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## Research Article

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# Development and Validation of a Physiologically Based Pharmacokinetic Model for Riociguat and Its Active Metabolite M1

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**Abstract:** Riociguat, a soluble guanylate cyclase stimulator for pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension, has complex pharmacokinetics involving enterohepatic circulation (EHC), multiple metabolic pathways, and dual renal/biliary elimination. We developed and validated the first physiologically based pharmacokinetic (PBPK) model for riociguat and its active metabolite M1 using PK-Sim 12.2, incorporating EHC based on biliary excretion and gallbladder emptying. The model was built with literature physicochemical/ADME parameters and clinical data from seven studies (0.5–2.5 mg single oral doses), then externally validated; over 90% of predictions fell within 0.5–2.0-fold error. Food-effect simulation predicted  $C_{\max}$  reduction to 53.4% and  $T_{\max}$  prolongation from 1.05 to 3.40 h, consistent with clinical reports. In virtual Child-Pugh A/B populations, riociguat  $AUC_{\text{inf}}$  increased by approximately 23% and 35%. DDI simulations showed clarithromycin increased  $AUC_{\text{inf}}$  by 37.5% (clinical: 41.5%), while rifampicin reduced it by 85.9%. This mechanistic PBPK platform showed robust predictive performance across healthy, food-effect, hepatic impairment, and DDI scenarios, supporting dose optimization and DDI risk assessment for riociguat, with clinical translational value.

**Keywords:** Riociguat; Physiologically based pharmacokinetic modeling; Enterohepatic circulation; Hepatic impairment; Drug–drug interaction; Pulmonary hypertension

## 1. Introduction

Pulmonary hypertension is a widespread, progressive pulmonary vascular disease with high mortality. It is characterized by sustained increases in pulmonary vascular resistance and pulmonary vascular remodeling, which increase cardiac afterload and ultimately lead to right heart failure and death <sup>[1]</sup>.

Riociguat, a soluble guanylate cyclase (sGC) stimulator approved in 2013, exhibits unique binding site selectivity and enzyme activation properties compared to other sGC stimulators or vasodilators. It is indicated for the treatment of pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH), improving exercise capacity and delaying clinical deterioration. It remains one of the first-line targeted therapies for pulmonary hypertension<sup>[2]</sup>. It is also the only drug currently approved for the treatment of both PAH and CTEPH <sup>[3, 4]</sup>. Its mechanism of action is independent of endogenous NO levels; rather, it directly stimulates sGC and enhances its sensitivity to NO, activating the NO-sGC-cGMP signaling pathway and thereby exerting vasodilatory and antiproliferative effects<sup>[5, 6]</sup>.

After oral administration, riociguat is rapidly absorbed, with a time to peak concentration ( $T_{max}$ ) of approximately 0.5–1.5 h. Its half-life differs substantially between nonsmoking and smoking populations<sup>[7]</sup>. Riociguat exhibits linear pharmacokinetic characteristics over the dose range of 0.5–2.5 mg, and food has a significant effect on its absorption<sup>[8]</sup>. Plasma protein binding of riociguat is approximately 95%. It is primarily metabolized by multiple hepatic enzymes, including CYP1A1, CYP3A4, and CYP3A5, to generate the major active metabolite M1. M1 is further metabolized by UGT1A1 and UGT1A9 to M3 or M4, which are subsequently excreted via urine or feces<sup>[9]</sup>. The chemical structures of riociguat and its

metabolite M1 are shown in Figure 1.

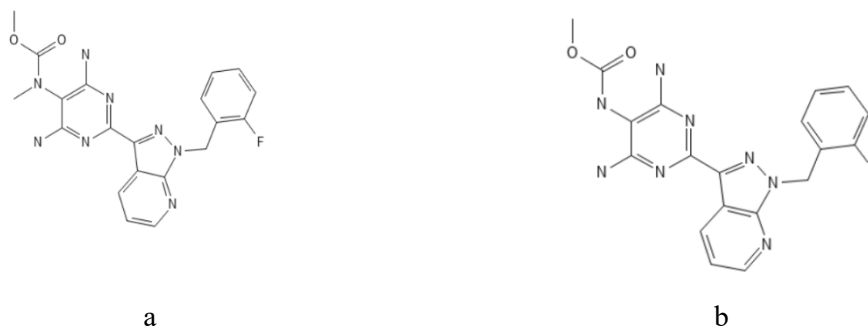


**Figure 1. Chemical structures of (a) riociguat and (b) its metabolite M1**

Riociguat exhibits a unique dual-route elimination profile. In a radiolabeled mass balance study, approximately 33–45% of riociguat and its metabolites were excreted renally, while approximately 48–59% were excreted via the biliary/fecal route, with substantial amounts of unchanged riociguat and its metabolite M1 detected in feces<sup>[9]</sup>. This provides the physiological basis for enterohepatic circulation (EHC) of riociguat and its metabolites—after biliary secretion into the intestine, a portion of the drug can be reabsorbed and re-enter the systemic circulation, forming a "recirculation" process. Furthermore, clinical pharmacokinetic data have shown that after oral absorption reaches its peak, plasma concentrations of riociguat and its metabolite M1 decline rapidly, followed by a prolonged terminal half-life<sup>[10, 11]</sup>. This prolonged terminal half-life may also be related to EHC, although this has not been fully confirmed. Alternatively, it may be attributed to other factors, such as deep intestinal absorption or irregular gastric emptying patterns<sup>[12, 13]</sup>. Furthermore, interindividual variability in EHC—including bile flow, intestinal transit time, and transporter expression levels—may be an important source of the marked interindividual variability in riociguat exposure.

The Drug-Drug Interaction (DDI) profile of riociguat primarily stems from three aspects. First, riociguat is metabolized by multiple cytochrome P450 (CYP) enzymes, including CYP1A1, CYP3A4, CYP2C8, and CYP2J2. The involvement of multiple enzymes in its metabolism implies that inhibition or induction of any single metabolic pathway is unlikely to fully explain the overall DDI profile; however, the presence of multiple compensatory

pathways also means that perturbation of a single pathway has a relatively limited impact on overall exposure. Second, riociguat is a substrate for efflux transporters such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP)<sup>[14]</sup>. These transporters play important roles in drug absorption and excretion. Third, riociguat and its metabolites are eliminated via both renal and biliary routes, and alteration of either elimination route can be compensated by the other pathway. These characteristics make it difficult to predict the DDI risk of riociguat using conventional models. Additionally, hepatic impairment is a common comorbidity in Pulmonary hypertension<sup>[15, 16]</sup>. Moreover, since the hepatobiliary route is one of the major elimination pathways for riociguat, hepatic impairment may significantly affect its plasma concentrations. To investigate the impact of different hepatic disease states on the pharmacokinetics of riociguat, we aimed to extend the PBPK model to patients with mild and moderate hepatic impairment.

For the reasons described above, conventional empirical pharmacokinetic models may not adequately capture the interindividual variability in riociguat disposition, necessitating a mechanistic modeling approach that integrates physiological parameters.

Physiologically based pharmacokinetic (PBPK) modeling is a mathematical approach that predicts drug disposition mechanistically based on human anatomical and physiological parameters and drug physicochemical properties<sup>[17, 18]</sup>. PBPK models can integrate population-specific physiological variability information, providing a scientific basis for dose extrapolation from adults to special populations such as hepatic impairment<sup>[19]</sup>. In this context, the development of a PBPK model for riociguat holds important clinical translational significance.

Therefore, this study aimed to investigate the EHC hypothesis and characterize the PK profile of riociguat accordingly, and further to simulate the effects of hepatic impairment on plasma concentration–time profiles as well as the DDI effects of riociguat with CYP enzyme inhibitors or inducers.

## 2. Materials and Methods

### 2.1. Modeling Software

This study used PK-Sim® 12.2 for PBPK model development. GetData Graph Digitizer (Version 2.20) was used to extract plasma concentration–time data from published literature. PK-Sim is a core component of the Open Systems Pharmacology Suite, an open-source platform for mechanistic modeling in pharmacokinetics and pharmacodynamics. Compared with other mainstream PBPK modeling platforms, PK-Sim offers the advantages of being open-source, freely available, and functionally comprehensive.

### 2.2. Clinical Data

Systematic literature searches were conducted in Web of Science, PubMed, and CNKI using the keywords "Riociguat" AND "Pharmacokinetics" to identify clinical trial reports describing riociguat pharmacokinetics. Studies that reported complete plasma concentration–time profiles of riociguat in humans were included. After full-text review and screening, studies without complete concentration–time data were excluded. Ultimately, seven studies that reported complete riociguat concentration–time profiles were identified and formed the dataset for PBPK model development. The baseline characteristics of the included studies are summarized in Table 1.

**Table 1. Baseline characteristics of studies included for PBPK model development**

| Study                              | Population               | N  | Sex<br>(M/F) | Dose regimen       | Age<br>(years) | BMI<br>(kg/m <sup>2</sup> ) |
|------------------------------------|--------------------------|----|--------------|--------------------|----------------|-----------------------------|
| <b>Frey 2016a</b> <sup>[20]</sup>  | Healthy White            | 16 | 11/5         | Single oral 1 mg   | 44-69          | 27.5±2.7                    |
| <b>Frey 2016b</b> <sup>[21]</sup>  | Healthy White            | 15 | 15/0         | Single oral 2.5 mg | 23-45          | 25.7±2.4                    |
| <b>Frey 2016c</b> <sup>[10]</sup>  | Healthy White<br>& Black | 9  | 9/0          | Single oral 2.5 mg | /              | /                           |
| <b>Frey 2016d</b> <sup>[11]</sup>  | Healthy White            | 16 | 11/5         | Single oral 2.5 mg | 35-69          | 27.6±3.1                    |
|                                    | Healthy White            | 16 | 10/6         | Single oral 1 mg   | 37-67          | 27.3±2.1                    |
| <b>Zhao 2015</b> <sup>[22]</sup>   | Healthy Chinese          | 6  | 6/0          | Single oral 1 mg   | 28-43          | 22.5±1.6                    |
|                                    | Healthy Chinese          | 6  | 6/0          | Single oral 2 mg   | 29-43          | 22.6±1.9                    |
| <b>Saleh 2016</b> <sup>[23]</sup>  | Healthy White            | 30 | 15/15        | Single oral 1 mg   | 22-45          | /                           |
|                                    | Healthy White            | 25 | 25/0         | Single oral 2.5 mg | 20-45          | /                           |
| <b>Becker 2016</b> <sup>[24]</sup> | Healthy White            | 12 | 12/0         | Single oral 2.5 mg | 21-41          | 23.3±2.0                    |

### 2.3. Modeling Workflow

The physiological parameters used for PBPK model development, such as organ blood flow and tissue partition coefficients, were based on the European population database embedded

in the software. Drug physicochemical properties, Absorption, Distribution, Metabolism, Excretion (ADME) parameters, and clinical PK data were obtained from published literature. Using the built-in healthy adult physiological parameters and clinical trial data, PBPK models for riociguat and its metabolite M1 were developed for single oral doses of 0.5 mg, 2 mg, and 2.5 mg. Model parameters were iteratively optimized using the Monte Carlo algorithm against clinically observed PK data to achieve the best fit. After model optimization, fully independent datasets—i.e., clinical studies not used for parameter fitting—were employed for external model validation. Subsequently, the validated healthy adult PBPK model was applied to DDI simulations and extrapolated to hepatic impairment populations.

Since PK-Sim does not include built-in physiological parameter sets for hepatic impairment, we adopted a "top-down" physiological parameter adjustment approach based on literature-reported changes in hepatic impairment relative to healthy adults. Key parameters adjusted included hepatic blood flow, liver volume, glomerular filtration rate, plasma protein levels, metabolic enzyme abundance, and hematocrit. These adjustments were systematically incorporated into the healthy physiological parameters to generate virtual populations with mild, moderate, and severe hepatic impairment according to Child-Pugh classification. A PBPK model for hepatic impairment was then established based on this virtual population to explore the effects of liver dysfunction on the pharmacokinetics of riociguat and its active metabolite M1, and to provide preliminary dose adjustment recommendations for hepatically impaired patients.

## **2.4. Modeling of Enterohepatic Circulation**

Riociguat has been identified as a substrate for the efflux transporters P-gp and BCRP<sup>[14]</sup>. When constructing the PBPK model in PK-Sim®, these transporters were incorporated into the model structure to describe their role in biliary excretion at the canalicular membrane of hepatocytes. Concomitant drugs that inhibit the activity of these transporters may reduce the biliary excretion of riociguat and its metabolites, leading to increased plasma concentrations. Given the relatively rapid metabolism of riociguat in vivo, the following assumptions were made regarding gallbladder emptying kinetics in the model: gallbladder emptying was

activated at 3 h post-dose and every 8 h thereafter to simulate normal meal patterns, with no emptying activity during the initial hours post-dose to reflect the fasting state, consistent with experimental study conditions.

## 2.5. Model Evaluation and Optimization

To verify the reliability of the model, sensitivity analysis was performed using the sensitivity analysis tool in PK-Sim to calculate the sensitivity value (S) for each parameter. Sensitivity is defined as the ratio of the relative change in an output parameter to the relative change in an input parameter, calculated according to the following formula:

$$S = \frac{\Delta PK_j / PK_j}{\Delta p_i / p_i} = \frac{\Delta PK_j}{\Delta p_i} \cdot \frac{p_i}{PK_j}$$

where  $PK_j$  is the baseline value of the  $j$ -th pharmacokinetic parameter, and  $\Delta PK_j$  is the change in that parameter following the perturbation of the input parameter;  $p_i$  is the baseline value of the  $i$ -th input parameter, and  $\Delta p_i$  is the small change applied to that parameter. In this study,  $AUC_{inf}$  (area under the plasma concentration–time curve from time zero to infinity) and  $C_{max}$  (maximum plasma concentration) were used as output parameters to calculate the relative changes  $\Delta AUC_{inf} / AUC_{inf}$  and  $\Delta C_{max} / C_{max}$  as sensitivity values for each input parameter.

High-sensitivity parameters or those involved in key ADME processes were optimized using the Monte Carlo algorithm to improve model accuracy. To verify model reliability, we validated the optimized PBPK model using independent datasets derived from clinical studies not used for parameter optimization, which differed in dosing regimen, subject demographics, and sampling time points from the model-building dataset. Predictive performance was evaluated by calculating the fold error (FE) between observed and predicted concentrations for the independent datasets. FE was calculated according to the following formula:

$$FE = \frac{C_{Predicted}}{C_{Observed}}$$

where  $C_{Predicted}$  is the PBPK model-predicted concentration and  $C_{Observed}$  is the clinically observed concentration. If  $0.5 < FE < 2$ , indicating that the difference between predicted and observed concentrations is within 2-fold, the model can be considered to have good predictive performance with sufficient accuracy and reliability, and can be used for subsequent PBPK modeling in hepatic impairment populations.

## **2.6. Hepatic Impairment Simulation**

Since PK-Sim does not include built-in physiological parameter sets for hepatic impairment populations, this study adopted the cirrhosis physiological parameter framework established by Edginton et al<sup>[25]</sup>. A "top-down" physiological parameter adjustment approach was employed to construct virtual hepatic impairment populations. First, a systematic literature search was conducted to collect data on physiological parameter changes in hepatic impairment populations of varying severity relative to healthy adults. Based on Child-Pugh A and B classifications, changes in parameters including hepatic blood flow, portal venous blood flow, functional liver volume, hepatic CYP450 enzyme abundance, plasma albumin, hematocrit, and glomerular filtration rate were incorporated to systematically adjust the healthy physiological parameters, generating three physiological parameter sets corresponding to mild, moderate, and severe hepatic impairment. Subsequently, the adjusted physiological parameters were input into PK-Sim to construct virtual populations at each severity level and establish the corresponding PBPK models. Finally, the model was externally validated using published clinical pharmacokinetic data from hepatically impaired patients, and model predictive performance was assessed by comparing FE values between predicted and clinically observed concentrations, ensuring model reliability and accuracy

## **3. Result**

### **3.1. PBPK Model Parameter Settings**

The molecular weight, pka, logP, and unbound fraction ( $f_u$ ) of riociguat and M1 were obtained from Drugbank. Riociguat is primarily metabolized by CYP3A4 and CYP1A1, and cleared via biliary and renal routes. In a radiolabeled ADME study, total recovery of riociguat and its metabolites was approximately 90–95% of the administered dose, with 33–45% excreted renally and 48–59% excreted via the biliary/fecal route. Unchanged riociguat accounted for approximately 9–44% of the dose in feces (partially in the form of the active metabolite M1) and approximately 4–19% in urine. The active metabolite M1 is further metabolized by UGT1A1 and UGT1A9<sup>[9]</sup>. All relevant modeling parameters and their final fitted values are presented in Tables 2 and 3, and the drug clearance pathways are shown

schematically in Figure 2.

**Table 2. PBPK model parameters for riociguat**

| Parameter                        | Initial value      | Final fitted value           |
|----------------------------------|--------------------|------------------------------|
| Molecular weight                 | 422.4              | 422.4                        |
| pka                              | 4.3                | 4.3                          |
| logP                             | 2.34               | 2.39                         |
| Solubility                       | 0.004 mg/mL        | 0.004 mg/mL                  |
| Intestinal permeability          | Thelen method      | $2.55 \times 10^{-5}$ cm/min |
| Partition coefficients           | Rodgers&Rowland    | Rodgers&Rowland              |
| Cellular permeability            | PK-Sim standard    | PK-Sim standard              |
| B/P                              | 0.7 <sup>[9]</sup> | 0.7                          |
| fu                               | 0.05               | 0.0307                       |
| CL <sub>int</sub> , CYP1A1 (L/h) | 1                  | 1.74                         |
| CL <sub>int</sub> , CYP3A4(L/h)  | 1                  | 4.01                         |
| K <sub>m</sub> (μmol/L) P-gp     | 1                  | 1                            |
| K <sub>cat</sub> (1/min) P-gp    | 0                  | 2.32                         |
| K <sub>m</sub> (μmol/L) BCRP     | 1                  | 1                            |
| K <sub>cat</sub> (1/min) BCRP    | 0                  | 0.28                         |
| Lag time                         | 0                  | 2min                         |
| Dissolution shape                | 0.92               | 1                            |
| Dissolution time (50%dissolved)  | 240min             | 6min                         |
| EHC continuous fraction          | 0.4                | 0.45                         |
| Gallbladder ejection fraction    | 0.65               | 0.65                         |

**Table 3. PBPK model parameters for M1**

| Parameter               | Initial value      | Final fitted value           |
|-------------------------|--------------------|------------------------------|
| Molecular weight        | 408.4              | 408.4                        |
| pka                     | 4.4                | 4.4                          |
| logP                    | 2.2                | 2.39                         |
| Solubility              | 0.004 mg/mL        | 0.004 mg/mL                  |
| Intestinal permeability | Thelen method      | $1.36 \times 10^{-5}$ cm/min |
| Partition coefficients  | Rodgers&Rowland    | Rodgers&Rowland              |
| Cellular permeability   | PK-Sim standard    | PK-Sim standard              |
| B/P                     | 0.7 <sup>[9]</sup> | 0.7                          |
| fu                      | 0.03               | 0.039                        |

|                                  |   |      |
|----------------------------------|---|------|
| CL <sub>int</sub> , UGT1A1 (L/h) | 1 | 1.31 |
| CL <sub>int</sub> , UGT1A9(L/h)  | 1 | 3.39 |

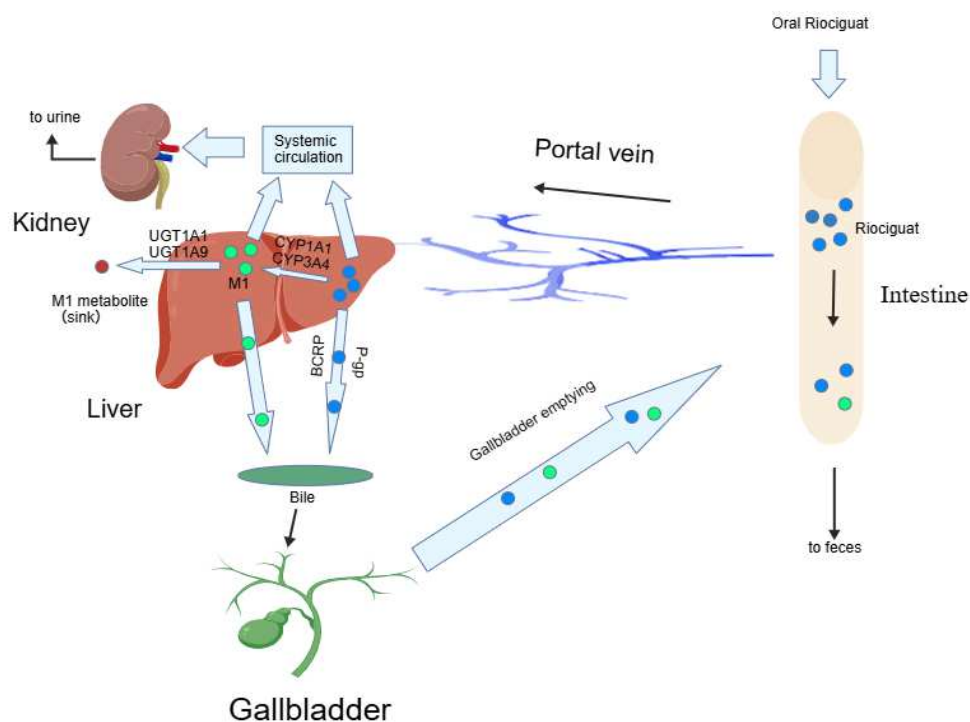


**Figure 2. Schematic diagram of the drug clearance pathways of riociguat in the PBPK model,**  
**Created with BioGDP.com<sup>[26]</sup>**

### 3.2. PBPK Model Development and Validation

Based on clinical data, a physiologically based pharmacokinetic (PBPK) model for riociguat was successfully developed in this study, and its predictive performance was systematically evaluated using an independent clinical dataset. The PBPK model-predicted curves and clinically observed data for riociguat at various doses, validated with the independent dataset, are presented in Figure 3. Given that EHC may cause secondary peaks in the plasma concentration–time curve, and to avoid interference with the estimation of absorption-phase peak parameters, the first peak concentration ( $C_{\max-1}$ ) and its corresponding time to peak ( $T_{\max-1}$ ) were used as the pharmacokinetic parameters for model validation, rather than the absolute maximum values over the entire time course. The corresponding pharmacokinetic parameters and fold errors (FE) are summarized in Table 4. Subsequently, the model-predicted plasma concentrations from the PBPK model in all healthy subjects were compared with the observed values (as shown in Figure 4). The results indicated that the

majority of clinically observed data fell within the 0.5- to 2.0-fold FE range, demonstrating that the model possesses good predictive capability for the pharmacokinetic behavior of this drug.

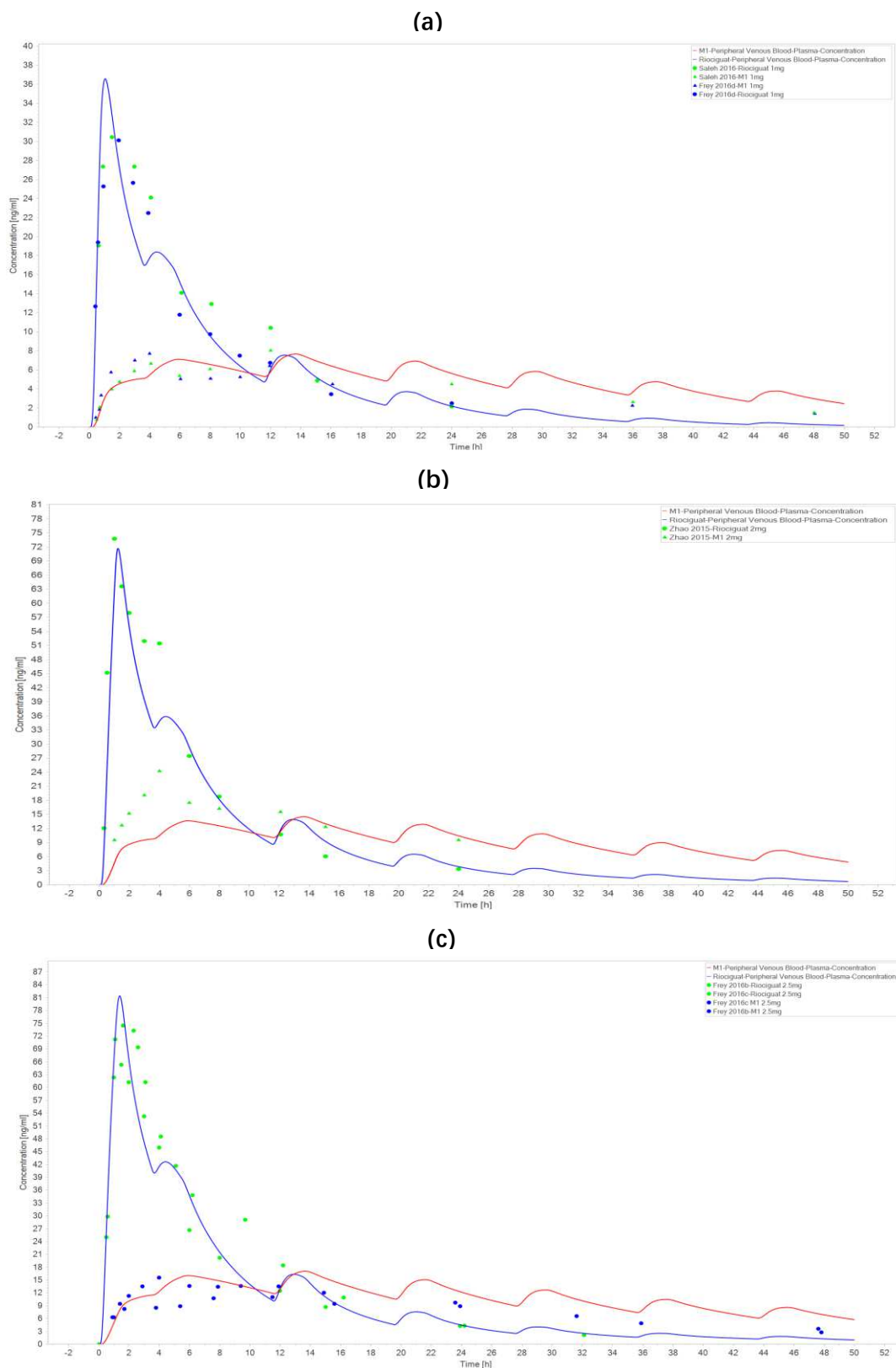


**Figure 3. Plasma concentration–time profiles of riociguat and its metabolite M1 following single oral administration of (a) 1 mg, (b) 2 mg, and (c) 2.5 mg riociguat. Curves represent PBPK model-predicted concentrations, and points represent mean clinically observed concentrations**

**Table 4. Model-predicted vs. observed pharmacokinetic parameters of a PBPK model externally validated in healthy adults**

| Study              | Type   | Riociguat                   |                           |                        | M1                          |                           |                        |
|--------------------|--------|-----------------------------|---------------------------|------------------------|-----------------------------|---------------------------|------------------------|
|                    |        | AUC <sub>inf</sub> , µg·h/L | C <sub>max-1</sub> , µg/L | T <sub>max-1</sub> , h | AUC <sub>inf</sub> , µg·h/L | C <sub>max-1</sub> , µg/L | T <sub>max-1</sub> , h |
| Single oral 1 mg   | Pre-   | 250.08                      | 36.59                     | 1.05                   | 273.55                      | 7.13                      | 5.85                   |
| Frey 2016d         | Obser- | 284.5                       | 30.12                     | 1.95                   | 214.94                      | 7.71                      | 3.98                   |
|                    | FE     | 0.88                        | 1.21                      | 0.54                   | 1.27                        | 0.92                      | 1.47                   |
| Saleh 2016         | Obser- | 285.14                      | 30.46                     | 1.5                    | 231.67                      | 6.69                      | 4.1                    |
|                    | FE     | 0.88                        | 1.20                      | 0.70                   | 1.18                        | 1.07                      | 1.43                   |
| Single oral 2.0 mg | Pre-   | 480.23                      | 71.77                     | 1.25                   | 520.30                      | 14.55                     | 5.70                   |
| Zhao 2015          | Obser- | 512.38                      | 73.75                     | 1.00                   | 666.53                      | 24.27                     | 4.00                   |
|                    | FE     | 0.94                        | 0.97                      | 1.25                   | 0.78                        | 0.60                      | 1.43                   |
| Single oral 2.5 mg | Pre-   | 567.03                      | 81.45                     | 1.4                    | 610.4                       | 16.04                     | 5.8                    |
| Frey 2016b         | Obser- | 520.08                      | 65.35                     | 1.5                    | 465.53                      | 15.53                     | 4                      |
|                    | FE     | 1.09                        | 1.25                      | 0.93                   | 1.31                        | 1.03                      | 1.45                   |
| Frey 2016c         | Obser- | 659.96                      | 74.53                     | 1.6                    | 465.87                      | 13.65                     | 9.2                    |
|                    | FE     | 0.86                        | 1.09                      | 0.88                   | 1.31                        | 1.18                      | 0.63                   |

**Note:** Pre-, model-predicted values; Obser-, actual observed values; FE: fold error; C<sub>max-1</sub>: first peak concentration; T<sub>max-1</sub>: time to first peak

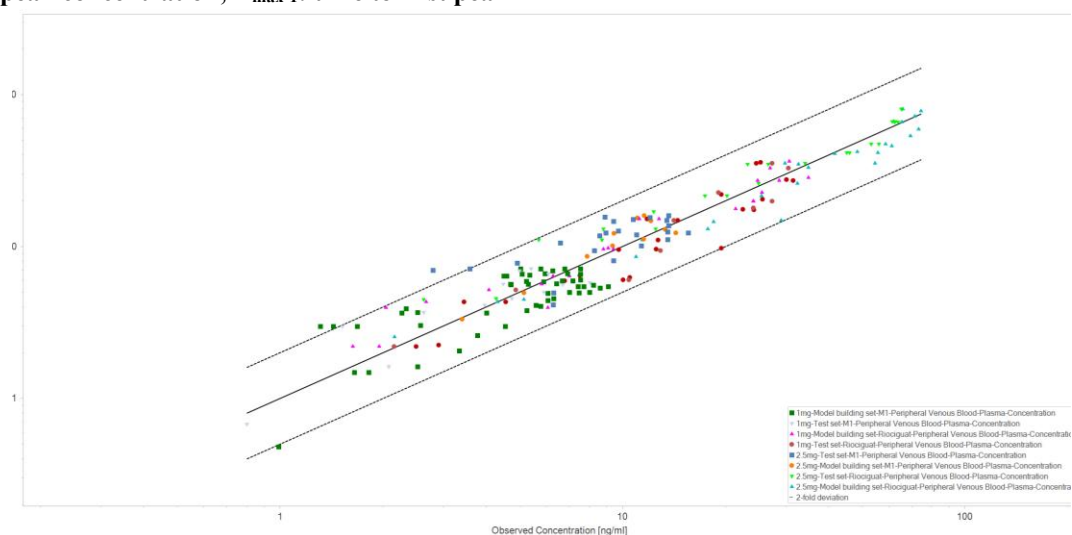


**Figure 4. Comparison of observed mean plasma concentrations and model-predicted values for riociguat and its metabolite M1 following a single oral 1 mg dose. Dashed lines represent the 2-fold error range (i.e., predicted values within 0.5- to 2-fold of observed values)**

### 3.3. Effect of Food on the Pharmacokinetics of Riociguat

In PBPK modeling, successful prediction of food effects is an important line of evidence supporting model reliability. To validate the robustness of the riociguat PBPK model developed in this study, we compared the predicted changes in postprandial exposure with published clinical food-effect data. Specifically, we simulated plasma concentration–time profiles of riociguat (1 mg, single oral dose) under fasting conditions and following a high-fat meal to evaluate the effects of food on the rate ( $C_{\max}$ ,  $T_{\max}$ ) and extent ( $AUC_{\text{inf}}$ ) of absorption, thereby confirming the model’s ability to describe pharmacokinetic changes in the fed state. The PBPK model-predicted curves and key pharmacokinetic parameters for riociguat and its metabolite M1 following co-administration with a standard meal are presented below. Becker et al. [8] and Frey et al. [9] showed that food has no significant effect on the  $AUC_{\text{inf}}$  of riociguat, but does affect the rate of absorption ( $C_{\max}$ ,  $T_{\max}$ ). Specifically, Becker et al. [8] reported that when riociguat was administered within 5 minutes after completion of a high-fat meal, the  $C_{\max}$  of riociguat decreased to 64.7% of control values, and  $T_{\max}$  was prolonged from 1 h to 4 h, which is generally consistent with the PBPK model predictions. The predicted plasma concentration–time curves of riociguat co-administered with a high-fat meal are shown in Figure 5, and the corresponding pharmacokinetic parameters are summarized in Table 5.

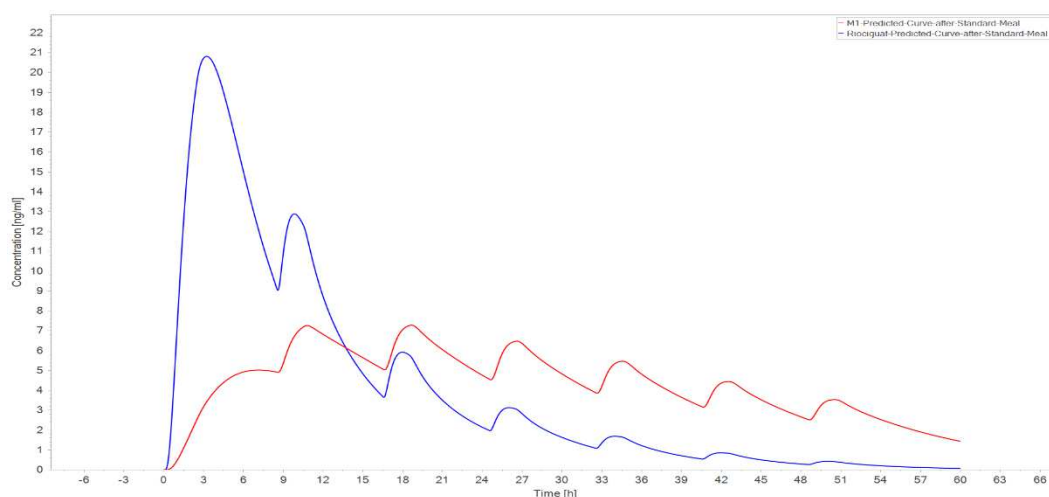


**Figure 5. Predicted plasma concentration–time profiles of riociguat co-administered with a high-fat meal**

**Table 5. Pharmacokinetic parameters of riociguat and M1 following co-administration with a high-fat meal**

| Treatment                     | Riociguat                      |                            |                         | M1                             |                            |                         |
|-------------------------------|--------------------------------|----------------------------|-------------------------|--------------------------------|----------------------------|-------------------------|
|                               | AUC <sub>inf</sub><br>(μg·h/L) | C <sub>max</sub><br>(μg/L) | T <sub>max</sub><br>(h) | AUC <sub>inf</sub><br>(μg·h/L) | C <sub>max</sub><br>(μg/L) | T <sub>max</sub><br>(h) |
| Riociguat alone               | 250.08                         | 36.59                      | 1.05                    | 273.35                         | 7.69                       | 13.65                   |
| Riociguat after Standard-Meal | 249.14                         | 20.82                      | 3.25                    | 275.61                         | 7.29                       | 18.65                   |

### 3.4. PBPK Model Development for Hepatic Impairment

Since PK-Sim does not include built-in physiological parameter sets for hepatic impairment populations, this study adopted the cirrhosis physiological parameter framework established by Edginton and Willmann <sup>[25]</sup> to construct virtual hepatic impairment populations. The final physiological parameter changes for mild and moderate hepatic impairment are presented in Table 6. Figure 6 shows the predicted plasma concentration–time profiles in hepatically impaired individuals, along with the observed riociguat plasma concentration data. Notably, the clinical study by Frey et al<sup>[11]</sup> reported that the unbound fraction (fu) of riociguat in patients with mild hepatic impairment was similar to that in controls, whereas fu in patients with moderate hepatic impairment was higher than in controls. To reasonably reflect this difference in the model, a plasma protein scaling factor—which has a significant impact on C<sub>max</sub> and AUC<sub>inf</sub>—was introduced and adjusted accordingly to capture the changes in unbound drug concentrations in hepatically impaired patients: the factor was kept unchanged for the mild hepatic impairment group, and increased to 1.1 for the moderate hepatic impairment group to fit the modest elevation in unbound riociguat concentration observed in moderate hepatic impairment. The final differences in physiological parameters between healthy individuals and patients with mild and moderate hepatic impairment are summarized in Table 6. As shown in Figure 6, riociguat accumulates in Child-Pugh A and Child-Pugh B individuals compared with healthy subjects, resulting in increased plasma exposure.

**Table 6. Adjusted physiological parameters in mild and moderate hepatic impairment relative to healthy subjects**

| Parameter                                          | Healthy | Child-Pugh | Child-PughB |
|----------------------------------------------------|---------|------------|-------------|
| Hepatic blood flow (L/min) <sup>a</sup>            | 1       | 1.4        | 1.62        |
| Renal blood flow (L/min) <sup>a</sup>              | 1.07    | 0.94       | 0.69        |
| Other organs blood flow (L/min) <sup>a</sup>       | 1       | 1.75       | 2.25        |
| Pancreas blood flow (relative) <sup>a</sup>        | 1       | 0.4        | 0.36        |
| Skin blood flow (relative) <sup>a</sup>            | 1       | 0.4        | 0.36        |
| Small-intestine blood flow (relative) <sup>a</sup> | 1       | 0.4        | 0.36        |
| Spleen blood flow (relative) <sup>a</sup>          | 1       | 0.4        | 0.36        |
| Stomach blood flow (relative) <sup>a</sup>         | 1       | 0.4        | 0.36        |
| Liver volume (L) <sup>a</sup>                      | 1.91    | 1.32       | 1.05        |
| Hematocrit <sup>a</sup>                            | 0.47    | 0.39       | 0.37        |
| GFR (ml/min/1.73m <sup>2</sup> ) <sup>a</sup>      | 96.56   | 97.04      | 68.06       |
| Plasma protein scaling factor                      | 1       | 1          | 1.1         |
| Hepatic CYP3A4 (μmol/L/liver) <sup>a</sup>         | 4.32    | 4.32       | 2.13        |
| Hepatic CYP1A1 (relative) <sup>b</sup>             | 1       | 0.83       | 0.63        |
| Hepatic UGT1A1 (relative) <sup>c</sup>             | 1       | 1          | 0.7         |
| HepaticUGT1A9 (relative) <sup>b</sup>              | 1       | 1          | 0.48        |

<sup>a</sup> Based on Edginton et al<sup>[25]</sup>, <sup>b</sup> Based on Drozdik et al<sup>[27]</sup>, <sup>c</sup> Based on Duthaler et al<sup>[28]</sup>.

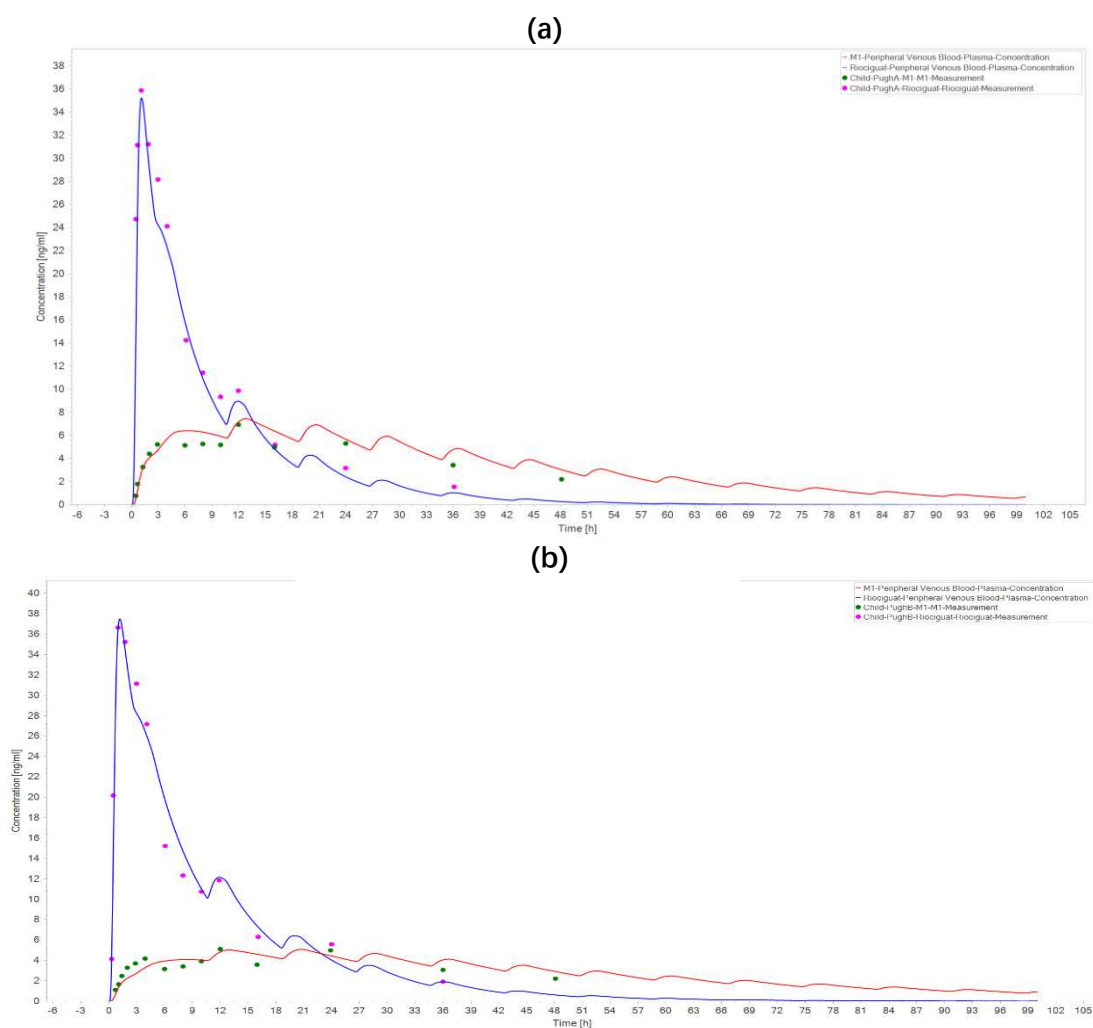


**Figure 6. Plasma concentration–time profiles of riociguat and its metabolite M1 following a single oral 1 mg dose of riociguat in (a) Child-Pugh A and (b) Child-Pugh B individuals. Red and green circles represent the observed mean plasma concentrations of riociguat and M1, respectively, while the blue and red curves represent the PBPK model-predicted concentration profiles of riociguat and M1, respectively**

### 3.5. DDI Simulations of Riociguat with Clarithromycin and Rifampicin

Clarithromycin is a strong reversible inhibitor of CYP3A4 and exerts moderate-to-potent inhibitory effects on P-gp and BCRP <sup>[29]</sup>. Since riociguat is partially metabolized by CYP3A4 and is a substrate for P-gp and BCRP, clarithromycin may increase riociguat plasma concentrations through dual mechanisms: inhibition of CYP3A4-mediated metabolic clearance and inhibition of P-gp/BCRP-mediated efflux. Rifampicin is a strong inducer of CYP3A4 <sup>[30]</sup>. By inducing CYP3A4 enzyme activity, rifampicin accelerates the oxidative metabolic clearance of riociguat, leading to reduced plasma concentrations. Based on these

interaction mechanisms, we simulated DDIs using the PBPK model. The specific simulation regimens were as follows: for clarithromycin, the simulation was designed according to the clinical study protocol, with clarithromycin 500 mg twice daily administered for 4 days, followed by a single 1.0 mg dose of riociguat on day 5; for rifampicin, the simulation was designed as rifampicin 600 mg once daily for 4 days, followed by a single 1.0 mg dose of riociguat. The DDI simulation results for riociguat are shown in Figure 7, with the corresponding pharmacokinetic parameters summarized in Table 7. The DDI simulations showed that co-administration with clarithromycin increased the  $AUC_{inf}$  of riociguat following a single dose by 37.5%, while co-administration with rifampicin decreased the  $AUC_{inf}$  to 14.1% of the control value. A clinical trial by Becker et al. <sup>[31]</sup> reported that clarithromycin co-administration increased riociguat  $AUC_{inf}$  by 41.5% (90% CI: 22.9%–62.9%), which is generally consistent with the model predictions, further supporting the reliability of the model. However, to date, no dedicated clinical study on the interaction between rifampicin and riociguat has been published, and further clinical investigation is warranted.

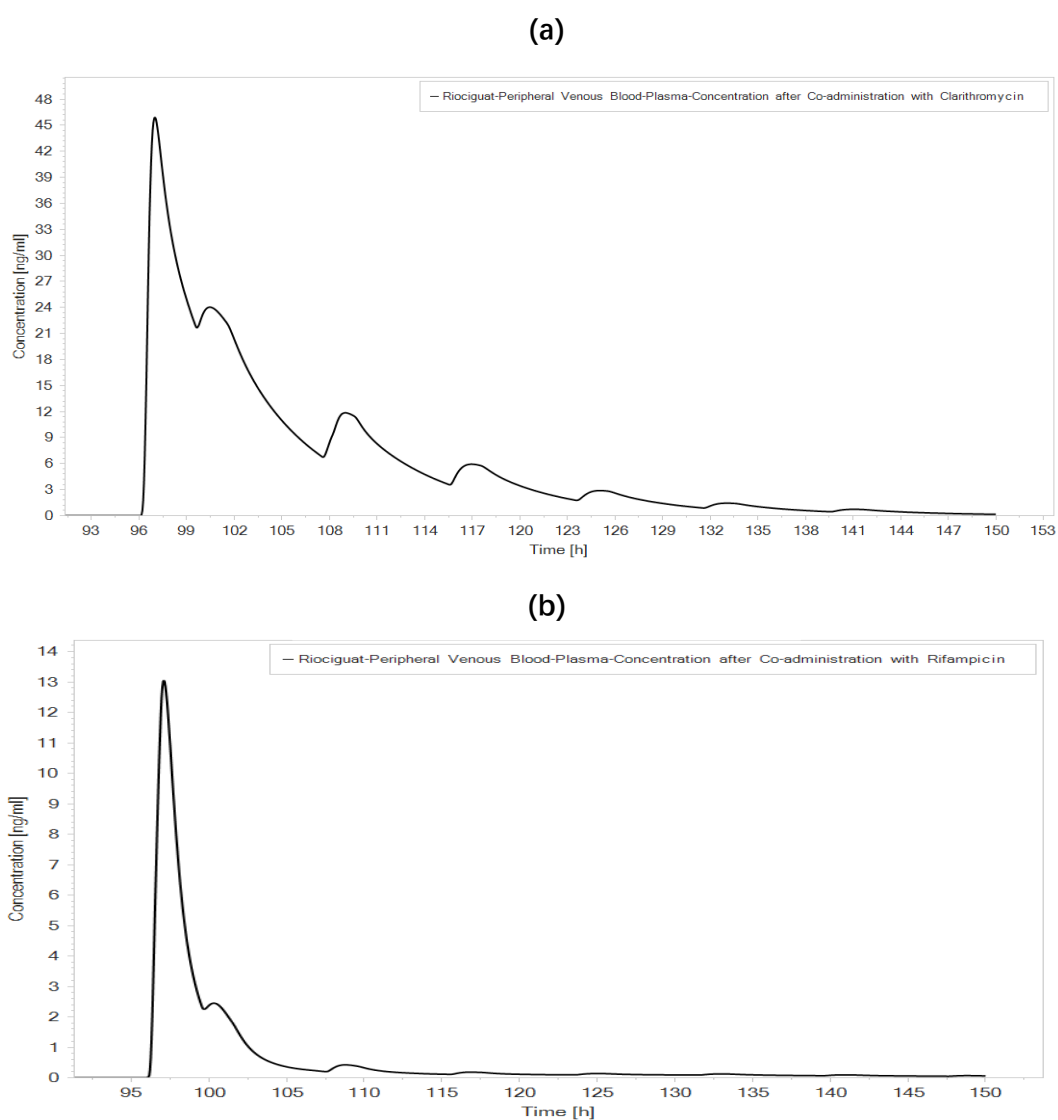


**Figure 7. PBPK-Simulated plasma concentration–time profiles of riociguat following a single oral 1 mg dose after 4 days of pretreatment with (A) clarithromycin or (B) rifampicin**

**Table 7. Pharmacokinetic parameters of riociguat alone and in combination with clarithromycin or rifampicin**

| Treatment                     | AUC <sub>inf</sub><br>( $\mu\text{g}\cdot\text{h/L}$ ) | C <sub>max</sub><br>( $\mu\text{g/L}$ ) | T <sub>max</sub><br>(h) | AUC <sub>inf</sub> ratio vs.<br>alone (%) | C <sub>max</sub> ratio vs.<br>alone (%) |
|-------------------------------|--------------------------------------------------------|-----------------------------------------|-------------------------|-------------------------------------------|-----------------------------------------|
| Riociguat alone               | 250.08                                                 | 36.59                                   | 1.05                    | 100                                       | 100.00                                  |
| Riociguat +<br>Clarithromycin | 343.84                                                 | 45.93                                   | 1.00                    | 137.5                                     | 125.50                                  |
| Riociguat<br>+Rifampicin      | 35.34                                                  | 12.83                                   | 1.10                    | 14.1                                      | 35.10                                   |

## 4. Discussion

Riociguat, as a targeted therapy for pulmonary hypertension, has been shown in clinical trials to significantly improve exercise capacity, hemodynamics, and functional class, reduce NT-proBNP levels, and decrease the incidence of clinical worsening events in patients with PAH <sup>[32]</sup>. Although riociguat is now widely used in clinical practice, not all of its pharmacokinetic characteristics have been fully elucidated, particularly in patients with hepatic impairment. In this study, we developed a PBPK model for riociguat, leveraging multiple clinical studies with riociguat plasma concentration data to enhance the verification and predictive capability of the model. Although PBPK modeling is more mechanistic than classical pharmacokinetic modeling <sup>[33]</sup>, existing PBPK models still have certain limitations: the absorption process is described using a first-order absorption model, rather than a more complex mechanism-based model incorporating solubility, particle size, permeability, and gastrointestinal physiological characteristics <sup>[34]</sup>. In this study, we successfully predicted the plasma concentration–time profiles of riociguat under different dosing regimens and, at the same time, introduced EHC to better fit the double-peak phenomenon observed in clinical trials for riociguat and its metabolites. Riociguat is eliminated via both biliary and renal routes. After biliary secretion into the intestine, a portion of the drug is reabsorbed via EHC and returns to the systemic circulation, a process that directly contributes to the pronounced tailing of the terminal elimination phase observed in clinically measured concentration–time curves, and consequently leads to an apparent prolongation of the terminal half-life. Furthermore, since the exact meal times of the study participants were unknown, we assumed that meals occurred at 3 h post-dose and every 8 h thereafter to simulate normal meal patterns; however, this may not perfectly reflect the actual meal schedules of clinical trial participants, potentially contributing to discrepancies between the PBPK curves and observed values. Previous studies have shown that changes in drug-metabolizing enzyme activity and hepatic blood flow under conditions of hepatic impairment can affect the pharmacokinetic behavior of

drugs<sup>[35]</sup>. The underlying reason is that hepatic impairment may reduce the liver's capacity for drug uptake and metabolism; therefore, the hepatic metabolic process of riociguat may vary with liver function status. In this study, we employed a "top-down" physiological parameter adjustment approach to construct virtual hepatic impairment populations. Based on Child-Pugh classification, changes in parameters including hepatic blood flow, portal venous blood flow, functional liver volume, hepatic CYP450 enzyme abundance, plasma albumin, hematocrit, and glomerular filtration rate relative to healthy adults were incorporated to systematically adjust the healthy physiological parameters, generating two physiological parameter sets corresponding to mild and moderate hepatic impairment. Subsequently, the adjusted physiological parameters were input into PK-Sim to construct virtual populations at each severity level and establish the corresponding PBPK models. Finally, the model was externally validated using published clinical pharmacokinetic data from hepatically impaired patients to ensure model reliability and accuracy. According to the study by Frey et al.<sup>[11]</sup>, in Child-Pugh B patients who received a single oral dose of riociguat 1.0 mg, the total exposure ( $AUC_{inf}$ ) of riociguat increased by approximately 50%, which was statistically significant; whereas in Child-Pugh A patients, total drug exposure was similar to, though slightly higher than, that in the control group. For the metabolite M1, although hepatic impairment may slow its formation and elimination, the overall exposure ( $AUC_{inf}$ ) showed only minor differences among the groups. Using the PBPK model, the predicted riociguat exposure in mild and moderate hepatic impairment increased by 12% and 36%, respectively, compared with healthy individuals, which is consistent with the observed trends. Considering the considerable variability in hepatic dysfunction among patients with the same Child-Pugh score, the PBPK model predictions for hepatically impaired patients can be considered reasonably reliable.

When applying the model to predict drug exposure in hepatic impairment populations, several important limitations should be noted. First, the majority of the base modeling data were derived from male subjects; therefore, the predictive extrapolability of the model to female patients is limited. Second, the age of the modeling individuals (30 years) was substantially lower than that of the subjects in hepatic impairment studies (35–70 years). Furthermore,

plasma protein levels may vary across different disease states, which in turn may affect the unbound drug fraction<sup>[36]</sup>. For highly protein-bound drugs such as riociguat, even small changes in the unbound fraction may have a significant impact on drug exposure due to the large bound proportion. Sensitivity analysis also confirmed that the unbound fraction of riociguat has an important influence on its plasma exposure and peak concentration ( $C_{max}$ ). Therefore, actual measurement of the unbound fraction in patients with cirrhosis may improve the predictive accuracy of riociguat pharmacokinetics. Additionally, the use of GetData Graph Digitizer (Version 2.20) to extract plasma concentration–time data from published figures inevitably introduces some deviation from the original clinical trial data, which represents an important source of modeling error. Finally, due to the lack of clinical data on the unbound fraction of riociguat in Child-Pugh C patients, we were unable to construct a PBPK model for this population; development of such a model remains contingent upon further clinical investigation.

When investigating the DDI profile of riociguat, we simulated the pharmacokinetic changes following co-administration of riociguat with clarithromycin and rifampicin using the established PBPK model. The simulation results showed that clarithromycin, through dual mechanisms—inhibition of CYP3A4-mediated metabolic clearance and inhibition of P-gp/BCRP-mediated biliary efflux—increased the AUC of a single oral 1.0 mg dose of riociguat by 37.5%, which is in close agreement with the clinically observed value of 41.5% (90% CI: 22.9%–62.9%) reported by Becker et al. in healthy subjects, confirming the model's reliable predictive capability for complex DDI scenarios involving multiple mechanisms. In contrast, rifampicin, by inducing CYP3A4 enzyme activity, accelerated the oxidative metabolic clearance of riociguat, and was predicted to reduce the AUC to 14.1% of the control value (i.e., an 85.9% reduction). Although dedicated clinical studies on the interaction between rifampicin and riociguat are currently lacking, this simulation result is consistent with the precautionary information regarding strong CYP3A4 inducers in the riociguat prescribing information, suggesting a significant risk of reduced efficacy upon co-administration, thus warranting close monitoring of clinical response and consideration of alternative therapeutic options. Collectively, these findings demonstrate that the PBPK model

developed in this study successfully integrates the multiple elimination mechanisms mediated by metabolic enzymes and transporters for riociguat. The prediction error for the clarithromycin DDI was less than 4 percentage points, fully validating the model's predictive reliability in complex DDI settings. Meanwhile, for strong inducers such as rifampicin for which clinical DDI data are not yet available, the model offers a mechanism-based quantitative prediction approach that may, to some extent, compensate for the lack of clinical evidence<sup>[37]</sup>. As more clinical DDI data accumulate in the future, the model can be further optimized and validated to provide more comprehensive decision support for the clinical management of riociguat combination therapy.

## 5. Conclusion

In this study, we successfully developed the first physiologically based pharmacokinetic model for riociguat that integrates EHC mechanisms, and systematically evaluated its predictive performance in healthy individuals, hepatically impaired patients, and drug–drug interaction scenarios. The model adequately described the plasma concentration–time profiles of riociguat and its active metabolite M1 over the 0.5–2.5 mg dose range. In external validation, more than 90% of predicted values fell within the 0.5- to 2-fold error range of observed values, demonstrating excellent predictive accuracy and robustness. The model also successfully simulated food effects, predicting that postprandial  $C_{\max}$  decreased to 53.4% of fasting levels and  $T_{\max}$  was prolonged from 1.05 h to 3.40 h, which is generally consistent with clinically reported values ( $C_{\max}$  reduced to 64.7% of control and  $T_{\max}$  prolonged to 4 h), further confirming its reliable description of absorption kinetics. Virtual hepatic impairment populations constructed using the “top-down” physiological parameter adjustment approach predicted that following a single oral 1 mg dose of riociguat, drug exposure ( $AUC_{\text{inf}}$ ) increased by approximately 12% and 36% in Child-Pugh A and B patients, respectively, compared with healthy individuals, consistent with observed clinical trends and suggesting that dose adjustment may be warranted in patients with moderate hepatic impairment. Drug–drug interaction simulations showed that clarithromycin (a CYP3A4/P-gp/BCRP inhibitor) increased riociguat  $AUC_{\text{inf}}$  by 37.5%, in close agreement with the reported value of 41.5%,

whereas rifampicin (a CYP3A4 inducer) reduced AUC<sub>inf</sub> by 85.9%, indicating that close monitoring of plasma concentrations and clinical response is advisable during co-administration.

In summary, this PBPK model provides a reliable mechanistic platform for dose optimization in special populations such as hepatic impairment and for DDI risk assessment of riociguat, with clear clinical translational value. Future studies incorporating data from Child-Pugh C patients, as well as physiological parameters from female and elderly populations, will further expand the applicability and generalizability of the model.

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