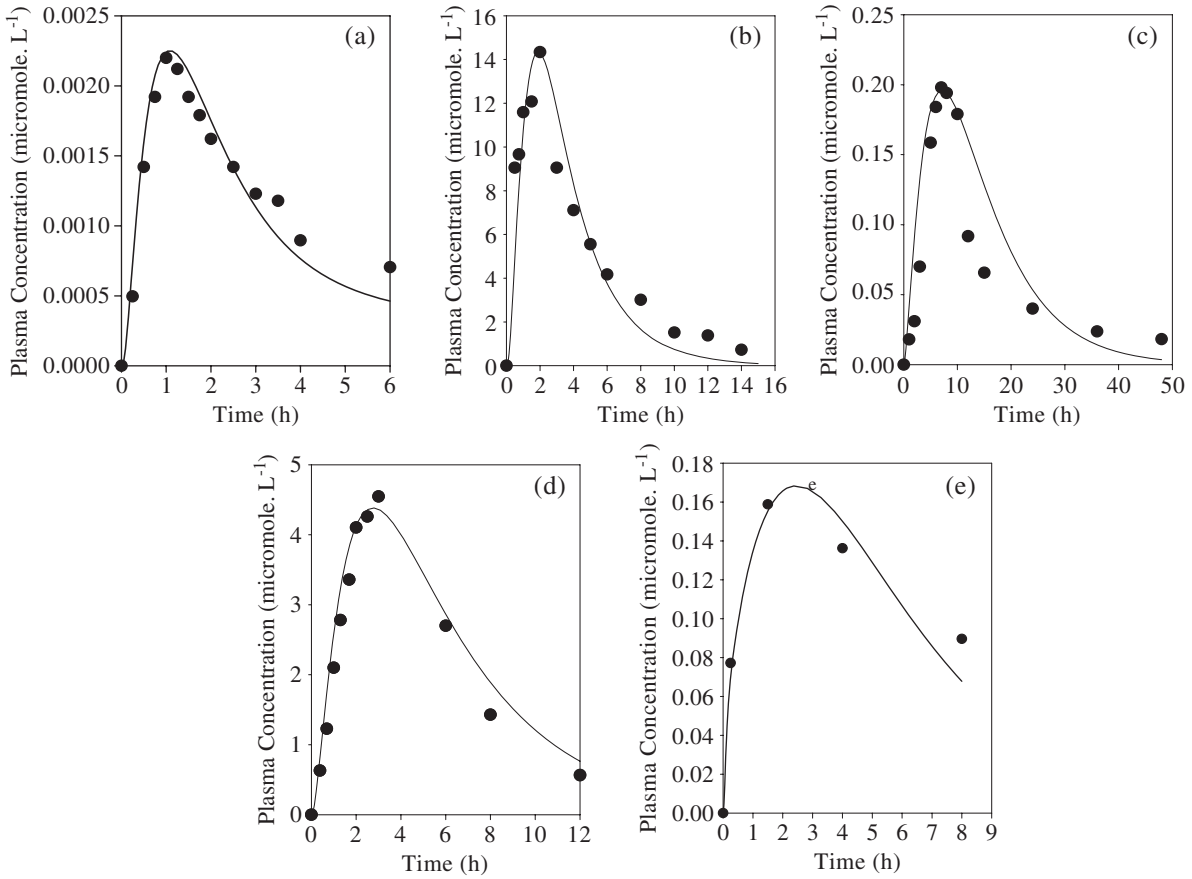


and their desired values for the chosen drug. The results suggest that V_{max} and K_m for transport in kidney, V_{max} and K_m for transport in liver, V_{max} and K_m for metabolism in liver, are the key parameters for all drugs (Figure 6). The bars in each plot are arranged in descending order of importance of the corresponding kinetic parameter based on mean sensitivity coefficients (refer to figure legend for detail). However, kinetic parameters for transport in renal excretion are also found to have significant effect on disposition of lisinopril in a healthy volunteer and as well as on statin group of drugs (both pravastatin and rosuvastatin) in rat. Further, V_{max} and K_m for efflux in both portal vein and kidney are found to have significant effect on pharmacokinetic behaviour of both pravastatin and rosuvastatin. These results further suggest that V_{max} and K_m for transport in muscle can play an important role in disposition of a drug in human.

4. Discussion

In the present study a framework was demonstrated for the estimation of kinetic parameters and sensitivity analysis. The existing PBPK model was enhanced by incorporating the mechanistic knowledge of drug disposition involving transporter proteins and metabolic enzymes. Since there is a good agreement between the model predictions and experimentally determined values for most of the drugs, the model structure appears to be sufficient. However, there is a slight discrepancy between the experimental values and model predictions at late stage for the drugs metformin and lisinopril. This can be attributed to the lack of precision in

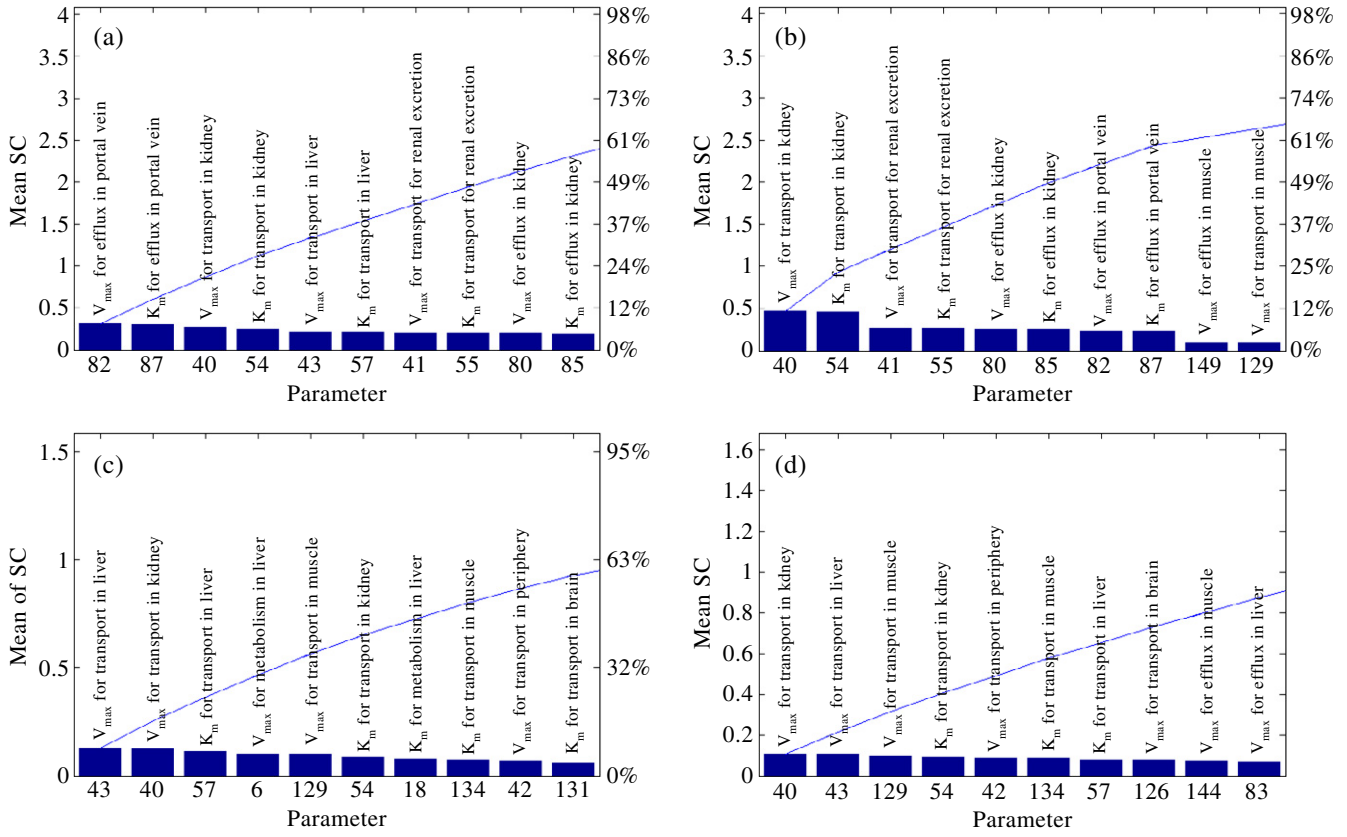
Figure 5: Model validation for five representative drugs. Experimental data (symbols) and model predictions (curves) for the plasma concentration of drugs in humans and animals. 5a: Plasma concentration-time profile for orally administered digoxin (0.5 mg.) to ten healthy volunteers (five male and five female)[42]; 5b: Metformin concentration in plasma after administration of 1 g of metformin to a healthy volunteer[43]; 5c: Representative plasma concentration vs. time profile of lisinopril in a healthy volunteer after a single 20 mg oral dose[44]; 5d: plasma concentration of methyldopa obtained from 25 healthy volunteers after an oral administration of 500 mg tablet[45]; 6e: Plasma concentration of rosuvastatin radioactivity was measured after single oral administration of 14C rosuvastatin at 5 mg/kg in the male rat[25].



estimation of biological parameter values. Here, we report a strategy to capture the key drug specific parameters which have significant effect on disposition profile of a drug in human and animals. In the present study, a global sensitivity analysis technique was applied on PBPK model to find out relatively important or sensitive drug dependent parameters.

The dominant effect of kinetic parameters i.e. V_{max} and K_m for active transport of pravastatin in liver agrees well with the experimental finding. Pravastatin is a HMG-CoA reductase inhibitor and is well known for its lipid-regulating effect in the liver. Therefore, liver selective uptake for theses drugs is a desirable property[15]. Na^+-independent multispecific anion transporter using ATP as the driving force is known to play a key role for uptake of pravastatin by rat hepatocytes[21,22]. Recently, the organic anion transporting polypeptide (OATP2)[23,24] and liver specific organic anion transporter (LST-1)[25,26] have been shown to be responsible for the uptake of pravastatin by the liver of rats and humans. From our analysis V_{max} for transport in kidney was also found to be an important parameter. This finding is commensurate with the experimental results demonstrated by Yamazaki *et al.*, With an i.v. bolus administration of pravastatin in rats, liver accounted for the major uptake, followed by kidney. At the same time, after portal vein administration the distribution to the liver is much larger than that of kidney due to extensive first-pass removal by the liver. Pravastatin also appears to be a substrate of multi drug resistance-associated transporter protein (MRP2) which is an efflux protein expressed in the liver, as the biliary excretion of pravastatin goes

Figure 6: Global sensitivity analysis results. Results for global sensitivity analysis of drugs when applied to the different sets of parameters. The primary y axis represents mean sensitivity coefficients (SC). SC is defined as change in concentration of of drugs in organ j (C_j) resulting from change in ith input parameter (P_i): $SC = \frac{\Delta C_j}{\Delta P_i}$. The secondary y axis represents the cumulative percentage of contribution towards variability of system output from ten most significant parameters indicated in the figure. The number under the bar of x-axis represents the index corresponding to a specific parameter used in the model. The bars in each plot are arranged in descending order of their mportance based on SC. The results shown are for four representative drugs: (A) Pravastatin, (B) Rosuvastatin, (C) Digoxin and (D) Metformin.



down with the decrease of MRP2 in the rats.[27] This also agrees well with our findings, which shows that $V_{\max}$ and K_m for efflux in liver are also sensitive parameters. Further, finding of $V_{\max}$ and K_m for active transport in kidney as important parameters for rosuvastatin may be attributed to the fact that, following administration of rosuvastatin, the liver shows the highest rsouvastatin uptake, followed by kidney as second highest[27].

Digoxin is mainly excreted from the circulation by kidney. P-glycoprotein (P-gp) is very well known as a digoxin pump and localized at the apical side of the nephron[28]. But, to access Pgp, digoxin has to cross the basolateral membrane from the circulation. Human OATP4C1/rat Oatp4c1 are believed to be primarily responsible for digoxin transport at the basolateral membrane in the kidney[28], as the other members of OATP family are neither expressed at the basolateral side of the kidney nor capable of transporting digoxin. On the other hand, the hepatic uptake of digoxin can be attributed to Oatp2 (slc21a5). It has been demonstrated that Oatp2 specifically transports the cardiovascular drug digoxin with high affinity[29–31]. As digoxin is also known to be a substrate for ATP-dependent drug efflux pump P-gp, which is located in the canalicular membrane of the hepatocytes[32], it is postulated that digoxin is actively taken up by hepatocytes via Oatp2 and secreted into biliary canaliculi via P-gp[33]. Digoxin is also extensively metabolized by cytochrome P4503A (CYP3A) in rat[34–36], which is commensurate with our finding of $V_{\max}$ for metabolism in liver as one of the important parameters. The current findings from

Table 4: A representative set of estimated drug specific parameter values for Digoxin in human

Organ/Tissue	Transport		Metabolism		Efflux	
	V_{max}	K_m	V^a_{max}	K^b_m	V_{max}	K_m
Stomach		16.5	1.1240	4886.7		
Small Intestine	0.2760	16.5	0.32	4886.9		
Large Intestine			0.32	2702.0		
Lung-T			15.727	2702.0	0.9941	16.9
Lung-V	27.1549	16.5				
Kidney-T	3.0 (hepatic excretion)	16.5	10.0	2702.0	0.8843	16.5
Kidney-V	200.38	16.5				
Brain-T			1.2	1600.0	0.5	16.5
Brain-V	4.7	3.5				
Heart-T			2.2	1600.0	0.6	16.5
Heart-V	4.7	3.5				
Adipose-T			1.2	1600.0	0.2	16.5
Adipose-V	10.7	3.5				
Muscle-T			0.2	1600.0	0.5	16.5
Muscle-V	4.7	3.5				
Skin-T			0.5	1600.0	0.1	16.5
Skin-V	4.7	3.5				
Liver-T	0.1522 (enterohepatic recirculation)	16.5	25.67	2702.0	1.471	16.5
Liver-V	29.0	16.5				
Portal vein					0.2463	16.5
Periphery-T			2.2	1600.0	0.6	7.5
Periphery-V	4.7	3.5				

[a] unit of V_{max} is micromole.L^{-1}.min^{-1}; [b] unit of K_m is micromole.L^{-1};

Table 5: A representative set of estimated drug specific parameter values for pravastatin in rat

Organ/Tissue	Transport	Metabolism		Efflux	
	V_{max}	V^a_{max}	K^b_m	V_{max}	K_m
Stomach		1.124	4886.8		
Small Intestine	0.2760	0.32	4886.9		
Large Intestine		0.32	2702.0		
Lung-T		12.72	2702.0	18.994	14.9
Lung-V	27.15				
Kidney-T	6.5 (hepatic excretion)	10.00	2702.0	21.984	16.5
Kidney-V	170.38				
Brain-T		5.5	1000.0	0.5	16.5
Brain-V	7.1				
Heart-T		4.5	1000.0	0.6	16.5
Heart-V	5.1				
Adipose-T		1.5	1000.0	1.1	16.5
Adipose-V	5.1				
Muscle-T		1.5	1000.0	1.1	16.5
Muscle-V	5.1				
Skin-T		1.5	1000.0	1.1	16.5
Skin-V	5.1				
Liver-T	0.224 (enterohepatic recirculation)	17.67	2702.0	1.47	16.5
Liver-V	29.0				
Portal vein				2.0	14.5
Periphery-T		0.5	1000.0	0.5	16.5
Periphery-V	2.1				

[a] unit of V_{max} is micromole.L^{-1}.min^{-1}; [b] unit of K_m is micromole.L^{-1}; K_m for transport in all the organs is estimated to 16.5 micromole.L^{-1};

the sensitivity analysis of model used for metformin are commensurate with the experimental results cited by Wang *et al.*,[37] The increase in the saturable uptake of the biguanides (metformin) by rOCT1 transfection suggests that the biguanides are the substrates of rOCT1[37]. Therefore, distribution of metformin into the liver or the hepatic uptake of metformin by liver may be attributed to the possible involvement of organic cation transporter 1 (OCT1). On the other hand, neither the distribution of metformin in kidney nor the renal excretion showed any significant difference in OCT1 present or absent mice, suggesting that OCT1 is not the major transporter involved in the renal uptake of metformin. Recently it has been shown that rOCT2 and rOCT3 is also expressed in the basolateral membrane of kidney[38,39] and may be responsible for renal uptake and/or secretion of cationic compounds. In a recent study using OCT3 (−/−) mice, no significant difference was observed in the disposition of 1-methyl-4-phenylpyridinium[40]. Based on this finding it may be proposed that OCT2 is most likely responsible for the uptake and control for renal excretion of metformin[37].

V_{max} and K_m for renal excretion as sensitive parameters for lisinopril seems to be reasonable as it is known that the major route of lisinopril elimination is through renal excretion[41]. However, our finding of Vmax and Km for metabolism in liver as sensitive parameters is not commensurate with the experimental results, as it is reported that lisinopril does not undergo metabolism and is excreted unchanged entirely in the urine.

5. Conclusions

The model developed in the present work has significantly improved ability to predict the uptake and disposition of drugs and toxins in a more diverse range of patients than is currently possible. The model includes a "customized" database of appropriate parameters to represent a diverse set of subpopulations accounting for differences such as sex and age, and genotype. These analyses and predictions will help in making an early selection of compounds for development based on pharmacokinetic properties as well as for advancing personalized medicine.

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Nomenclature

C_{1i}	Concentration of drug in vascular compartment of organ i. 1 represents vascular compartment
C_{2i}	Concentration of drug in tissue compartment of organ i. 2 represents tissue compartment
$V_{vascular,i}$	Volume of vascular compartment of organ i
$V_{tissue,i}$	Volume of tissue compartment of organ i
$F_{in,i}$	Volumetric flow rate of blood into organ i
$F_{out,i}$	Volumetric flow rate of blood out of organ i
RT_i	Rate of transport in organ i
RE_i	Rate of efflux in organ i
RD_i	Rate of diffusion in organ i
RM_i	Rate of metabolism in organ i
$V_{max,TR}$	Maximum reaction rate for transport in organ i
$K_{m,TR}$	Michaelis-menten constant for transport in organ i
$V_{max,Efflux}$	Maxium reaction rate for efflux in organ i
$K_{m,Efflux}$	Michaelis-Menten constant for efflux in organ i
$V_{max,Meta}$	Maximum reaction rate for metabolism in organ i
$K_{m,Meta}$	Michaelis-Menten constant for metabolism in organ i
$I_{tissue,i}$	Concentration of inhibitor in tissue compartment of organ i
$I_{vascular,i}$	Concentration of inhibitor in vascular compartment of organ i
K_i	Diffusion coefficients,
SA_i	Surface area of organ i,

Competing interests

The author(s) declare that they have no competing interests

Authors' contributions

DD contributed to modeling and simulation and manuscript preparation. PD contributed conceptually in developing and designing the model. PPW proposed the model, supervised the work and coordinated the manuscript preparation. All authors read and approved the final manuscript.

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