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ARTICLE

PBPK modeling of recombinant factor IX Fc fusion protein (rFIXFc) and rFIX to characterize the binding to type 4 collagen in the extravascular space

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Abstract

Patients with severe and sometimes moderate hemophilia B are prophylactically treated with factor IX concentrates to prevent bleeding. For some time now, various extended terminal half-life (EHL) recombinant factor IX concentrates are available allowing less frequent administration during prophylaxis in comparison to standard half-life recombinant FIX (rFIX). Especially, recombinant FIX-Fc fusion protein (rFIXFc; Alprolix®) exhibits a rapid distribution phase, potentially due to binding to type IV collagen (Col4) in the extravascular space. Studies suggest that the presence of extravascular rFIXFc is protective against bleeding as without measurable FIX activity in plasma, and no extra bleeding seems to occur. The physiologically based pharmacokinetic (PBPK) model for rFIXFc which we describe in this study, is able to accurately predict the observed concentration-time profiles of rFIXFc in plasma and is able to quantify the binding of rFIXFc to Col4 in the extravascular space after an intravenous dose of 50 IU/kg rFIXFc in a male population. Our model predicts that the total AUC of rFIXFc bound to Col4 in the extravascular space is approximately 19 times higher compared to the AUC of rFIXFc in plasma. This suggests that rFIXFc present in the extravascular compartment may play an important role in achieving hemostasis after rFIXFc administration. Further studies on extravascular distribution of rFIXFc and the distribution profile of other EHL-FIX concentrates are needed to evaluate the predictions of our PBPK model and to investigate its clinical relevance.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The extravascular concentration pool of factor IX might play a crucial role in the hemostasis.

WHAT QUESTION DID THIS STUDY ADDRESS?

Can we quantify the binding of FIX in the extravascular spaces when patients received recombinant factor IX Fc fusion protein (rFIXFc) using PBPK modeling?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The AUC of extravascular rFIXFc was approximately 19 times higher compared to the AUC of rFIXFc in plasma.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

No extra bleeding seems to occur in the absence of rFIXFc plasma concentrations, which potentially can be attributed to the high extravascular rFIXFc concentrations. With this information, we might be able to further optimize treatment in patients with hemophilia B.

INTRODUCTION

Hemophilia B patients are characterized by a deficiency of coagulation factor IX (FIX) resulting in bleeding, typically in joints and muscles.[1] Patients with severe and sometimes moderate hemophilia B receive FIX prophylaxis to prevent bleeding by maintaining a plasma FIX trough level of at least >1 IU/dL.[2] For some time now, biochemically modified FIX concentrates with extended terminal half-lives (EHL-FIX concentrates) are available for prophylaxis.[3] One of these concentrates consists of recombinant FIX coupled with the human IgG1 Fc domain (rFIXFc; Alprolix®). Compared to standard terminal half-life recombinant FIX concentrate (rFIX; Benefix), rFIXFc has been shown to have a four to five times longer terminal half-life,[3] resulting in less frequent dosing to maintain target FIX trough concentrations, thereby improving patient burden of administration frequency and quality of life.

Especially, rFIXFc has been demonstrated to exhibit a rapid distribution phase, which is possibly due to binding to the neonatal Fc-receptor (FcRn) and to type IV collagen (Col4) in the extravascular space (Figure 1). Col4 is a major component of the basement membrane, which is a differentiated extravascular space that provides support and structural integrity to various tissues and organs, including blood vessels.[4] Endothelial cells line the inner surface of blood vessels and form a continuous layer known as the endothelium. The basement membrane, which lies beneath the endothelium, is composed of various components, including Col4.[5] The extravascular concentration pool of both rFIX and rFIXFc is approximately 17–20 times greater compared to plasma according to studies[6,7] Studies suggest that the presence of extravascular rFIXFc is protective against bleeding as without measurable FIX activity in plasma, and no extra bleeding seems to occur.[7] Despite these findings, current empirical population pharmacokinetic (PK) models for FIX only describe FIX

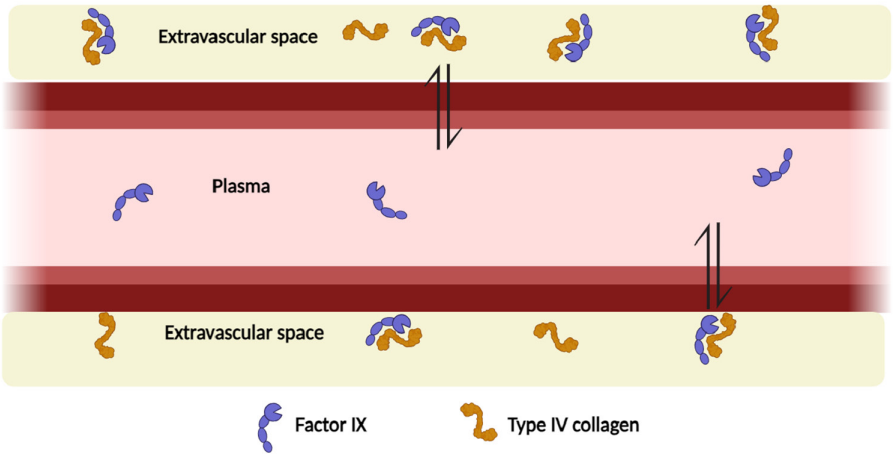

FIGURE 1 Schematic representation of a blood vessel showing the distribution of Factor IX (FIX) in plasma and the extravascular space. FIX circulates in plasma. In the extravascular space, FIX binds to Type 4 collagen in the extracellular matrix.

concentrations over time in plasma, as FIX concentrations are typically measured in the blood and not in the extravascular space.[3] Therefore, a better understanding of the distribution and PK of rFIXFc in the extravascular space is important for optimization of the treatment of hemophilia B patients with this and other EHL-concentrates.

Physiologically based pharmacokinetic (PBPK) modeling is a powerful tool that allows for the prediction of drug distribution and PK in various tissues, including the extravascular space.[8] Unlike empirical population PK models, PBPK models are based on mechanistic understanding of drug absorption, distribution, metabolism, and elimination in the body, and can take into account physiological factors and physicochemical characteristics of the drug. Therefore, PBPK modeling offers a potential solution to the current limitations of FIX PK modeling, allowing for the prediction of rFIXFc distribution and PK in both plasma and extravascular space.

In this study, we aim to develop a PBPK model for rFIXFc using the PK-Sim platform. Our goal is to predict the PK of rFIXFc in plasma and investigate the distribution and binding of rFIXFc to Col4 in the extravascular space of various tissues. By exploring the distribution and PK of rFIXFc in the extravascular space, we will gain new insights into the potential impact of these factors on the hemostatic characteristics of the drug. Additionally, we aim to assess the applicability of the developed PBPK model for other FIX concentrates such as rFIX. With this study, we hope to begin to acquire a better understanding of the most optimal use of EHL-FIX concentrates in people living with hemophilia B.

METHODS

Software

The whole-body scale PBPK modeling of rFIXFc and rFIX was performed using PK-Sim (version 11 – build 150, Open Systems Pharmacology).[9] Plasma concentration-time curve data for rFIX were obtained from literature using GetData Graph Digitizer (Version 2.26.0.20). Data analysis and graphics were performed with the R (Version 4.1.1) and R Studio (version 1.4.1717).

PBPK model development of rFIXFc

The base model for large molecule drugs in the software package has been described previously.[8] Fifteen organs were included in the model structure to represent a virtual human. Each tissue compartment is subdivided into a plasma compartment, vascular endothelium compartment, an endosomal compartment, an interstitial fluid or extravascular compartment, and an intracellular space compartment (Figure 2). In the endosomal compartment, a generic mechanistic model for FcRn-drug binding and complex recycling was included. The transit of drugs around the body and between organs is mediated via plasma flow into tissues and then returned via plasma flow except for the portion undergoing lymphatic drainage into a lymph node compartment, which then returns back to plasma. More details about the PBPK model and mass transfer process can be found in the supplementary. Moreover, an abbreviation list is included in the supplement.

For development of the PBPK model for rFIXFc, physicochemical properties and clinical observations of rFIXFc were collected from published literature and the European public assessment report (EPAR).[10]

Using the PK-Sim software, we developed a PBPK model for rFIXFc in a virtual Caucasian male patient of 30 years with a height of 176 cm and body weight of 73 kg receiving an intravenous (IV) dose of 50 IU/kg rFIXFc (approximately 0.6 mg/kg).[10] According to the EPAR specification, 1 mg of rFIXFc contains 55–84 IU rFIXFc activity. In our model, we used 1 IU of rFIXFc activity corresponding with 12 µg of protein. Concentrations of rFIXFc activity are expressed in nanomolar (nM); molecular weight of rFIXFc is 98 kDa, Therefore, in our model, we used 1 IU/mL of rFIXFc is equal to 122 nM. However, FIX has a normal activity of 90 nM,[11] therefore we opted for the conversion of 1 IU/mL of rFIXFc is equal to 122 nM, as this was the closest to the FIX activity of 90 nM.

To account for the rapid distribution phase of rFIXFc, we incorporated binding to Col4 in the extravascular space. The tissue expression distribution of Col4 (relative expression amount) in the model was informed by the PK-Sim® expression database based on array profiles.[12] It has been reported that FIX may also bind to the vascular endothelium.[13] Therefore, we also included a binding site for rFIXFc in the vascular space as a vascular endothelium binding partner (VEBP) to replicate the mechanics of binding to vascular endothelium. A generic enzyme was added to the model to simulate the degradation of rFIXFc in plasma. The reaction was implemented as first-order reaction described by the catalytic rate constant Kcat. Moreover, we incorporated the FcRn pathway into our model to account for the recycling of rFIXFc through these receptors. The model parameters related to the binding of rFIXFc to Col4 and VEBP, available Col4 and VEBP in tissues, enzymatic degradation, and recycling through FcRn receptors were calibrated using observed clinical data of rFIXFc in plasma. Functionalities provided in PK-Sim

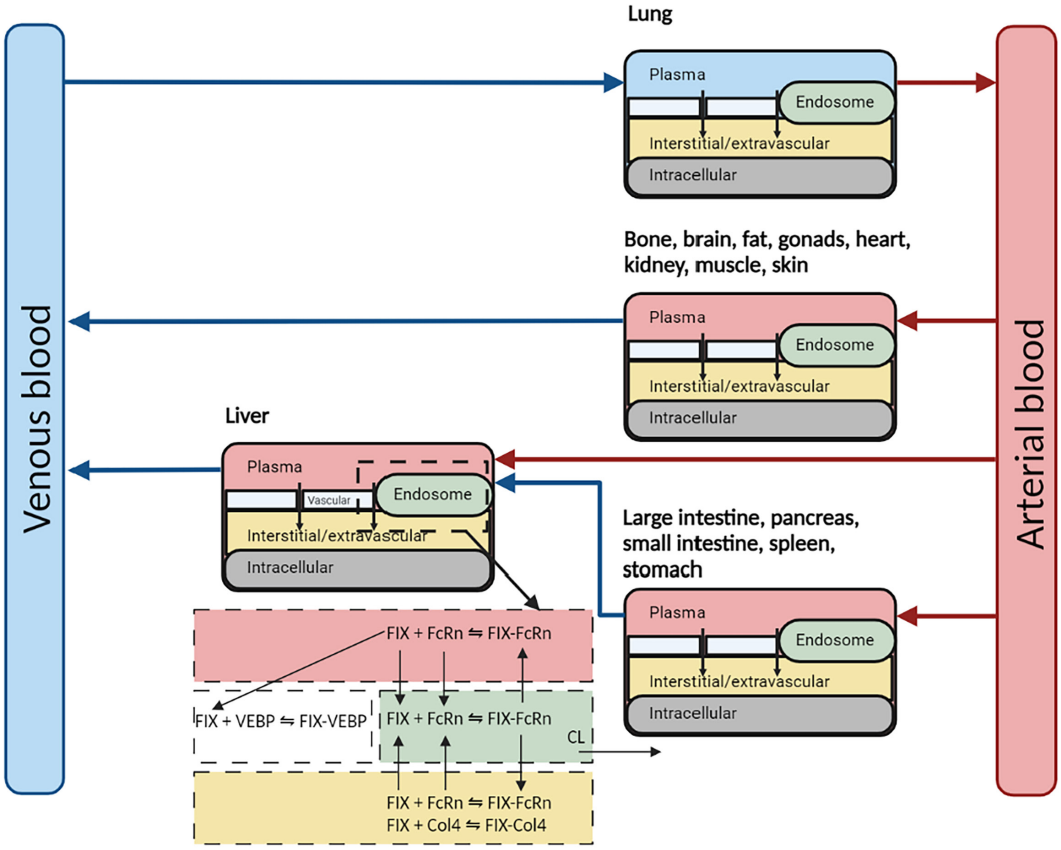

FIGURE 2 Schematic overview of the physiologically based pharmacokinetic model. Each of the organ compartments consist of a vascular space (red or blue), containing blood serum and blood cells, an endothelial barrier (white, interrupted by small and large pores), interstitial/extravascular space (yellow), which contains the type 4 collagen (Col4). Factor IX (FIX) is able to bind reversibly to the Col4 in the extravascular space and FIX has binding partner in the vascular endothelium (VEBP). In the endosomal space (green), the reversible binding of rFIXFc to the neonatal Fc receptor (FcRn) is included.

for model parameter estimation (Monte-Carlo optimization method) and local sensitivity analysis were applied. A detailed description of the sensitivity analysis is provided in the supplement.

Model verification

To verify the accuracy of our PBPK model, we compared the simulated rFIXFc PK profiles with the observed clinical data from the EPAR.[10] A virtual male population was generated, consisting of 1000 subjects ranging in age from 12 to 60 years. Each individual in the population was characterized by their height, weight and BMI. The population predictions were plotted as the median with a 95% prediction interval. If the observed concentrations and its standard deviation fall within the 95% prediction interval in the majority of calculations, it indicates that the model provides a reasonable estimate of the observed data variability.

Applicability of developed PBPK model for rFIX

To assess the applicability of the developed PBPK model for other FIX concentrates such as rFIX, we predict plasma rFIX concentrations after IV administration of rFIX. In the rFIXFc model, the FcRn pathway was disabled and the molecular weight was adjusted to 55 kDa for rFIX.[14] Here, we aim to understand the impact of FcRn-mediated recycling to the PK of rFIXFc. If the model accurately predicts the PK of rFIX when disabling FcRn recycling, then it indicates that other parameters and mechanisms incorporated in the model (aside from FcRn recycling) are sufficient to predict the PK of rFIXFc.

The predicted rFIX plasma concentrations were then compared by using observed clinical data provided by Suzuki et al[15] where patients received a median dose of 55 IU/kg of rFIX (approximately 0.25 mg/kg, since 1 mg of rFIX contains at least 200 IU/dL rFIX activity).[16] The model performance was assessed in which the observed

clinical data had to be within a two-fold error of the PBPK model prediction.

Prediction of extravascular rFIXFc concentration in major tissues

To further understand the PK behavioral characteristics of rFIXFc in the extravascular tissues, we simulated the extravascular rFIXFc binding to Col4 over time of 50 IU/kg rFIXFc in major organs in a Caucasian male patient of 30 years with a height of 176 cm and body weight of 73 kg. The extravascular tissues concentrations of major organs in the human body were simulated, including bone, brain, gonads, heart, intestinal mucosa, (small and large) intestines, kidney, liver, lung, muscle, pancreas, skin, spleen and stomach.

RESULTS

PBPK modeling and model evaluation

A PBPK model was developed for rFIXFc using the base model for large molecules in PK-Sim with modifications to feature the binding of rFIXFc to Col4. Figure S1 displays a schematic overview of the model development. The model was optimized by fitting the model to the reference plasma concentration-time profiles through estimation of drug-specific parameters relevant for the elimination and distribution.

Parameter sensitivity analysis

A sensitivity analysis was performed to evaluate model parameters that influence on the PK of rFIXFc in plasma. In summary, the total body clearance was most sensitive to Kcat and to a lesser degree the binding to FcRn, while the hydrodynamic radius was the most influential on the volume of distribution. Albeit important for the overall model performance, the binding to Col4 and VEBP was identified to have less influence on the plasma PK of rFIXFc. Further information and details on the sensitivity analysis method and results are displayed in the supplement and Figure S2.

Simulations of rFIXFc and rFIX in plasma

Observations and simulated rFIXFc plasma concentration-time profiles using the final model are shown in Figure 3. We simulated 1000 virtual males with ages ranging from 12 to 60 years that matched the population of the observed clinical trial.[10] In Figure 3a, the

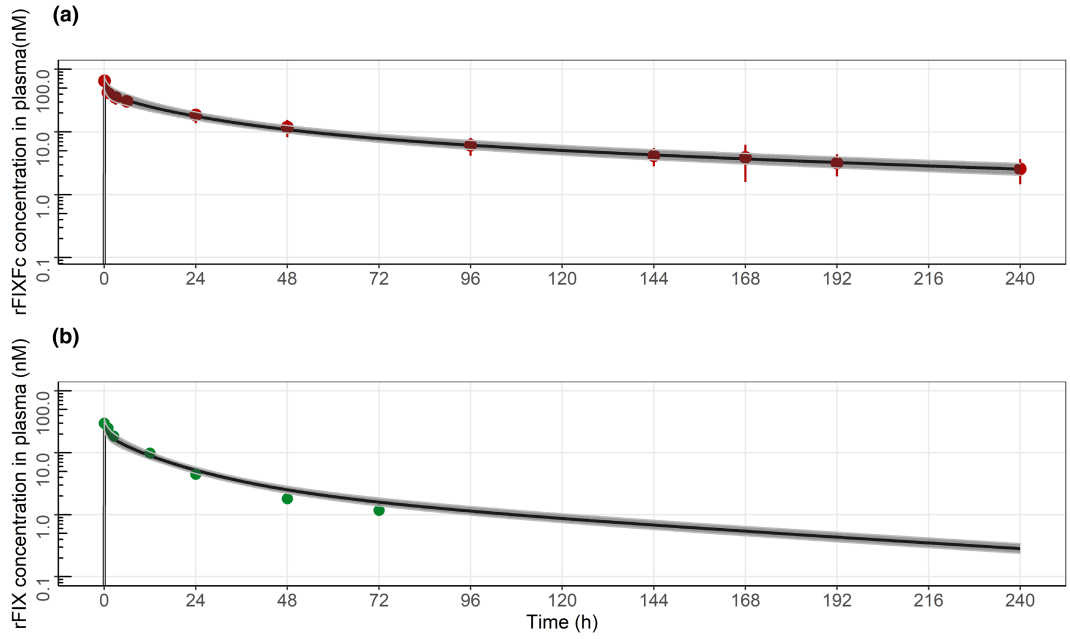

FIGURE 3 Population predicted of rFIXFc and rFIX concentrations in plasma over time. (a) Observed rFIXFc and predicted rFIXFc concentrations in plasma over time in a virtual male population of 12–60 years old after single bolus dose of 50 IU/kg (0.6 mg/kg). Observations were obtained from the EPAR[10] (b) Observed rFIX and predicted rFIX concentrations in plasma over time in a virtual male population 12–60 years old on after single bolus dose of 50 IU/kg (0.25 mg/kg). Observations were obtained from Suzuki et al.[15] V Black solid line: population predictions, red circles: median observed rFIXFc concentrations and standard deviation, green circles: median observed rFIX concentrations, gray shaded area: 95% prediction interval.

TABLE 1 Parameters of the final PBPK model.

Parameter	Final value	Reference
rFIXFc		
Molecular weight	98 kDa	Drugbank
Hydrodynamic radius	3.6 nm	Estimated
K_D^{FcRn}	1.42 nM	Estimated
K_D^{Col4}	5.26 μM	Estimated
K_D^{VEBP}	0.04 μM	Estimated
rFIX[1]		
Molecular weight	55 kDa	Drugbank
Hydrodynamic radius	3.4 nm	Estimated with inbuild calculator
Binding partners (concentration)		
Collagen IV extravascular	3.83 μM	Estimated
Endothelium vascular	20.0 μM	Estimated
Enzyme		
Enzymatic activity (Kcat)	0.061 1/day	Estimated

Note: The K_D^{FcRn} for rFIX was set to 9999 M, indicating no binding to the FcRn receptor. Values for K_D^{Col4} and K_D^{VEBP} were the same as for rFIXFc. rFIXFc = Alprolix®.

Abbreviations: K_D^{FcRn}, Dissociation constant for FcRn binding in endosomal space; K_D^{Col4}, Dissociation constant for binding in extravascular space; K_D^{VEBP}, Dissociation constant for vascular endothelium binding partner (VEBP).

observed rFIXFc and predicted rFIXFc concentrations in plasma over time after single bolus dose of 50 IU/kg (0.6 mg/kg) in a virtual male population are displayed. After refining the model, the observed rFIXFc concentrations were adequately predicted by the model. Table 1 summarizes the model parameters that were used and estimated in the final PBPK model. The observed rFIXFc concentrations and most of its standard deviations are within the 95% prediction interval. This shows that the PBPK model is able to the predict rFIXFc concentrations in a male population between 12 and 60 years old. For rFIXFc, a CL of 2.49 mL/h/kg and a Vss of 370 mL/kg was obtained. The calculated terminal half-life was 142 h. The binding of rFIXFc to the FcRn receptor played a crucial role in accurately characterizing the concentration profiles of rFIXFc in plasma, as the Kd to Fc-Rn receptor was estimated as 1.42 nM.

To assess the applicability of the developed PBPK model for rFIX, we disabled the FcRn recycling mechanism and used the molecular weight to rFIX (55 kDa), in all other respect the same parameterization was applied. In Figure 3b, the median observed rFIX and predicted rFIX concentrations in plasma over time in a virtual male population of 12–60 years is displayed. Most median observed rFIX concentrations are within the 95% prediction interval, although the median observed rFIX concentrations at $t = 48$ and 72 h are somewhat lower compared to the model prediction. For rFIX, we obtained a CL of 7.51 mL/h/kg and a Vss of 339 mL/kg, while the terminal half-life was 44 h.

PBPK model predictions of rFIXFc concentrations in tissues of extravascular space

The rFIXFc PBPK model quantified the binding of rFIXFc to Col4 in the extravascular space. Figure 4 displays the extravascular rFIXFc concentrations in tissues and Table 2 displayed the PK parameters in plasma and tissues. The results showed that area under the curve (AUC) of rFIXFc to Col4 in the extravascular space was approximately 19 times higher compared to the plasma concentration. The highest concentrations of extravascular rFIXFc bound to Col4 were found in the spleen and lungs. The peak rFIXFc concentrations and AUC in the extravascular spleen and lungs were 154 and 26.2 nmol, and 10.1 and 7.9 μmol*h/L, respectively. The peak rFIXFc concentrations in the extravascular lungs were lower compared in the extravascular spleen. It is important to note that the concentration of rFIXFc in the extravascular space of the lungs decreased very slowly over time, resulting in a high AUC. Moreover, the extravascular rFIXFc peak in the spleen is higher (154 nmol) compared to the plasma rFIXFc peak (61 nmol).

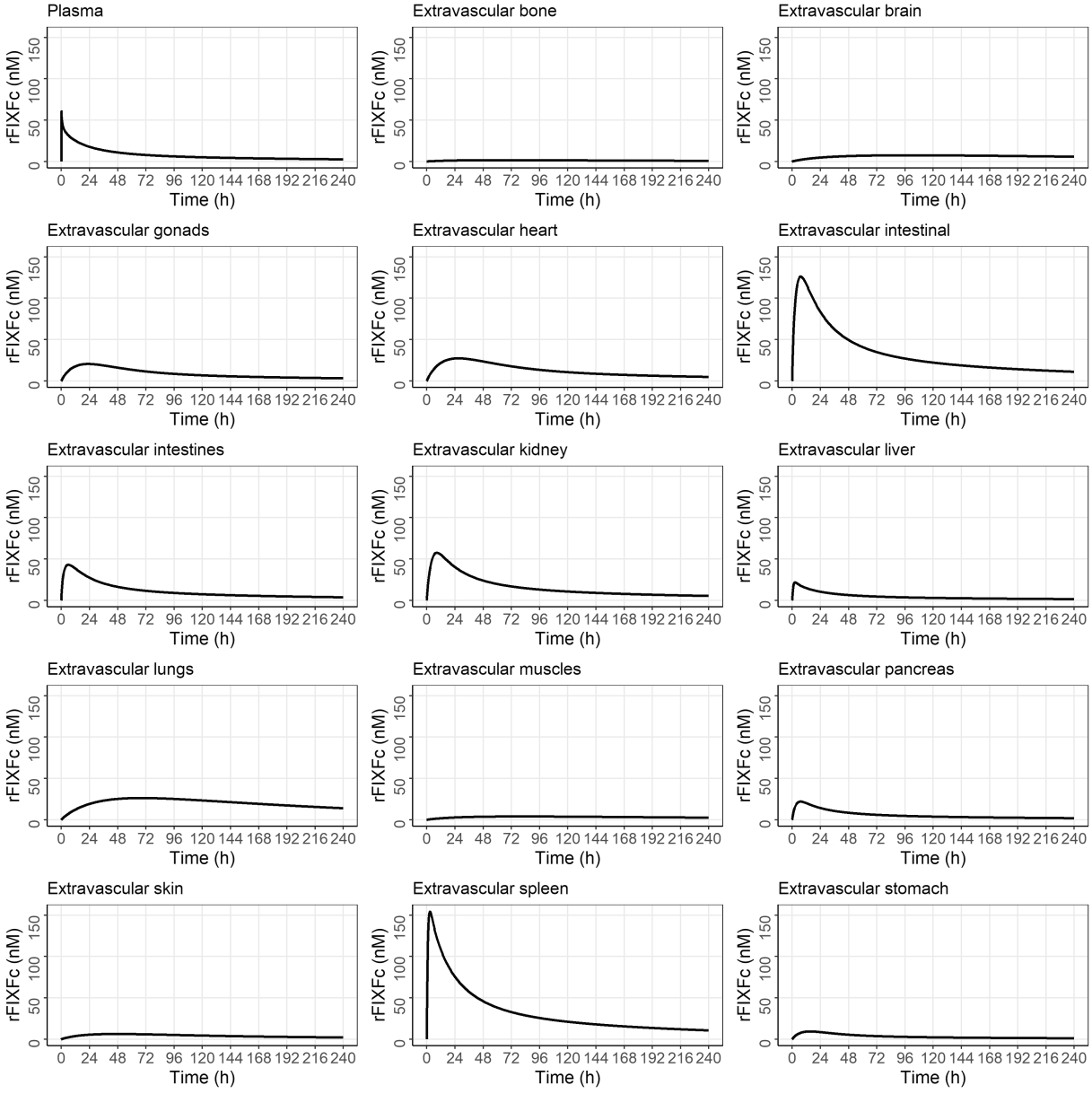

FIGURE 4 Predicted rFIXFc concentration over time in plasma and extravascular tissues after an intravenous bolus of 50 IU/kg rFIXFc. Black solid line: predicted population median curve.

DISCUSSION

PBPK modeling is a valuable tool for assessing drug distribution in various tissues and organs. It can be applied to predict drug distribution in previously unexplored scenarios, such as binding in extravascular spaces of tissues, with the aim of enhancing our understanding of potential associations between extravascular concentrations and drug effects. In this study, we developed a PBPK model for rFIXFc. Our model was able to predict the PK of rFIXFc in plasma. We also quantified the binding of rFIXFc to Col4 in the extravascular space of various tissues. Moreover, the rFIXFc model was able to predict the plasma concentration-time profile of rFIX.

Previous studies have acknowledged the potential importance of extravascular rFIXFc for hemostasis and protection form bleeding.[17,18] However, to the best of our knowledge, no studies have quantified the concentration of rFIXFc in the extravascular space of tissues in a human population. Our study provides novel insights in this area by predicting that the AUC of rFIXFc in the extravascular space of tissues is approximately 19 times higher than in plasma, suggesting that extravascular rFIXFc plays a significant role in the local control of bleeding. Our study also revealed the highest AUC of extravascular rFIXFc to be present in the spleen, lungs, intestinal mucosa, and kidney. These higher tissue concentrations of rFIXFc suggests that they may be

TABLE 2 Plasma and tissue exposure after 50 IU/kg rFIXFc.

Parameter	AUC (0–>240 h) (nmol × h/L)	Peak rFIXFc (nM)	T_{max} (h)
rFIXFc intravascular			
Plasma	2455	60.9	0.05
rFIXFc extravascular			
Bone	431.5	1.557	76.5
Brain	3601	7.430	94.00
Gonads	2715	20.46	20.50
Heart	3996	27.28	26.50
Intestinal mucosa	17,549	27.28	8.00
Intestines	3996	210.6	5.75
Kidney	4952	57.55	9.50
Liver	1402	21.84	2.25
Lungs	7933	26.22	79.50
Muscles	1421	3.921	81.25
Pancreas	1785	22.05	6.75
Skin	1340	6.247	47.50
Spleen	10,131	154.4	2.25
Stomach	998.2	9.320	14.75
Total rFIXFc in extravascular tissues	49,052	–	–

Note: rFIXFc = Alprolix®.

Abbreviations: AUC, area under the concentration versus time curve; C_{max}, maximum concentration; T_{max}, time to reach maximum concentration.

particularly responsive to treatment with rFIXFc, and that the drug may have a more pronounced effect on hemostasis in these tissues compared to other organs with lower concentrations of extravascular rFIXFc.

Previous studies investigated the distribution of rFIX and rFIXFc concentrations in animals. Van der Flier et al. observed that both rFIX and rFIXFc distributes outside the plasma compartment in certain tissues at higher plasma levels, indicating that rFIX and rFIXFc act similar.[19] They found highly perfused tissues such as the heart, liver, and lungs signaled a high extravascular level of rFIXFc. While Herrmann et al. found that the highest homogenate plasma FIX level was in the liver, kidney, and lungs in rodents, with a high homogenate plasma FIX level also observed in the lungs after 72 h.[20] Our study similarly describes a high level of rFIXFc in the extravascular space of the lungs, not only supporting the potential importance of extravascular rFIXFc for local hemostatic control but also verifying the accuracy and applicability of our PBPK model. However, our PBPK model also predicted the highest extravascular rFIXFc concentration in the spleen. Contrastingly, Herzog et al. found the highest distribution of rFIX to be in the liver, while the distribution to the spleen was much lower compared to the liver.[21]

In our PBPK model for rFIXFc, the distribution to the extravascular space in the liver was much lower compared to the distribution to other organs. The expression distribution of Col4 (relative expression amount) was provided by the PK-Sim® expression database based on array profiles.

The relative expression of Col4 was widely distributed in most organs of humans, with highest expression occurs in the spleen, followed by the lungs, which is reflected by the predictions made by the PBPK model. However, clinical measurements were only obtained from human plasma and not from human extravascular space. Van der Flier et al. used single-photon emission computed tomography/computed tomography (SPECT/CT) technique to investigate the bio distribution of rFIXFc in mice. These SPECT/CT images show the distribution of rFIXFc in various organs. However, we cannot quantify these SPECT/CT images to molar concentrations.[19] Our PBPK model therefore seems to be the only tool now available to estimate the binding of rFIXFc to Col4. Further studies may be needed to investigate the impact of tissue-specific PK on the efficacy and safety of rFIXFc. At low rFIXFc plasma concentrations, extravascular rFIXFc may be particularly important for achieving hemostasis, since it may provide a local source of the drug that can rapidly be available at the site of injury.

The simulated terminal half-life for rFIXFc and rFIX in plasma was calculated as, respectively, 142 h and 44 h. Our PBPK model considered the binding of rFIXFc to FcRn receptors, which enabled us to capture the prolonged half-life effect. This modeling approach allowed us to simulate the PK behavior of rFIXFc and rFIX in a dynamic and mechanistic manner, taking into account relevant physiological processes and receptor interactions.[22] When comparing our findings with the study by Diao et al., Koopman et al. and Björkman et al., half-lives of respectively 82, 88 h for rFIXFc and 22 h for rFIX[23–25] were reported, which are notably lower than our calculations. Tardy et al. observed a median half-life of 50 h for rFIX in a group of patients aged 13–75 years. This half-life closely aligns with our own findings of 44 h. Notably, their study extended the sampling period beyond 72 h, while Björkman et al. restricted sampling to a maximum of 72 h. This divergence in sampling duration may account for the disparity in reported half-life values. Moreover, in our study, PK-Sim calculated the half-life based on the terminal 10% of the data points, which might also have contributed to the observed discrepancy compared to other reported half-lives.[26] While the previous mentioned studies determined the half-life based on the elimination rate, which provides a different perspective on the PK of rFIXFc and rFIX. As our model accurately describes the dynamics of the terminal phase of the plasma profile, it is obvious that this discrepancy between the simulated and observed half-lives is caused by the different approaches for calculation. Furthermore, it is important to consider the complexity of the PK profile of rFIXFc and the potential limitations of the population PK models used in previous studies.[23,27,28] Many existing population PK models for rFIXFc are based on two- or three-compartment models, which assume distinct phases in the drug concentration-time profile. However, our findings suggest that a three-compartment model may not adequately capture the complete PK behavior of rFIXFc. Therefore, it was necessarily to include an extra binding partner site for rFIXFc to adequately describe the rFIXFc plasma levels. Our PBPK modeling approach allowed us to simulate the PK behavior of rFIXFc and rFIX in a more mechanistic manner. By considering relevant physiological processes and receptor interactions, our model successfully captured the prolonged half-life effect of rFIXFc through its binding to FcRn receptors.

In our PBPK model, we estimated the Kd of rFIXFc to Col4 as 5.3 μM in the extravascular compartment, while the available Col4 concentration in the extravascular compartment was estimated as 3.8 μM. It is important to consider other studies that have investigated the binding affinity of rFIXFc to Col4. One study focused on mice and reported a Kd of 40 nM for rFIXFc, with an available Col4 concentration of 574 nM[7]. We obtained different values regarding the Kd for rFIXFc and the available Col4 concentration, although a direct comparison of mice data to human data is challenging due to species differences. However, an extra binding site of rFIXFc was necessarily to implement in order to describe the rFIXFc over time in plasma adequately. Machado et al.[29] concluded that there might be other binding partners besides Col4 for rFIXFc. In our PBPK model, we included a binding partner in the vascular endothelium site (concentration 13.6 μM) and a Kd of 0.03 μM, which suggested a strong binding of rFIXFc to this binding partner in the vascular endothelium.

Although the developed model shows overall good performance, there are several limitations. Firstly, the model adopts a tissue expression pattern of Col4 binding sites to the extravascular space according to the array profile included in the PK-Sim database, while the actual distribution of Col4 may be slightly different from what is predicted by the array profiles. Secondly, our study did not include a direct comparison of our model predictions with experimental data from the extravascular space of tissues, since only plasma observations were available. Therefore, further validation of our predictions in these tissues is needed. Finally, the binding affinity of rFIXFc to Col4 and the available concentration of Col4 in the tissues was estimated in our PBPK model based on plasma rFIXFc observations. It was notable that the Kd constant for rFIXFc to Col4 was found to be higher compared to the available Col4 in the tissues. Nonetheless, we observed high concentrations of rFIXFc in the extravascular tissues based on parameter estimations. We refined the PBPK model by fitting it to observed data, enabling us to estimate the Kd constant and the available concentration of Col4. It is important to note that the estimation of binding parameters in PBPK models involves simplifications and assumptions. Furthermore, conducting a sensitivity analysis to investigate the parameters that significantly impact predictions is a crucial step in validating a PBPK model. A model is considered reliable if small variations in parameter values result in prediction changes for a dose metric that are within the expected range of its experimental measurement variability.[30] The PBPK model here was shown to be sensitive to parameter changes. The hydronymic radius significantly influences the rate of extravasation, affecting the distribution of the drug between the plasma and extravascular spaces. K_D^{FcRn} showed a medium sensitivity on the clearance. The K_D^{FcRn} has an inverse effect on the clearance as this processes protects the entities from the generic implementation of endosomal clearance.[8] Not including this binding would result in a higher clearance. K_D^{VEBP} and K_D^{Col4} showed, respectively, medium and low sensitivity.

The binding to Col4 and VEBP facilitates a localized distribution in the respective biophases, impacting not only the extravascular but also the intravascular dynamics of the drug. Despite low sensitivity for overall plasma PK, binding processes were necessary to capture the full dynamics in the plasma concentration-time profile. This is also in line with literature highlighting the importance of the binding of rFIXFc to Col4.[6,31,32] Overall, in addition to the biological rationale, these processes improve model performance in terms of capturing plasma concentration dynamics. PBPK models aim to capture the overall behavior of drugs within the body, considering multiple physiological factors and interactions. However, the accuracy and reliability of specific parameter estimates, such as binding affinity, can be influenced by the availability and quality of data, as well as the model assumptions.

In conclusion, our study demonstrates the utility of PBPK modeling for predicting the PK of rFIXFc in plasma and for quantifying the binding of rFIXFc to Col4 in the extravascular space of various tissues. Furthermore, our study highlights the potential importance of extravascular rFIXFc for achieving hemostasis and optimizing treatment in people living with hemophilia B. Future studies should focus on further characterizing the distribution and PK of rFIXFc in the extravascular space of tissues, as well as the potential impact of these factors on the hemostatic effects of the drug. In addition, future studies should investigate other EHL FIX concentrates and their potential binding to Col4.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. M.E.C. and R.A.A.M. designed the research. M.E.C. and E.S. performed the research. M.E.C., E.S. and R.A.A.M. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

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

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