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ORIGINAL ARTICLE

Physiologically based pharmacokinetic modelling of treprostinil after intravenous injection and extended-release oral tablet administration in healthy volunteers: An extrapolation to other patient populations including patients with hepatic impairment

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Aims: Pulmonary arterial hypertension (PAH) is characterized by increased pulmonary arterial pressure, resulting in right ventricular overload, right heart failure and eventually death. Treprostinil is a prostacyclin analogue that is used in the treatment of PAH. As an orphan drug, limited information is available regarding its disposition and its use in special populations such as elderly, paediatric and pregnant patients. The objective of the current study was to develop a robust physiologically based pharmacokinetic (PBPK) model for treprostinil intravenous injection and extended-release tablet as the first step to optimize treprostinil pharmacotherapy in patients.

Methods: PBPK model was built using Simcyp simulator which integrated physico-chemical properties, observed or predicted parameters for drug absorption, distribution and elimination for treprostinil, and population specific physiological characteristics. Three clinical trials after intravenous infusion and nine studies after oral administration of treprostinil extended-release tablet in healthy volunteers were used to develop and validate the model. The simulated PK profiles were compared with the observed data. Extrapolation of the model to patient populations including patients with hepatic impairment was conducted to validate the predictions.

Results: Most of the observed data were within the 5th and 95th percentile interval of the prediction. Most of the percentage error in the PK parameters were within ±50% of the corresponding observed parameters. The developed model predicted the lung exposure of treprostinil to be approximately 0.17 times of concentration in plasma.

Conclusion: Predicted absorption, distribution, and metabolic enzyme kinetics gave an insight into the disposition of treprostinil in humans. Extrapolation of the established model to patient populations with hepatic impairment successfully documented the model reliability. The developed model has the potential to be used

in the PK predictions in other special patient populations with different demographic, physiological and pathological characteristics.

KEYWORDS
extended-release tablet, hepatic impairment, modelling and simulation, physiologically based pharmacokinetic modelling, treprostinil

1 | INTRODUCTION

Treprostinil is a prostacyclin analogue used in the treatment of pulmonary arterial hypertension (PAH).[1] It is available as an injection that is administered as a continuous infusion via intravenous or subcutaneous routes (Remodulin), as intermittent nebulization for inhalation (Tyvaso), and as an extended-release tablet via oral route (Orenitram).[2] Treprostinil undergoes significant metabolism in the liver, primarily mediated by the cytochrome P450 enzyme **CYP2C8**. Cytochrome P450 (**CYP2C9**) also plays a role in the metabolism of treprostinil, but its contribution is relatively small.[3] Only 3.7% of the dose is recovered in the urine as the unchanged parent drug when administrated subcutaneously according to the package insert, while 0.19% of the dose is excreted in the urine after oral administration.[4] As an orphan drug, limited information on enzyme kinetics, and inter-subject variability is available in the literature.

Physiologically based pharmacokinetic (PBPK) modelling allows mechanistic-modelling and simulation of the processes of drug absorption, distribution, metabolism and excretion, and is increasingly being used in drug research and development.[5] Unlike classical PK models, the compartments in the PBPK model represent the real organs and tissues and each organ/tissue is connected by the circulatory system. The transit of the drug in the body complies with mass balance and is determined by multiple factors, such as the physicochemical properties of the compound, unbound concentration of the drug in the circulation, composition of tissue/organ, blood flow, and other factors.[6] Disease induced physiological and pathological changes in patients, are likely to alter the PK of drugs, and may lead to under-treatment or adverse effects.[7] As a result, regulatory organizations, including both the Food and Drug Administration and European Medicines Agency, have issued a series of guidelines for evaluating the PK in special patient populations and translating the finding into dosage recommendations and labelling. A PBPK model can be used to extrapolate plasma concentration–time profiles from healthy subjects to different patient populations and allows prediction of PK behaviour in special populations, such as elderly, paediatric and pregnant patients, and in patients with renal and/or hepatic impairment, while avoiding the ethical and practical obstacles that arise in performing such studies in the real world.[5,8] The PBPK models are helpful in exploring the underlying mechanisms of drug dispositional changes as well.[9]

The objective of this study is to build a PBPK model for treprostinil after intravenous injection and extended-release oral tablet administration, in order to understand the PK behaviour of treprostinil and apply the developed model to patients with hepatic impairment.

What is already known about this subject

- Pulmonary arterial hypertension is characterized by increased pulmonary arterial pressure, resulting in right ventricular overload, right heart failure and eventually death. Treprostinil is a prostacyclin analogue used in the treatment of pulmonary arterial hypertension. Limited pharmacokinetic data exist on this drug.

What this study adds

- As an orphan drug, limited information is available on its disposition in humans and its use in various patient populations. A robust physiologically based pharmacokinetic model predicting the plasma concentration after intravenous injection and extended-release tablet of treprostinil was established and validated with 12 clinical trials using single or multiple doses of treprostinil.
- Kinetics data on enzymes responsible for the metabolism of treprostinil were predicted by the retrograde model.
- Extrapolation of the established model to patient population including patients with hepatic impairment was performed successfully.

Clinical significance

- The developed model can be further applied to predict drug–drug interactions and pharmacokinetics in other special patient populations.

2 | MATERIALS AND METHODS

2.1 | Data source and clinical trial demographics

Physicochemical characteristics and human clinical PK data were searched in NCBI database and Google Scholar using the key words

"treprostinil" and "treprostinil and pharmacokinetics". In total, 3 PK studies after intravenous infusion[10–12] and nine PK studies after oral administration of the treprostinil extended-release tablet[3,4,12–16] in healthy volunteers; as well as 3 PK profiles in patients with hepatic impairment,[3] and 4 PK profiles in patients with PAH or systemic sclerosis[17,18] were retrieved. The PK profiles were digitized using WebPlot Digitizer version 3.8 (https://automeris.io/WebPlotDigitizer/). PK parameters were tabulated to summarize the inter study variability. Table 1 summarizes the subject demographics available from the clinical trials, as well as the studies used for the model development and model validation. Physicochemical properties and other parameters identified are presented in Table 2. A blood to plasma partition coefficient of 0.55 (minimal value in Simcyp simulator) was derived from a ratio of whole blood to plasma area under the concentration–time curve (AUC) or maximum concentration (Cmax) in mass balance study of treprostinil following single dose oral administration in healthy subjects.[4]

2.2 | PBPK model development and validation after IV infusion and oral administration

PBPK model simulations were conducted using Simcyp Population-based Simulator (Version 17 release 1, Certara, Sheffield, UK). Healthy population (Caucasian) incorporated in the software was used as a large proportion of participants in the reported clinical trials were white subjects (Table 1). One hundred virtual subjects with the corresponding demographic and physiological characteristics matched with the clinical study were used for simulation, in 10 trials with 10 subjects in each trial. The general workflow of treprostinil model development after infusion in healthy population consisted of the following steps. The initial PBPK model was built by inputting drug properties (Table 1) and PK parameters obtained from literature. The clearance of 43 L/h was the average clearance value after intravenous administration obtained from 3 reports.[4,10,11] As per the information in the label for treprostinil sodium injection and extended-release

TABLE 1 Clinical studies used in physiologically based pharmacokinetic model development and verification

No.	Subject	Dose regimen (dose, infusion time)	*n* (female%)	Age range (y)	Reference
Single dose of infusion in healthy volunteers					
1[a]	8 white, 3 black, 4 Hispanic	0.00225 mg/kg, 2.5 h	15 (47)	18–49	10
2[b]	26 white, 8 black, 17 others	0.0432 mg/kg, 72 h	51 (40)	18–63	11
3[b]	Unknown (default white)	0.2 mg, 4 h	24 (default 50)	Unknown (default 18–65)	4
Single dose of extended-release tablet in healthy volunteers					
1[a]	29 white, 2 unknowns	1 mg, fed	31 (50)	18–55	13
2[b]	6 white, 2 black	1 mg, fed	8 (37.5)	37–64	12
3[b]	5 white, 3 black	1 mg, fed	8 (0)	39–49	3
4[b]	17 white, 2 black	0.5 mg, fed	19 (53)	20–54	15
5[b]	Unknown (default white)	1 mg, fed	24 (default 50)	Unknown (default 18–65)	4
6[b]	Unknown (default white)	1 mg, fed	24 (default 50)	Unknown (default 18–65)	4
Multiple dose of extended release tablet in healthy volunteers					
1[b]	17 white, 2 black	0.5 mg × 22, tid, fed	19 (53)	20–54	14
2[b]	17 white, 4 black, 1 Indian	1 mg × 9, bid, fed	22 (22)	20–54	15
3[b]	14 white, 4 black	1 mg × 9, bid, fed	18 (17)	20–52	16
Single dose of extended-release tablet in hepatic impairment subjects					
1[b]	6 white, 2 black[c]	1 mg, fed	8(38)	43–54	3
2[b]	7 white, 1 black[d]	1 mg, fed	8(25)	45–60	3
3[b]	6 white[e]	1 mg, fed	6(0)	50–56	3
Multiple dose of extended-release tablet in patients					
1[b]	49 white, 2 black, 19 others	2 mg × 11, bid, fed	70(74)	18–65	17
2[b]	49 white, 2 black, 19 others	6 mg × 11, bid, fed	70(74)	18–65	17
3[b]	17 white, 2 black	2 mg × 15, bid, fed	19(84)	34–65	18
4[b]	17 white, 2 black	4 mg × 15, bid, fed	19(84)	34–65	18

[a]model development;
[b]model verification;
[c]patients with mild hepatic impairment;
[d]patients with moderate hepatic impairment;
[e]patients with severe hepatic impairment.
bid, twice daily; tid, 3 times daily

TABLE 2 Physio-chemical and blood binding data used in physiologically based pharmacokinetic modelling

Parameter	Input value	Source/reference
MW(g/Mol)	390.5	https://www.drugbank.ca/drugs/DB00374
Compound type	Monoprotic acid	https://www.drugbank.ca/drugs/DB00374
pKa	3.76	4
Log Po:w	3.0	4
B/P	0.55	4
F_u in plasma	0.09	Treprostinil injection package insert

B/P, blood to plasma partition coefficient; fu, plasma fraction unbound; logPo:w, logarithm of the octanol to water partition coefficient; MW, molecular weight; pKa, negative logarithm of the acid dissociation constant.

extended release tablets, after subcutaneous and oral administration, 3.7 and 0.19% of the administered dose was observed in the urine, the renal clearance was assumed to be 2% of the total in vivo clearance (0.9 L/h). An average 6% of the dose did not appear either in urine or in the faeces. 1% of the parent drug was found in the faeces following subcutaneous administration, and biliary clearance was assumed to be 1% of the total body clearance. Therefore, additional systemic clearance accounted for 7% of the total in vivo clearance value (3.0 L/h). Full PBPK model with Rodgers and Rowland method[19] was used to predict the tissue:plasma partition coefficient (Kp). The predicted volume of distribution at steady state (Vss) was 0.1 L/kg, which is lower than the reported average data 0.4 L/kg.[10,11] The initially predicted PK profile and parameters were compared with the observed data, which showed significant deviations. Vss was calculated with the following equation:

$$\mathrm{Vss} = \mathrm{Vp} + \mathrm{Ve} \times \mathrm{E:P} + \sum \mathrm{Vt} \times \mathrm{Kp}.$$

where E:P is the erythrocyte–plasma partition coefficient, and Kp is the tissue–plasma partition coefficient for a specific tissue. Error in Kp is likely to be responsible for the underprediction. Using the sensitivity analysis, we can assess the quantitative changes of Vss with respect to changes in input variables (e.g. Kp). Parameter sensitivity analysis was performed to understand the root cause of the deviations, which showed that the Kp scalar and the adipose Kp have the most significant effect on the Vss. The parameter estimation was conducted to optimize the values to the observed data using Nelder–Mead method, in which weighted least squares is utilized by minimizing an objective function. Kp values for other organs and tissues remained the same as the predicted values. Simulated PK profile was compared with the observed data to verify the model performance. Distribution parameters are summarized in Table 3.

To understand the metabolic contribution of treprostinil, retrograde model was used to calculate the intrinsic hepatic clearance (CL_{int}) of each enzyme based on information on human in vivo intravenous (CL_{iv}) clearance values, renal clearance, biliary clearance and additional systemic clearance.[20] In this case, additional systemic clearance was adjusted to 2.6 L/h (6% of the total clearance) and biliary clearance was included in the elimination component. The relative contributions of CYP2C8 and CYP2C9 were 90 and 10%, respectively, when using retrograde model to predict the enzyme kinetics data. The final values in the elimination component are summarized in Table 4.

TABLE 3 Distribution parameters for treprostinil profile

Parameter	Value
Vss (L/kg) predicted	0.42
Vss (L/kg) observed[a]	0.40
Tissue: Plasma partition coefficients (Kp)	
Adipose[b]	1.20
Bone	0.08
Brain	0.05
Gut	0.13
Heart	0.13
Kidney	0.11
Liver	0.08
Lung	0.17
Pancreas	0.06
Muscle	0.05
Skin	0.22
Spleen	0.09
Kp scalar[b]	0.7

[a]average reported value of Wade et al.[10] and Laliberte et al.[11];
[b]The adipose compartment Kp value and Kp scalar were optimized using the Simcyp parameter estimation module, and the Nelder–Mead method was used for the minimization. Rodgers and Rowland method was used for prediction.

PK data from 2 model-naïve clinical studies were utilized in order to evaluate model predictions. The comparison of the observed data with the simulated data and the percentage errors were calculated. After finalizing the PBPK model of treprostinil following IV infusion, a PBPK model for oral administration of treprostinil extended-release tablet was built. The advanced dissolution, absorption and metabolism model was used to predict the rate and extent of oral absorption in healthy population. The dissolution profile of treprostinil from the extended-release tablet was input into the Simcyp.[21] Polar surface area of 86.99 Å and hydrogen bond donors of 3 were used to predict the human jejunum permeability[22] and the predicted value was 0.46×10^{-4} cm/s. The fraction of the unbound drug within the

TABLE 4 Clearance parameters used in physiologically based pharmacokinetic modelling

Parameters	Value
CL_{int} (μL/min/pmol) of CYP 2C8	20.5
CL_{int} (μL/min/pmol) of CYP 2C9	0.75
fu_{mic}	1
Cl_{int} (bile) (μL/min/10^6 cells)	2.04
CL_R (L/h)	0.9
Additional systemic clearance (L/h)	2.6

CL_{int}, in vitro intrinsic clearance; CYP, cytochrome P450; fu_{mic}, fraction of unbound drug in the in vitro microsomal incubation; Cl_{int} (bile), biliary clearance; CL_R, renal clearance.

enterocyte ($f_{u,gut}$, 0.936) were predicted using physicochemical properties, blood binding and tissue composition. Plasma unbound fraction in the oral model was adjusted to 0.04 as per data in treprostinil extended-release tablet package insert. The absorption rate scalar for the colon was optimized to be 0.05 by using parameter estimation.

2.3 | Simulation of treprostinil PK following oral administration in subjects with hepatic impairment

To evaluate the application of the developed model in patients with hepatic impairment, treprostinil extended-release tablet disposition was simulated. Three general cirrhosis population with Child–Pugh class of mild (A), moderate (B) and severe (C) incorporated in the Simcyp simulator were used to mimic the physiological changes in the subjects. The model predicted PK parameters were compared with the data in published reports in subjects with hepatic impairment.[3]

2.4 | Simulation of treprostinil PK following oral administration in patients with PAH or systemic sclerosis

Two available PK studies were used to evaluate the model performance against reported clinical data. Shah et al.[18] reported the PK study using escalating doses of oral treprostinil in patients with systemic sclerosis without organ failure. Another reported PK study was in patients with PAH.[17] Simulated steady-state PK profiles were compared with the observed data in patients. Healthy population was used because of the nonavailability of specific populations in the Simcyp simulator for patients with systemic sclerosis or PAH and the metabolic capacity is not known to be altered in these conditions.

2.5 | Model evaluation

Visual check of the plots of observed *vs.* simulated profiles was performed for the evaluation of the model. The 5th and 95th percentile intervals were calculated to simulate the intersubject variability. The percentage error was calculated for the Cmax, AUC and the time to reach Cmax (Tmax) according to the following equation:

$$\% \ \text{Error} = \frac{V_{Pred} - V_{Obs}}{V_{Obs}} X\,100$$

where V_{pred} is the PBPK-predicted value and V_{obs} is the observed value obtained from the published report. Given the immense inter-study variability for treprostinil PK in clinical trials, most of the prediction were be located around the line of unity, and a maximum deviation of ±50% was considered acceptable.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[23,24]

3 | RESULTS

3.1 | Treprostinil PK prediction and validation after intravenous infusion

In sensitivity analysis, the sensitivity index measures the difference in output per unit change in an input parameter value from its initial value. The sensitivity indices for Kp scalar and Kp for adipose were 0.10 and 0.28 respectively, which were higher than indices for other tissues. Therefore, parameter estimation was used to optimize the Kp scalar and Kp values for adipose to fit the observed data and the results are presented in Table 3.

The plasma concentration–time profile reported by Wade et al.[10] was used to build the PBPK model. Predictive performance of the model was evaluated by overlapping of the predicted profile over the observed data. Pharmacokinetic profiles from 2 model naïve studies were used to validate the model.[4,11] The data were generated for the doses and infusion times prescribed in the model-naïve trials. The results shown in Figure 1 demonstrate that the predicted concentration–time profiles after IV infusion in the healthy population well described the observed data. In addition, the 5th and 95th percentile intervals included most of the observed concentration-time data. Percentage error in prediction is presented in Figure 2. Additionally, treprostinil levels in lungs can be predicted using the developed model. A Kp of 0.17 for lung suggested that treprostinil exposure in lungs was lower than that of plasma.

3.2 | Treprostinil PK prediction and validation after oral administration of extended-release tablets

The plasma concentration-time profile reported by Kim et al.[13] was used to build the model following oral administration of the extended-

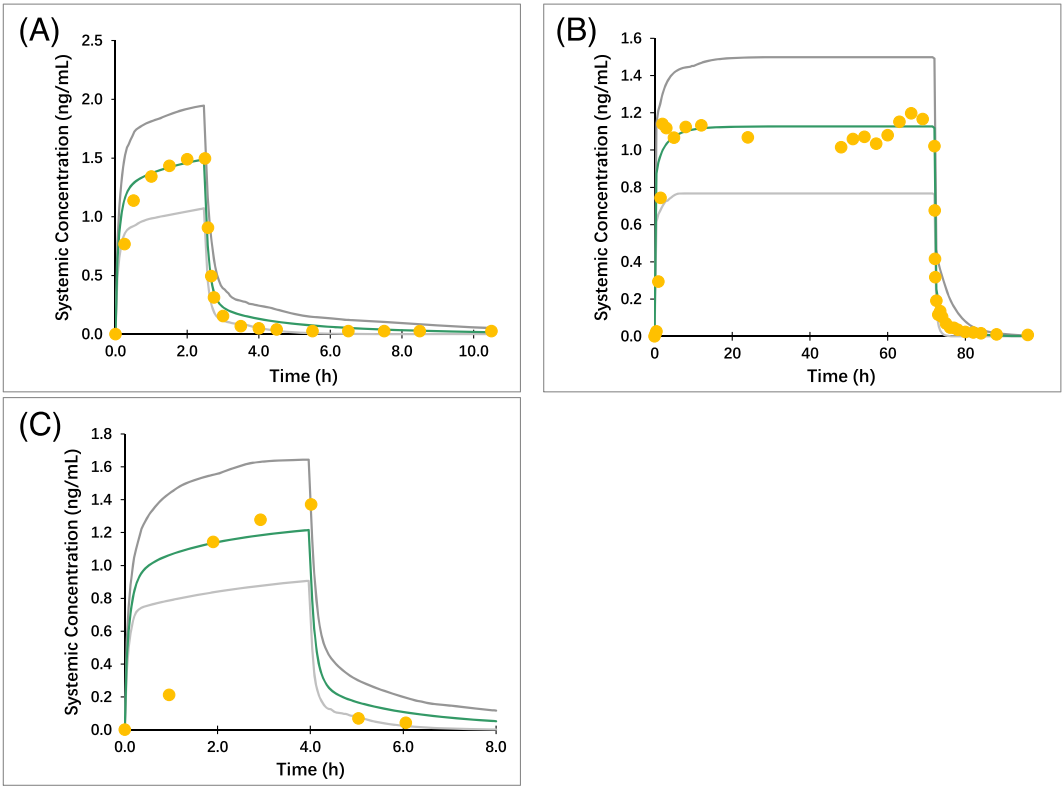

FIGURE 1 Predicted and observed concentration–time profiles following a single intravenous (IV) infusion of treprostinil. (A) Plot of the final model built in comparison with the observed data from Wade et al.[10] (B) Validation plot in comparison with the observed by Laliberte et al.[11] (C) Validation plot in comparison with the observed data from reference.[4] The middle line represents the simulated mean concentrations, the round dots represent observed data, and the lower and upper lines show the simulated 5th and 95th percentiles

release tablets. The pharmacokinetic profiles from 8 model naïve studies were used to validate the model. As shown in Figure 3A–F, the PK profiles with 2 single doses, namely 0.5 mg and 1 mg, were simulated. Clinical studies following multiple dose administration included 2 dosing regimens, namely 0.5 mg 3 times daily for 22 doses and 1 mg twice daily for 9 doses. (Figure 3G–I). The simulated PK profiles well described the observed values. In addition, the 5th and 95th percentile intervals of the prediction covered most of the observed concentration–time data. Percentage errors are shown in Figure 2.

3.3 | Extrapolation of the PBPK model to hepatically impaired patients

Simcyp simulator provides built-in cirrhotic populations with Child–Pugh class of mild (A), moderate (B) and severe (C) with a corresponding demographic characteristics and physiological changes. One hundred virtual cirrhosis patients from each class were used to predict the PK profile in patients with hepatic impairment. Demographic details of the patients were matched with those in the published reports. Predicted PK profiles generated for single dose oral administration of treprostinil extended-release tablets are similar to the observed data (Figure 4A-4C). Most of the observed data points were within the 5th and 95th percentile of the predictions suggesting that the developed model can be successfully extrapolated to subjects with liver dysfunction.

The contribution of CYP2C8 to the treprostinil elimination in healthy volunteers and in patients with mild, moderate, severe hepatic impairment was 81, 77, 72 and 70%, respectively. The decreased contribution of CYP 2C8 and CYP2C9 to the elimination of treprostinil is in line with the severity of liver dysfunction and its impact on CYPs.

3.4 | Simulation of treprostinil PK following oral administration in patients with PAH or systemic sclerosis

As shown in Figure 5, the predicted PK profiles at steady state are similar to the observed profiles after multiple doses of treprostinil extended-release tablets in patients. Most of the observed values fell within the 5th and 95th percentile range suggesting that this model can successfully be extrapolated to the patients with PAH or systemic sclerosis. Among the 4 studies, the percentage error for 3 studies were in the acceptable range.

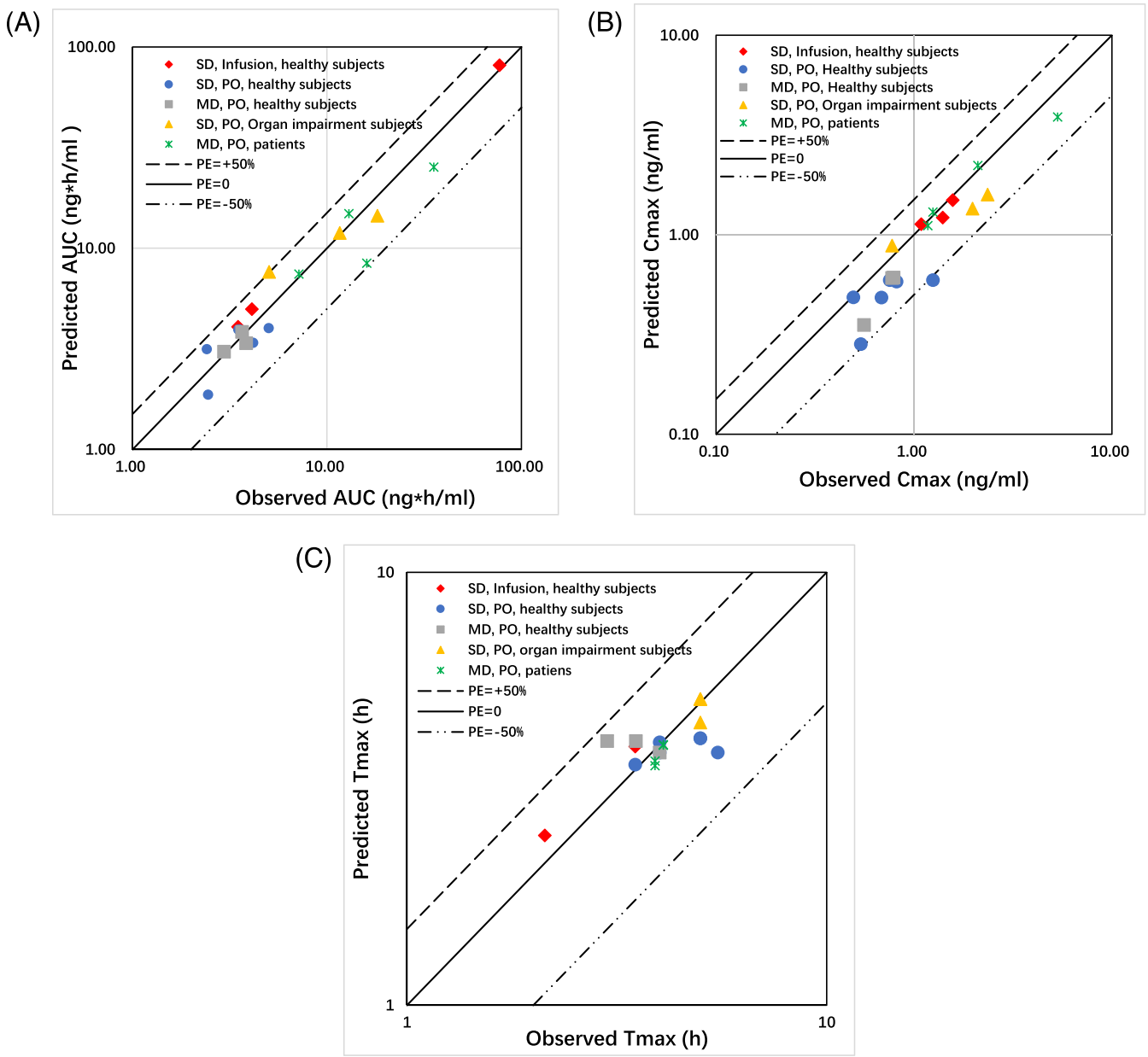

FIGURE 2 Comparison of the observed pharmacokinetic parameters with the predicted parameters for each dosing regimen in healthy individuals or subjects with organ impairment. (A) AUC, (B) Cmax, (C) Tmax. AUC, area under the concentration–time curve; Cmax, peak plasma concentration; Tmax, time to reach Cmax; SD, single dose; MD, multidose. PO, oral administration; PE: percentage error, which is calculated by $(V_{pred} - V_{obs})/V_{obs} \times 100\%$, where V_{pred} and V_{obs} are the simulated and observed mean Cmax, AUC or Tmax values for each clinical trial. Solid dots represent the observed value *vs*. predicted value. Solid line and dotted lines represent percentage errors of 0, +50 and −50%, respectively

4 | DISCUSSION

In the current study, we developed and validated PBPK models of treprostinil after intravenous infusion and oral administration of an extended-release tablet. The advanced dissolution, absorption and metabolism model for absorption, full PBPK model for distribution, and enzyme kinetics model for metabolism were incorporated into the global model. Three clinical studies using IV infusion and 13 studies after oral administration of treprostinil extended-release tablets were used for model validation. The predicted PK profiles in 100 virtual trial-matched population fitted well with the observed data. Most of the observed concentration-time levels were within the 5th and 95th percentile intervals. Percentage error of C_{max}, $AUC_{0\text{-}t}$ and T_{max} were between −50% and +50% with 3 exceptions in which the percentage errors were −53% and +92% for Cmax and +90% for AUC. The developed model successfully predicted the PK profiles in hepatically impaired subjects and patients with PAH or systemic sclerosis.

In the model developed, the in vivo clearance parameters were initially used. To better understand the metabolism of treprostinil, the recombinant system retrograde model was used to predict

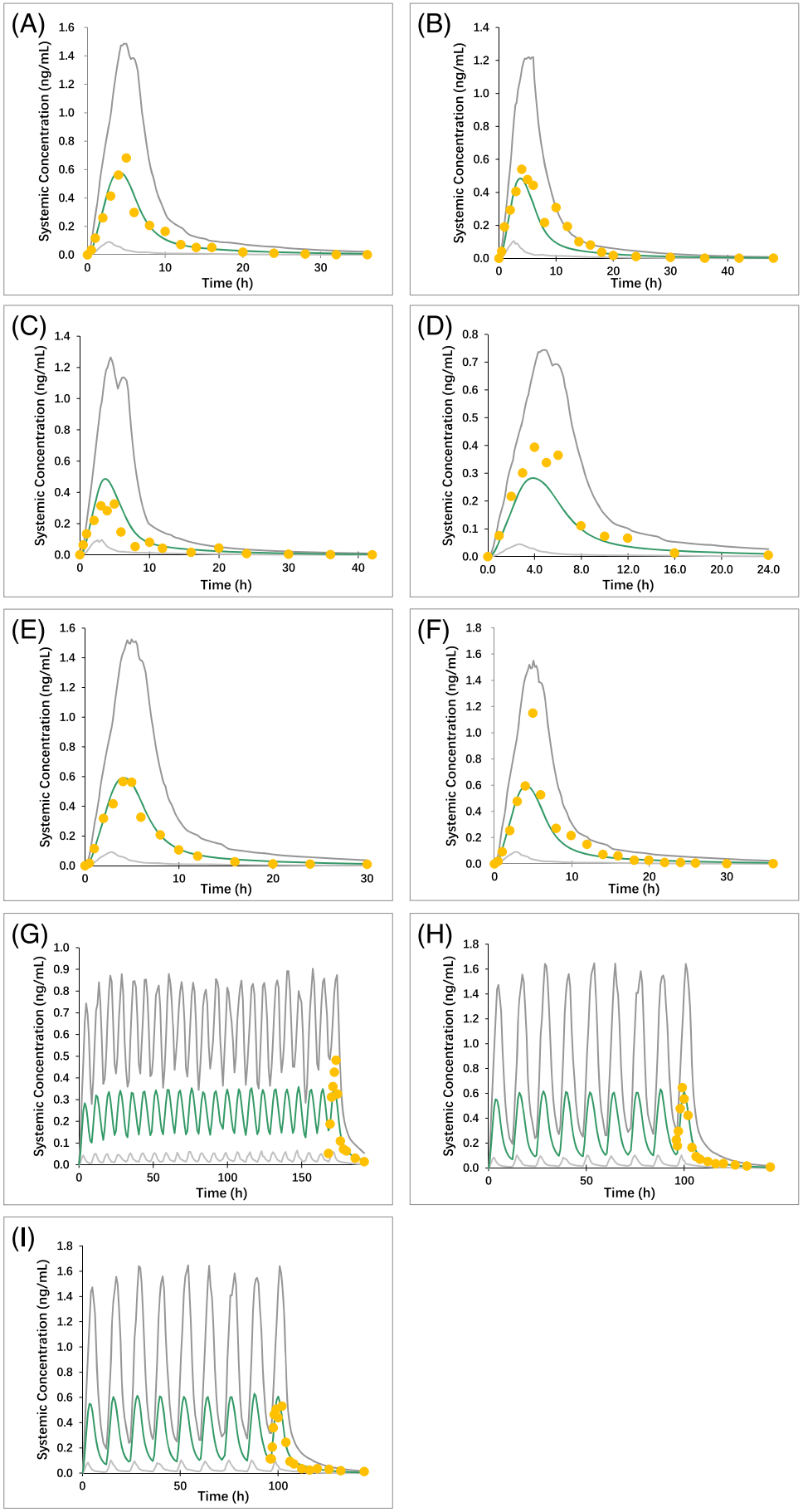

FIGURE 3 Predicted and observed concentration–time profiles following oral administration of treprostinil. (A) Plot of the final model built after single dose of 1 mg in comparison with the observed data from Ref.[13] (B) Validation plot after single dose of 1 mg in comparison with the observed from Ref.[12] (C) Validation plot after single dose of 1 mg in comparison with the observed data from Ref.[3] (D) Validation plot after single dose of 0.5 mg in comparison with the observed data from Ref.[14] (E) Validation plot after single dose of 1 mg in comparison with the observed data from Ref.[4] (F) Validation plot after single dose of 1 mg in comparison with the observed data from Ref.[4] (G) Validation plot after multiple dose of 0.5 mg × 22, 3 times daily in comparison with the observed data from Ref[14] (H) Validation plot after multiple dose of 1 mg × 9, twice daily in comparison with the observed data from Ref.[15] (I) Validation plot after multiple dose of 1 mg × 9, twice daily in comparison with the observed data from Ref.[16] The middle line represented the simulated mean concentrations, the round dots represent observed data, and the lower and upper lines show the simulated 5th and 95th percentile

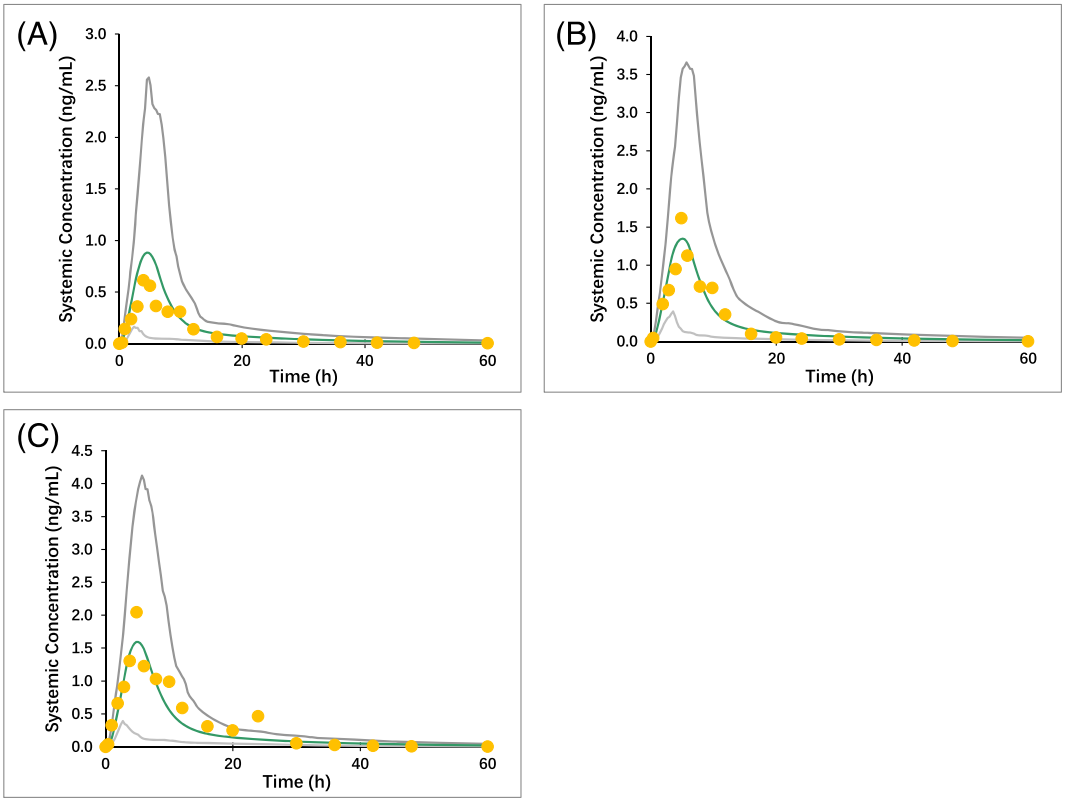

FIGURE 4 Predicted and observed concentration–time profiles following oral administration of treprostinil in subjects with hepatic impairment. (A) Validation plot in comparison with the observed data in patients with mild hepatic impairment from Ref.[3] (B) Validation plot in comparison with the observed in patients with moderate hepatic impairment from Ref.[3] (C) Validation plot in comparison with the observed data in patients with severe hepatic impairment from Ref.[3] The middle line represented the simulated mean concentrations, the round dots represent observed data, and the lower and upper lines show the simulated 5th and 95th percentile

the enzyme kinetics data. This was based on the information on the in vivo intravenous clearance (CL_{iv}) in humans, hepatic uptake and the elimination pathways involved.[20] This procedure is extremely useful in the absence of or availability of sparse in vitro enzyme kinetics data. Furthermore, an intrinsic biliary clearance can be predicted through back-extrapolation from a given in vivo biliary clearance information, defined as a percentage of CL_{iv}. Use of retrograde model requires information about relative contribution of the various elimination pathways (f_m), and renal and biliary clearance. Based on the unchanged drug excreted in the urine (average 2%) and faeces (1%) following subcutaneous administration of treprostinil (data obtained from package insert[4]), the contribution of renal and biliary clearance to the total in vivo clearance was assumed to be 2 and 1%, respectively. The elimination of treprostinil in humans is predominantly mediated by CYP2C8, with a minor contribution from CYP2C9.[3] Currently, parameters such as Km and Vmax or intrinsic hepatic clearance of CYP2C8 and CYP2C9, is not available for treprostinil. Using each recombinant enzyme at a time, in vitro studies indicated that the disappearance of approximately 95% of the treprostinil was due to CYP2C8 and approximately 22% of metabolism was associated with CYP2C9.[3] We hypothesized that CYP2C8 and CYP2C9 respectively accounted for 90 and 10% of the metabolism of treprostinil in vivo. With the predicted hepatic intrinsic clearance from retrograde model, we successfully simulated the treprostinil PK profiles at different doses and different routes of administration. Furthermore, the predicted AUC changes in the hepatically impaired subjects were consistent with the reported values[3] (Figure 3). The results in turn provided specific evidence that our assumptions about the contribution of CYP2C8 and CYP2C9 were appropriate. Changing the proportion metabolized by CYP 2C8 and 2C9 to 85:15 and 95:5 did not improve the model fit. In addition, since **UDP-glucuronosyltransferase** contributes minimally (2.54%) to the disappearance of parent drug, the impact of **UDP-glucuronosyltransferase** was not accounted for in this study.[4] The retrograde model was also able to predict the whole organ metabolic clearance.

The initial predicted Vss was 0.1 L/kg, which was much lower than the observed mean V_d of 0.4 L/kg.[10,11] The under-prediction indicated the uncertainty associated with the model parameters.[27,28] Kp scalar and adipose: plasma partition coefficient were optimized by parameter estimation module which used the nonlinear mixed effect methods. The increase in adipose: plasma partition coefficient indicated that the drug probably accumulates in space similar to fat in the body.

Treprostinil extended-release tablet is designed as an osmotic-controlled release oral delivery system,[21] which allows extended-release of the drug in the gastrointestinal tract. Percent dissolved

from 1 mg treprostinil diethanolamine osmotic tablet during 24 h was digitized using WebPlot Digitizer version 3.8. The in vitro dissolution data showed that 18.8, 39.4 and 59.0% of dose was released/dissolved at 2, 4 and 6 hours respectively. The remaining dose dissolved evenly and completely by 24 h with dissolved percentages of 70, 84, 91 and 100% at 8, 12, 20, and 24 h respectively.[21] Tmax was observed in 4–6 h following oral administration of the treprostinil tablet.[2] Given that 50% of the stomach contents are emptied into intestines in 2.5–3 hours in healthy volunteers,[27] the absorption of treprostinil mainly occurs in the duodenum, jejunum and ileum. Furthermore, the colon absorption rate scalar was set as low as 0.05 to accommodate for the decreased levels 4–5 hours following administration of treprostinil tablet suggesting negligible absorption from the colon. Treprostinil being a monoprotic acid with a pKa of 3.76, the percent ionized drug will increase in the colon, thus limiting the extent of absorption. The PBPK model can assimilate both formulation mediated and physiology mediated changes in absorption and simulate treprostinil PK profiles accurately.

The relative availability of treprostinil from the extended-release tablet compared to the oral solution is 70%.[4] Given that the bioavailability $F = Fa \times Fg \times Fh$, Fg and Fh are same for oral solution and tablet, we can deduce that at least 30% of the dose was not absorbed from gut following oral administration of treprostinil tablet. Even though Fa, fraction absorbed from treprostinil tablet is not known, the predicted Fa of 0.48 (0.46–0.51; CI) is consistent with the ~50% dissolved fraction observed in the dissolution studies around the Tmax.[4] The predicted Fg and Fh were 87% and 60%, which indicated that hepatic metabolism is responsible for the majority of the disappearance of treprostinil from the body. Gut metabolism plays a relatively minor role as CYP2C8 and CYP2C9 are expressed at lower levels in the gut compared to the liver.[28] The Ka predicted by the PBPK model, 0.20 L/h, is similar to the value Ka of 0.24 L/h obtained from compartment analysis of concentration-time data reported by Jenkins et al.[12] using Phoenix WinNonlin. The reason for the minor deviation could be related to the small number of subjects and the large inter-subject variability.[12]

Liver impairment-induced physiological changes have a direct impact on the pharmacokinetics of medications due to alterations in the activity of drug metabolizing enzymes, liver size, hepatic blood flow, and plasma protein synthesis.[29] Liver impairment induced decrease in drug clearance shows an associated increase in the drug exposure and risk of drug related toxicities.[30] Patients with hepatic impairment showed higher treprostinil AUC than the healthy volunteers when same dose is administered. The built model successfully

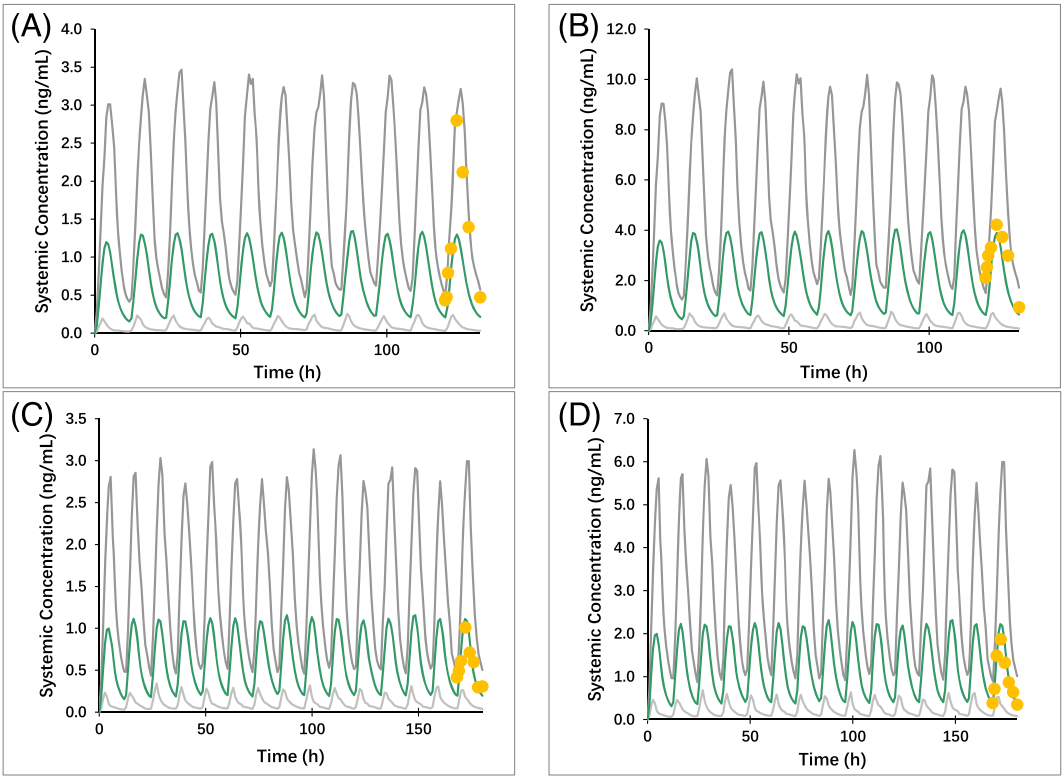

FIGURE 5 Predicted and observed concentration–time profiles following oral administration of treprostinil in patients with pulmonary arterial hypertension (PAH) or systemic sclerosis. (A) Validation plot in comparison with the observed data in PAH patients following 2-mg multiple oral doses of treprostinil from Ref.[17] (B) Validation plot in comparison with the observed in PAH patients following 6-mg multiple oral doses of treprostinil from Ref.[17] (C) Validation plot in comparison with the observed data in patients with systemic sclerosis following 2-mg multiple oral dose of treprostinil from Ref.[18] (D) Validation plot in comparison with the observed data in patients with systemic sclerosis following 4-mg multiple oral dose of treprostinil from Ref.[18] The middle line represented the simulated mean concentrations, the round dots represent observed data, and the lower and upper lines show the simulated 5th and 95th percentile

predicted the increase in drug levels in patients. The observed PK profiles were within the 5th and 95th percentile interval of the simulated PK profiles.[3] Reported mean AUC_{0-24} in subjects with mild, moderate and severe hepatic impairment following single dose oral administration of 1 mg treprotinil tablet were increased by approximately 2.2, 4.9 and 7.6 folds, respectively, in comparison to the healthy volunteers.[3] Additionally, the predicted AUCs in cirrhosis patients with Child–Pugh class A, B and C were 2.4-, 3.8- and 4.6-fold respectively higher than the healthy volunteers. The deviations in the prediction may be related to use of white population alone for the simulations.[3] Contribution of each elimination route to the total clearance after PO treprostinil extended-release tablet in patients with hepatic impairment were compared with healthy volunteers. Figure 6 shows that with an increase in the severity of liver dysfunction, the metabolic contribution of CYP 2C8 and CYP 2C9 is decreased.

Using the developed model, we were able to accurately predict the PK profiles in PAH or systemic sclerosis patients. Patient populations with PAH or systemic sclerosis are not available in the build-in population library in Simcyp simulator. A satisfactory prediction indicates the similarities between healthy population and the specific patient populations for treprostinil disposition. We also note that certain physiological differences may exist between the healthy population and patient populations, and further studies are needed to establish the databases to account for physiological changes observed in special patient populations.

With the PBPK model of treprostinil, we can predict the concentration in lung to be approximately 0.17 times of that in plasma. Given the high protein binding (91%) of treprostinil[4] and only unbound drug can permeate into the lung tissue, it is reasonable to accept that lung has the lower concentration than plasma. By directly vasodilating the pulmonary and systemic arterial vascular beds, treprostinil can improves systemic oxygen transport and increases cardiac output.[31]

Mass balance studies showed that 5 metabolites were detected in the urine with treprostinil glucuronide accounting for 10.2–15.5% of the subcutaneously administered dose.[32] While only 2.4% of the orally administered dose was detected as glucuronide conjugate in the urine.[4] Current retrograde model cannot incorporate both phase I and phase II metabolic pathway together to predict enzyme kinetics data, which may be a limitation of the current model. Furthermore, African American population accounted for a minor proportion in the clinical reports. Pharmacogenomic study showed a different distribution of CYP 2C alleles in African American population than in other populations.[33] Simcyp simulator version 17 does not have a library for African American population and the use of virtual Caucasian population only may in general lead to the discrepancy between the clinically observed and simulated data.

In conclusion, treprostinil PBPK model following intravenous infusion and oral administration was developed and validated with 16 model-naïve clinical studies. The built model can simulate the PK profiles in subjects with hepatic impairment and in patients with PAH or systemic sclerosis and facilitates our ability to understand the

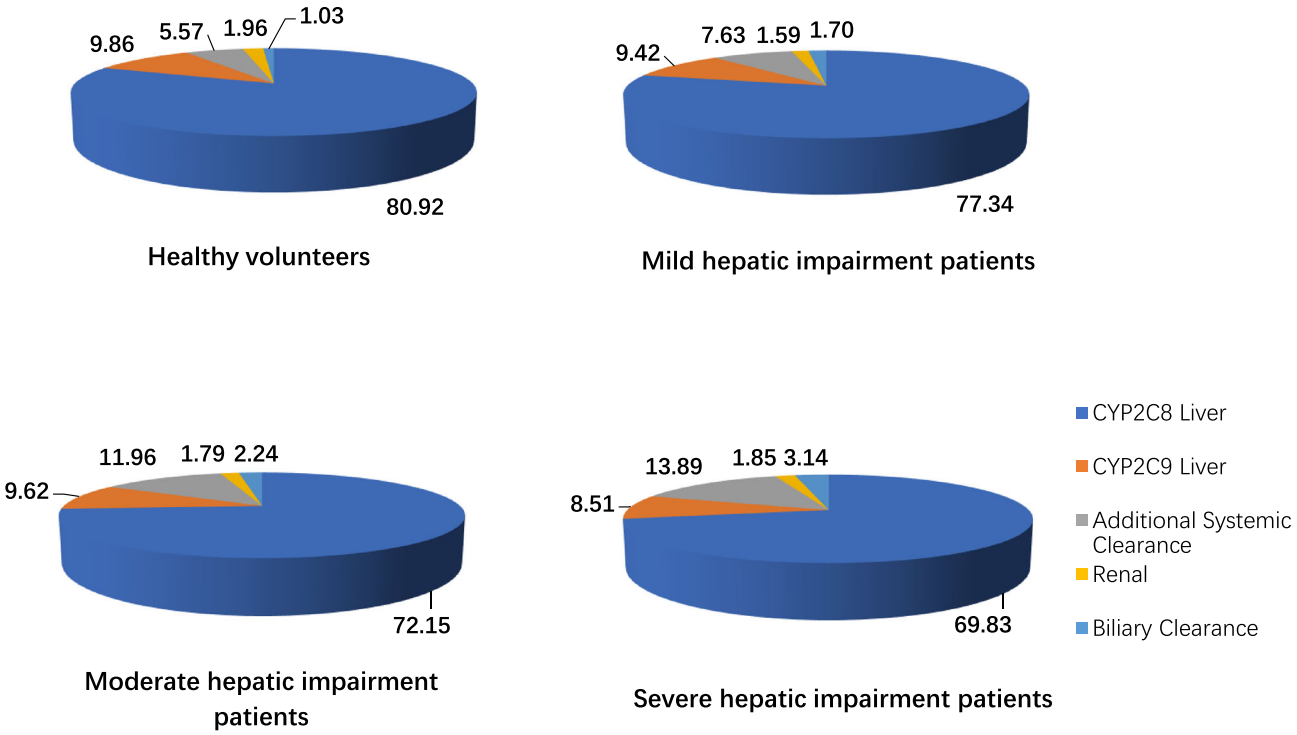

FIGURE 6 Comparison of the contribution of each elimination route to the total clearance after orally given treprostinil extended-release tablet in healthy volunteers and in patients with hepatic impairment. CYP, cytochrome P450. Demographic details of the patients were matched with reference[3]

disposition of treprostinil in the human body. It has the potential to predict the PK of treprostinil in other special populations, such as paediatric, geriatric and pregnant populations, and to predict drug–drug interactions as well.

COMPETING INTERESTS

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CONTRIBUTORS

Xuemei Wu and Raman Venkataramanan designed the research study and drafted the first draft of the manuscript. Xuemei Wu, Xiaohan Zhang and Ruichao Xu performed the research. Xiaohan Zhang, Imam Hussain Shaik and Raman Venkataramanan reviewed the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

All data included in this study will be available upon request from the corresponding author.

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REFERENCES

1. Zare P, Heller D. Treprostinil. StatPearls. 2019.
2. Kumar P, Thudium E, Laliberte K, Zaccardelli D, Nelsen A. A Comprehensive Review of Treprostinil Pharmacokinetics via Four Routes of Administration. *Clin Pharmacokinet*. Dec 2016;55(12):1495-1505. https://doi.org/10.1007/s40262-016-0409-0
3. Peterson L, Marbury T, Marier J, Laliberte K. An evaluation of the pharmacokinetics of treprostinil diolamine in subjects with hepatic impairment. *J Clin Pharm Ther*. Dec 2013;38(6):518-523. https://doi.org/10.1111/jcpt.12094
4. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/203496Orig1s000ClinPharmR.pdf. Accessed March 22, 2013.
5. Zhuang X, Lu C. PBPK modeling and simulation in drug research and development. *Acta Pharmaceutica Sinica B*. Sep 2016;6(5):430-440. https://doi.org/10.1016/j.apsb.2016.04.004
6. Jamei M, Marciniak S, Feng K, Barnett A, Tucker G, Rostami-Hodjegan A. The Simcyp population-based ADME simulator. *Expert Opin Drug Metab Toxicol*. Feb 2009;5(2):211-223. https://doi.org/10.1517/17425250802691074
7. Blot SI, Pea F, Lipman J. The effect of pathophysiology on pharmacokinetics in the critically ill patient--concepts appraised by the example of antimicrobial agents. *Adv Drug Deliv Rev*. Nov 20 2014;77:3-11. https://doi.org/10.1016/j.addr.2014.07.006
8. Zhao P, Zhang L, Grillo JA, et al. Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. *Clin Pharmacol Ther*. Feb 2011;89(2):259-267. https://doi.org/10.1038/clpt.2010.298
9. Jamei M. Recent Advances in Development and Application of Physiologically-Based Pharmacokinetic (PBPK) Models: a Transition from Academic Curiosity to Regulatory Acceptance. *Curr Pharmacol Rep*. 2016;2(3):161-169. https://doi.org/10.1007/s40495-016-0059-9
10. Wade M, Baker FJ, Roscigno R, DellaMaestra W, Hunt TL, Lai AA. Absolute bioavailability and pharmacokinetics of treprostinil sodium administered by acute subcutaneous infusion. *J Clin Pharmacol*. Jan 2004;44(1):83-88. https://doi.org/10.1177/0091270003261343
11. Laliberte K, Arneson C, Jeffs R, Hunt T, Wade M. Pharmacokinetics and steady-state bioequivalence of treprostinil sodium (Remodulin) administered by the intravenous and subcutaneous route to normal volunteers. *J Cardiovasc Pharmacol*. Aug 2004;44(2):209-214. https://doi.org/10.1097/00005344-200408000-00010
12. Jenkins A, Wang-Smith L, Marbury T, Laliberte K. Pharmacokinetics of treprostinil diolamine in subjects with end-stage renal disease on or off dialysis. *J Cardiovasc Pharmacol*. Apr 2013;61(4):272-276. https://doi.org/10.1097/FJC.0b013e31827e0fa9
13. Lim A, Wang-Smith L, Kates J, Laurent A, Kumar P, Laliberte K. The effect of different meal compositions on the oral bioavailability of treprostinil diolamine in healthy volunteers. *J Clin Pharm Ther*. Dec 2013;38(6):450-455. https://doi.org/10.1111/jcpt.12085
14. Jones A, Wang-Smith L, Pham T, Laliberte K. Pharmacokinetics of 3 times a day dosing of oral treprostinil in healthy volunteers. *J Cardiovasc Pharmacol*. Mar 2014;63(3):227-232. https://doi.org/10.1097/FJC.0000000000000039
15. Gotzkowsky SK, Dingemanse J, Lai A, Mottola D, Laliberte K. Lack of a pharmacokinetic interaction between oral treprostinil and bosentan in healthy adult volunteers. *J Clin Pharmacol*. Jul 2010;50(7):829-834. https://doi.org/10.1177/0091270009351173
16. Gotzkowsky SK, Kumar P, Mottola D, Laliberte K. Lack of a pharmacokinetic interaction between treprostinil diolamine and sildenafil in healthy adult volunteers. *J Cardiovasc Pharmacol*. May 2013;61(5):444-451. https://doi.org/10.1097/FJC.0b013e3182893d90
17. Hellawell JL, Bhattacharya S, Farber HW. Pharmacokinetic evaluation of treprostinil (oral) for the treatment of pulmonary arterial hypertension. *Expert Opin Drug Metab Toxicol*. Oct 2014;10(10):1445-1453. https://doi.org/10.1517/17425255.2014.958466
18. Shah AA, Schiopu E, Hummers LK, et al. Open label study of escalating doses of oral treprostinil diethanolamine in patients with systemic sclerosis and digital ischemia: pharmacokinetics and correlation with digital perfusion. *Arthritis Res Ther*. Apr 18 2013;15(2):R54. https://doi.org/10.1186/ar4216
19. Rodgers T, Rowland M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. *J Pharm Sci*. Jun 2006;95(6):1238-1257. https://doi.org/10.1002/jps.20502
20. Michelet R, Van Bocxlaer J, Allegaert K, Vermeulen A. The use of PBPK modeling across the pediatric age range using propofol as a case. *J Pharmacokinet Pharmacodyn*. Dec 2018;45(6):765-785. https://doi.org/10.1007/s10928-018-9607-8
21. Kidane A, Bhatt PP. Osmotic drug delivery system. *US 9,393,203 B2*. Jul.19 July 19, 2016
22. Winiwarter S, Bonham NM, Ax F, Hallberg A, Lennernas H, Karlen A. Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. *J Med Chem*. Dec 3 1998;41(25):4939-4949. https://doi.org/10.1021/jm9810102
23. Alexander SPH, Fabbro D, Kelly E, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Enzymes. *Br J Pharmacol*. Dec 2019;176(Suppl 1):S297-S396. https://doi.org/10.1111/bph.14752
24. Southan C, Sharman JL, Benson HE, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. *Nucleic Acids Res*. Jan 4 2016;44(D1):D1054-D1068. https://doi.org/10.1093/nar/gkv1037
25. Kalluri HV, Zhang H, Caritis SN, Venkataramanan R. A physiologically based pharmacokinetic modelling approach to predict buprenorphine pharmacokinetics following intravenous and sublingual administration. *Br J Clin Pharmacol*. Nov 2017;83(11):2458-2473. https://doi.org/10.1111/bcp.13368

26. Wu X, Zhang H, Miah MK, Caritis SN, Venkataramanan R. Physiologically Based Pharmacokinetic Approach Can Successfully Predict Pharmacokinetics of Citalopram in Different Patient Populations. *J Clin Pharmacol*. Apr 2020;60(4):477-488. https://doi.org/10.1002/jcph.1541
27. Camilleri M, Colemont LJ, Phillips SF, et al. Human gastric emptying and colonic filling of solids characterized by a new method. *Am J Physiol*. Aug 1989;257(2 Pt 1):G284-G290. https://doi.org/10.1152/ajpgi.1989.257.2.G284
28. Enayetallah AE, French RA, Thibodeau MS, Grant DF. Distribution of soluble epoxide hydrolase and of cytochrome P450 2C8, 2C9, and 2J2 in human tissues. *J Histochem Cytochem*. Apr 2004;52(4):447-454. https://doi.org/10.1177/002215540405200403
29. Rodighiero V. Effects of liver disease on pharmacokinetics. An update. *Clin Pharmacokinet*. Nov 1999;37(5):399-431. https://doi.org/10.2165/00003088-199937050-00004
30. Diep U, Chudow M, Sunjic KM. Pharmacokinetic Changes in Liver Failure and Impact on Drug Therapy. *AACN Adv Crit Care*. Summer 2017;28(2):93-101. https://doi.org/10.4037/aacnacc2017948
31. Lindegaard Pedersen M, Kruger M, Grimm D, Infanger M, Wehland M. The prostacyclin analogue treprostinil in the treatment of pulmonary arterial hypertension. *Basic Clin Pharmacol Toxicol*. Aug 12 2019;126(1):32-42. https://doi.org/10.1111/bcpt.13305
32. http://www.northernther.com/images/Canada_Remodulin_PM_09SEP03.pdf. Accessed Sep 9, 2003. (2):6–8.
33. Rajman I, Knapp L, Morgan T, Masimirembwa C. African Genetic Diversity: Implications for Cytochrome P450-mediated Drug Metabolism and Drug Development. *EBioMedicine*. Mar 2017;17:67-74. https://doi.org/10.1016/j.ebiom.2017.02.017

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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