A Systems Pharmacology Model for Predicting Effects of Factor Xa Inhibitors in Healthy Subjects: Assessment of Pharmacokinetics and Binding Kinetics

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Factor Xa (FXa) emerged as a promising target for effective anticoagulation and several FXa inhibitors are now available for the prevention of venous thromboembolism. However, in previously reported pharmacokinetic/pharmacodynamic (PK/PD) models, the complex coagulation processes and detailed information of drug action are usually unclear, which makes it difficult to predict clinical outcome at the drug discovery stage. In this study, a large-scale systems pharmacology model was developed based on several published models and clinical data. It takes into account all pathways of the coagulation network, and captures drug-specific features: plasma pharmacokinetics and drug-target binding kinetics (BKs). We aimed to predict the anticoagulation effects of FXa inhibitors in healthy subjects, and to use this model to compare the effects of compounds with different binding properties. Our model predicts the clotting time and anti-FXa effects and could thus serve as a predictive tool for the anticoagulant potential of a new compound.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC? Although many mathematical models have been developed in the last three decades to represent coagulation system dynamics, none of them was built to be applied to predict clinical coagulation behaviors of FXa inhibitors and to highlight the roles of PKs and drug-target BKs.

WHAT QUESTION DID THIS STUDY ADDRESS? Can we predict drug effects of FXa inhibitors by using the kinetic data from the drug discovery stage? Are BKs strong determinants of drug effects?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE Not only drug and target-related properties, but also the biological system-related parameters and species are strong determinants of drug effects. As such, this might enable us to understand the behavior of the system as a whole.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS The systems pharmacology model may be used to identify and validate targets in the coagulation cascade.

Orthopedic surgery, orthopedic trauma, acute coronary syndromes, and atrial fibrillation are well-documented risk factors for thromboembolic events, such as deep vein thrombosis, pulmonary embolism, and stroke.1 Prophylaxis with an anticoagulant drug can reduce these risks. Vitamin K antagonists, such as warfarin, heparins (including low molecular weight heparins), and parenterally administered direct thrombin inhibitors are used in the clinic to mitigate these risks. However, these anticoagulant therapies have some drawbacks. Warfarin can be administered orally; however, blood monitoring is required, it has a slow onset and offset of action thus rapid intervention is difficult, and is prone to have extensive food and drug interactions.1–3 Heparins have a rapid onset of action but must be administered parenterally and are thus neither convenient nor cost-effective for administration after hospital discharge.

During a thromboembolic event, a dysfunctionality of the coagulation process may result in the formation of an unwanted blood clot. The classic coagulation cascade includes extrinsic, intrinsic, and common pathways, which are assessed by prothrombin time (PT) and activated partial thromboplastin time (aPTT), respectively.5 Factor Xa (FXa) emerged as a promising target for effective anticoagulation because it acts at the convergence point of the intrinsic and extrinsic coagulation pathways. FXa catalyzes the conversion of prothrombin to thrombin6; one molecule of FXa results in the generation of more than 1,000 thrombin molecules.6 Inhibiting FXa may thus block this burst of thrombin-generation and diminish thrombin-mediated activation of coagulation and platelets. Indeed, several FXa inhibitors are now available for the prevention of venous thromboembolism. These direct FXa inhibitors are rivaroxaban (BAY 59-7939), apixaban (BMS-52247-01), and edoxaban (DU-176b). The pharmacokinetic and pharmacodynamic (PK/PD) properties of these drugs have recently been summarized.7 Both apixaban and rivaroxaban have predictable dose-dependent pharmacokinetic (PK) properties. In addition, the bioavailability of apixaban is ~50% and is not significantly influenced by dose or administration with meals. Rivaroxaban’s bioavailability ranges from 66–100%, depending on the dose and whether or not the drug is taken with a meal. Both agents are readily absorbed, achieving maximal plasma concentrations ~3 hours after administration. Protein binding is similar for the two compounds (apixaban ~87%; rivaroxaban ~93%). Apixaban and rivaroxaban also share...
similar pathways of elimination, including metabolism, mainly by cytochrome P450 3A4 (CYP3A4), as well as biliary and renal elimination, and have relatively similar terminal half-lives (apixaban $\sim$12 hours; rivaroxaban $\sim$10 hours). Rivaroxaban is administered once daily (q.d.), twice daily (b.i.d.), or a combination of q.d. and b.i.d., depending on the indication, whereas apixaban is administered b.i.d. for all indications.8

For the last decade, the concept of drug-target residence time or binding kinetics (BKs) has been in focus in the drug discovery and development literature,9 because there is mounting evidence that the often ignored kinetic aspects of the interaction between a small molecule drug and its protein target in the body are highly relevant for in vivo efficacy and clinical success. However, traditional early phase drug design and discovery campaigns often depend on equilibrium affinity (i.e., $K_i$) or structure–activity relationship. This approach does not seem to predict clinical efficacy very well, which is witnessed by the high levels of attrition during the translation of a lead’s in vitro activity into its in vivo and clinical evaluation.10 In addition, compounds with similar PK properties may differentiate their effects, owing to their distinguished binding properties. Drug-target BKs, which represents how quickly drug-target complexes form and dissociate, has not been considered a major contributor to the onset or duration of drug action in vivo as binding events.11,12 It might contribute to the high attrition, which is due to insufficient efficacy ($\sim$51%), whereas the PK issue is seldom reported ($<1\%$).13 To address this issue, our study aims to develop an in silico model for compound selection that is more predictive of clinical outcomes in much earlier phases of drug design and discovery.

The exploration of PKs and BKs of drugs focusing on the duration of effect has been described using a simple PK/PD model.11 In the traditional way of PK/PD modeling, the complex physiological processes involved in the action of drugs are coarse-grained and modeled as a series of interconnected “compartments,” but are not at the molecular level. To date, the study to elucidate the exact role of target BKs on the effects of small molecules is still relatively limited. Systems pharmacology model is a powerful tool for data and knowledge integration for hypothesis testing, as well as for providing a quantitative understanding of a pharmacological target or pathway and for testing “what-if” scenarios that may not be obtained experimentally. Moreover, it has been suggested that innovation may come from combining a systems biology and a PK/PD modeling approach; allowing to perform a quantitative analysis of the dynamic interactions between a drug (or drugs) and a biological system.14 In the present analysis, we aimed to investigate the application of an integrated PK, BK, and systems biology model to predict the anticoagulation effects of FXa inhibitors in healthy subjects and to use this model to compare the effects of FXa inhibitors with different binding properties. The current model predicts the clotting time and anti-FXa effects reasonably well and the role of PKs and BKs was further investigated by sensitivity analysis and simulations. We believe that the current model is a good starting point and has potential to serve as a tool for new compound and/or target selection in the management of coagulation.

**METHODS**

Our model is outlined in Figure 1, which consists of a biological network and a section related to drug action. The biological network is comprised of coagulation pathways (extrinsic, intrinsic, and common pathways of clotting), antithrombotic agents, and the mechanism of fibrinolysis.4,15 The drug-specific parts include the models for PKs and BKs. In summary, the development of the model can be subdivided into four key parts: (1) the use of reported PK models to simulate the plasma drug concentration after multiple-dose treatment16,17; (2) the use of in vitro drug-target BK data to describe the interaction of drug molecules
with their targets,\textsuperscript{18,19} and to predict how fast a drug effect will (dis)appear in a healthy subject; (3) the application of previously published systems biology models and parameters to assess the clotting time (PT and aPTT) and the extent of FXa inhibition; and (4) the definition of between-subject variability in healthy individuals using clinical data from the Leiden Thrombophilia Study.\textsuperscript{20–22}

Pharmacokinetics and pharmacodynamics
The PK data of rivaroxaban was from a total of 43 healthy white male subjects, who were aged 18–45 years and of normal body weight. The subjects received 5 mg rivaroxaban once, twice, or three times daily or 10, 20, or 30 mg rivaroxaban twice daily. The population PK of rivaroxaban was described by an oral, two-compartment model with first-order absorption.\textsuperscript{16} The simulation of drug concentration after multiple doses (5, 10, 20, and 30 mg twice daily) was conducted based on this PK model. Plasma protein binding of rivaroxaban was assumed to be 93\% (i.e., within the reported range of 92–95\% in man).\textsuperscript{23} In the pharmacodynamic study, healthy white male subjects, 20–45 years of age, were assigned to receive rivaroxaban (n = 8 per dose regimen) orally and the dosing regimens were 5, 10, 20, and 30 mg twice daily. The values of PT, aPTT, and the inhibition of FXa activity were evaluated to reflect the effect of rivaroxaban.\textsuperscript{24}

A total of 246 healthy subjects (white, Japanese, and Chinese) were included in the PK study of apixaban, the subjects received doses of 5, 10, and 20 mg in once and twice daily regimens. The final PK model was a two-compartment model with first-order absorption and elimination.\textsuperscript{17} The simulation of apixaban concentrations after multiple doses (2.5, 5, 10, and 25 mg twice daily) was conducted based on the reported PK model. Plasma protein binding of apixaban was set to be 87\%.\textsuperscript{19} In the pharmacodynamic study, 48 healthy subjects were included. Four apixaban dose panels were tested: 2.5, 5, 10, and 25 mg twice daily. Study medication was administered orally for seven days. Blood samples were taken to measure PT and aPTT.\textsuperscript{25} For more details, the reader is referred to the original reports.

As a validation step for the model, data of plasma concentration vs. drug effect was used.\textsuperscript{26–28} All the datasets were obtained from above-cited literature and digitized using GelData Graph Digitizer (version 2.22). In order to correct for the variability of the thromboplastin reagents used in different studies, all the values of PT and aPTT were converted to normalized ratios (relative change in PT and aPTT).

Target binding kinetics
FXa inhibitors bind to FXa whether it is free, on the prothrombinase complex (factor Xa-Va complex), or clot-bound. In vitro experimental kinetic data were available for the reaction of complexation to FXa.\textsuperscript{18,19} Association rate constants (kon) were calculated based on measured binding affinity (K) and dissociation rate constants (koff). Free drug concentration and target BKs were connected by using the following equation:

\[
C + T \overset{k_{on}}{\underset{k_{off}}{\rightleftharpoons}} CT
\]

where \(C\) is the free drug concentration and \(T\) is the density of target.

Systems biology model
Several coagulation models have previously been developed to describe the time courses of coagulation factors\textsuperscript{30,31} and somehow to predict the \textit{in vitro} blood coagulation tests\textsuperscript{32–34}.

In this study, the model equations partially overlap with equations used in the reported models.\textsuperscript{33,34} We started with a comprehensive coagulation model,\textsuperscript{34} but performed model reduction and extension. The components related to the vitamin K cycle and ATIII-heparin complex for simulating the profiles for drug therapies of warfarin, vitamin K, unfractionated heparins, and low molecular weight heparins were removed from the current model,\textsuperscript{34} whereas the models have been extended and adapted for the purpose of simulating the treatment of FXa inhibitors (i.e., the interactions of FXa inhibitors and FXa/FXa-FVa complex). The current model consists of 45 ordinary differential equations (45 species, 82 reactions, 116 kinetic parameters) that are based on the chemical kinetic theory (Figure 2). All the kinetic data and original conditions are from existing literature (Supplementary Tables S1 and S2). The model development and simulations were performed in MathWorks SimBiology toolbox (MATLAB 2014b). The rates of change of the concentration of factors in each compartment were described by a series of ordinary differential equations. Each component of the coagulation network was assumed to have a first order rate of degradation or elimination. The general equation used for enzyme catalyzed stimulation was based on a turnover model and assuming mass-balance principle via Michaelis-Menten kinetics\textsuperscript{32} Eq. 2. The rate of transformation of species caused by another factor C is given by:

\[
\frac{dS}{dt} = -\frac{V_{max}\times C}{K_m + C} \times S
\]

where \(V_{max}\) is the maximum possible rate at which a reaction can occur and \(K_m\) is the concentration of the catalyzing factor for which half of the \(V_{max}\) rate is achieved.

The PT test is one of the commonly used tests in clinical practice to diagnose the state of the blood coagulation system, which assesses the extrinsic and common pathways. It is the time (in seconds) that is taken to form a fibrin clot after adding calcium. In our model, the PT was assumed to represent the time necessary to convert 70\% of fibrinogen to fibrin monomer and the concentration of tissue factor was assumed to be 70 nM, which leads to the reported PT value of approximately 13 seconds.\textsuperscript{31} The aPTT test in contrast to the PT test, measures the activity of the intrinsic and common pathways of coagulation. It is performed in two stages. The first stage is the preincubation of a plasma sample with phospholipids and negatively charged materials. The second stage begins after the addition of calcium, which triggers a coagulation cascade resulting in thrombin generation and conversion of fibrinogen to fibrin.\textsuperscript{32} In our model, the initial concentration of factor XIIa in the contact
stage was set to $2.3 \times 10^{-6}$ nM, which is enough to cause the complete activation of factor XII and prekallikrein during the standard five minutes of the preincubation phase. In the aPTT test, the time is measured from the addition of calcium ions to the formation of a clot. Therefore, the clotting time in our model was assumed to be the time from the beginning of the second stage to convert 5% of fibrinogen to fibrin, which leads to the reported aPTT value of ~30 seconds. Factor Xa activity was determined by a two-step process, as described by Kubitza et al. (2005). In this study, the incubation time (activation time) for the first step was set to four minutes.

Population prediction

The population in our model was selected from healthy controls ($n = 473$), as described in the Leiden Thrombophilia Study. The individual factor levels were obtained for each of the 473 healthy subjects and translated to nanomolar (nM) concentrations using literature values for the average plasma concentration (Supplementary Tables S2 and S3). Systematic analyses of the contribution of the plasma factors to the output of PT, aPTT, and the inhibition of factor Xa was conducted by adjusting the range of factor levels. The initial condition of each factor was linearly spaced between ranges of ±50% around the fitted initial conditions. The ranges of prediction (90% prediction intervals) were obtained using "parameter scan" in the SimBiology software, in which the parameter values were generated using a multivariate normal distribution with unit covariance matrix and a mean vector corresponding to the fitted parameter values.

Sensitivity analysis

Sensitivity analysis is a powerful method for systematically determining which rate constants and concentrations in a model have significant influence on the overall behavior of the model. In this study, we performed a local sensitivity analysis to evaluate the impact of individual parameters and species on the interested state variables. The sensitivity calculation based on the model of PT, aPTT, and FXa inhibition was performed for three key state variables (amount of fibrin, cross-linked fibrin, and FXa, respectively). An approach of full dedimensionalization (Eqs. 3 and 4) was applied to evaluate the relative importance of specific species and rate constants with respect to the formation of clot. The fully normalized sensitivity ($s_{ij}(t)$) of the $j$th observable ($c_j(t)$) (includes the concentration of fibrin and FXa) with respect to a change in the $j$th rate constant ($k_j$) or initial condition of species ($x_{0,j}$) is computed using the complex-step method and given by:

$$s_{ij}(t) = \frac{k_j}{c_j(t)} \left( \frac{\partial c_j(t)}{\partial k_j} \right)$$

$$s_{ij}(t) = \frac{x_{0,j}}{c_j(t)} \left( \frac{\partial c_j(t)}{\partial x_{0,j}} \right)$$

To quantify the impact of parameter changes on the observables during the specified simulation duration, the sensitivities are integrated:

$$S_j = \frac{1}{T} \int_{0}^{T} dt \cdot |S_j(t)|$$

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Figure 2 Schematic of the coagulation network and drug action. CA, activator for the contact system; DP, degradation product; F, fibrin; Fg, fibrinogen; XF, cross-linked fibrin; II, prothrombin; Ila, thrombin; K, kallikrein; P, plasmin; Pg, plasminogen; PK, prekallikrein; TF, tissue factor; TFPI, tissue factor pathway inhibitor; ATIII, antithrombin-III; Xa:D, drug-FXa complex; Xa:Va:D, drug-FXa-FVa complex.
where $T$ is the final time point. The outputs of interest ($S_i$) represent the integral of sensitivity over time.

**RESULTS**

The model-based research was started with the simulation of multiple-dose PK based on the reported model, described in the Methods. The results of PK simulation are given in Supplementary Figure S1.

**Model-predicted FXa inhibitor effects on PT**

The predicted PT profiles and ranges after treatments with rivaroxaban and apixaban are shown separately in Figure 3a,b, 3(f1), 3(f4) and Supplementary Figure S2a,b.

From the prediction, both rivaroxaban and apixaban prolonged PT in a concentration-dependent incremental manner through their inhibition of free and bound FXa in virtual human studies. A relatively wide range of PT values was obtained in the population prediction (Supplementary Figure S2a,b). The observed PT values in clinical trials were selected for model validation. Based on the demographics of healthy subjects (age, sex, and body mass index) enrolled in the different clinical trials, the specific predictions were conducted. The predictions of PT for rivaroxaban and apixaban were in agreement with the observed data (Figure 3a,b, 3(f1), and 3(f4)).

**Model-predicted FXa inhibitor effects on aPTT**

The predicted time courses of aPTT and the intervals are displayed in Figure 3c,d and Supplementary Figure S2c,d, and the predicted concentration-dependent profile is shown in Figure 3(f2). The study shows a concentration-dependent
prolongation of aPTT in human plasma. Comparing with the predicted and observed aPTT values, the model described the level of aPTT for rivaroxaban (Figure 3c and 3(f2)). For apixaban, however, the model is overpredicting the actual observed aPTT values (see Figure 3d). A possible reason might be the difference in thromboplastin reagent concentrations used in the rivaroxaban and apixaban clinical trials.40 Consequently, this may hamper the prediction of coagulation effects of different compounds.

**Model-predicted FXa inhibitor effects on the activity of FXa**

The predictions of rivaroxaban and apixaban treatments on FXa activity are shown in Figure 3e, 3(f3) and Supplementary Figure S2e,f. Compared with PT and aPTT, smaller between-subject variability was observed in the simulation of FXa inhibition (Supplementary Figure S2e,f). The simulated results agree well with the observed values obtained in the rivaroxaban clinical trials (Figure 3e and 3(f3)).

**Sensitivity analysis**

The top sensitive parameters \(S_{ij}/C_{21} \geq 0.01\) are listed in Figure 4. Both target BKs \(k_{on}\) and \(k_{off}\) for drug-FXa interaction) and drug concentration have a high impact on the response of fibrin and FXa. To verify these results, several simulations for rivaroxaban were conducted whereby BK parameters and dosing intervals were varied (Figure 5). Remarkable changes were observed in the time profiles of PT, aPTT, and FXa inhibition when \(k_{on}\) was varied 10-fold (Figure 5(a1), 5(a2), and 5(a3)). The possible reason for the relatively low response of slow on-rate compounds is that the compound is being cleared before it will bind. The target turnover might be another reason, as the FXa concentration is decreased when slow on-rate compounds binds to the target. In Figure 5(b3), the time profiles of

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**Figure 4** Sensitivity analyses for the model of PT (a), aPTT (b), and FXa inhibition (c).
FXa inhibition with relatively low off-rates (10-fold varied \(k_{\text{off}}\)) almost overlay, because the target fraction bounds were all at high levels (whereas significantly low level of prediction were obtained when we used 100-fold higher level of \(k_{\text{off}}\)). This therefore illustrates that the kinetics themselves may be equally or even more important than the steady-state potency, because the prediction would be different when we select compounds with close value of \(K_i\) and distinct \(k_{\text{on}}\) and \(k_{\text{off}}\). However, obvious changes were observed in the time profiles of PT and aPTT when \(k_{\text{off}}\) was varied 10-fold (see Figure 5(b1) and 5(b2)). This shows that the signal pathways could enlarge the difference from BKs. This indicates the importance of drug BKs on the drug effects. PK profiles were also varied by simulating different dosing intervals (q.d. vs. b.i.d.). Based on our model, a twice-daily dosing regimen for rivaroxaban would maintain a favorable steady-state concentration and thus pharmacological effect. Finally, in order to highlight the role of drug-target BKs, we compared rivaroxaban (5-chloro-N-[(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl[thiophene-2-carboxamide] with its (R) enantiomer 5-R-rivaroxaban (5-chloro-N-[(5R)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl[thiophene-2-carboxamide]). No human clinical PK data of R-rivaroxaban are available; we therefore assumed that they would be similar to S-rivaroxaban. The BK properties of the two rivaroxaban enantiomers, however, are very different (Table 1). As a result, 5-R-rivaroxaban with an extremely low association rate constant (\(k_{\text{on}} = 0.0774 \text{nM}^{-1}\text{s}^{-1}\)) shows no effect on PT, aPTT, and FXa activity (Figure 6). This provides strong evidence for the significant influence of target BKs on drug effects.

**DISCUSSION**

In this study, we report the development of a systems pharmacology model based on previously published models of coagulation and literature-reported results on the PT, aPTT, and anti-FXa tests. In the in vivo system, FXa is generated through both the extrinsic tenase (tissue factor, Factor VII, and Ca\(^{2+}\) as an activating ion) and intrinsic tenase (Factors IXa, VIIIa, and X, and Ca\(^{2+}\) complexes). During the initial stage of the hemostatic event triggered by tissue factor, low levels of FXa and FIIa are generated. Once generated, the limited amount of FXa produced by the extrinsic tenase binds to active platelet membrane and convert picomolar amounts of prothrombin to thrombin. This thrombin then activates FVIII.
and FV allowing the initial formation of the intrinsic tenase and prothrombinase complexes. The burst phase of thrombin generation depends on the additional FXa generated via the intrinsic tenase complex. In addition, the \textit{in vivo} coagulation is affect by blood flow, which leads to an exchange of proteins between the fresh blood pool and clot. Therefore, it is time- and space-dependent.\textsuperscript{43} Although the clotting cascade is divided into intrinsic, extrinsic, and common pathways in the \textit{in vitro} PT and aPTT tests, which has little \textit{in vivo} validity, it remains a useful concept for interpreting the results of laboratory investigations.\textsuperscript{44} In this study, the model, which includes main elements of coagulation network, is used to describe and simulate the effects of drug therapies on the coagulation network and \textit{in vitro} blood coagulation tests (i.e., PT and aPTT). The \textit{in silico} model could possibly be applied for compound selection based on the properties of drug-target BKs that is more predictive of clinical outcomes in much earlier phases of drug design and discovery.

Drug-target BKs and system-related kinetic data are all measured separately \textit{in vitro}. It is necessary to understand all determinants of drug action for predicting a clinical drug effect from \textit{in vitro} experiments. These determinants include drug concentrations at the target site and BKs, but also other factors, such as competition with endogenous compounds that normally bind to this target as well as the impact of binding to the target to induce a signaling cascade (target activation). The exact role of these factors in the determination of drug effects is unfortunately still poorly understood. Until now, it is still difficult to apply published coagulation models to predict the results of laboratory coagulation tests for PT, aPTT, and anti-FXa assays simultaneously. One reason is that the involved pathways or numerical estimates of rate constants are uncertain.\textsuperscript{33} Even when biochemical pathways have been measured, a wide variety of values may exist for a given parameter. Thus, a model constrained on clinical data is critical, and this may help to (i) reject measured values as being physiologically implausible; (ii) allow some uncertain parameters to be estimated through modeling, and (iii) identify highly sensitive parameters or species that are rate-controlling, because these parameters or species will serve as key elements for the subsequent prediction. In this study, the model constraint was conducted based on the data from the single dose treatment of rivaroxaban in a phase I clinical trial.\textsuperscript{37}

In this study, we show that there are large variations in predicted time profiles of PT and aPTT in a healthy population. These variations are much larger than those observed for the percentage FXa inhibition. Consequently, it demonstrates that the anti-FXa level may provide a more accurate assessment of anticoagulation. This observation agrees well with the suggestion to preferably use an anti-FXa test and not a PT or aPTT test to evaluate anticoagulation.\textsuperscript{40,45} The percentage of FXa inhibition has a relatively small inter-individual variability because the anti-Xa assay is directly based on enzymatic inhibition, which can be accurately measured using well-defined chemical reagents that are not biologically derived. In addition, the sensitivity analysis also supported this finding because a larger number of species is involved in the PT and aPTT tests as compared to the anti-FXa assay. From the results of different studies, the model predicts PT reasonably well (both for rivaroxaban and apixaban). For rivaroxaban, satisfactory predictions were made for the aPTT test and anti-FXa assay. However, unlike the relatively standardized PT testing, there is no standardization of reagents used for aPTT testing. The variation in coagulometers, the lot-to-lot variation of a given manufacturer’s reagents, and the different buffers used in aPTT tests may significantly contribute to the varying results.\textsuperscript{25,40,46} It may be that for these reasons, the prediction for apixaban in the aPTT test was not good. Consequently, it may make the model difficult to use to predict the coagulation effects of various other anti-FXa compounds. In order to obtain a good prediction of the anticoagulant effect for FXa inhibitors, the exact methods for the assays used including detailed formation of the reagents applied are required, and the reagents and other impact factors need to be taken into consideration in the model for a better aPTT prediction.

A sensitivity assessment of model parameters and species used for the models predicting PT, aPTT, and anti-FXa was conducted to evaluate whether the model can be reduced. Overall, PT and aPTT models are much more

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**Table 1 In vitro binding kinetics of rivaroxaban and apixaban**

| Inhibitor          | Target          | $k_{on}$ (nM$^{-1}$ h$^{-1}$) | $k_{off}$ (h$^{-1}$) | Ref. |
|--------------------|-----------------|-------------------------------|----------------------|------|
| Rivaroxaban        | FXa             | 360                           | 144                  | 14   |
| (5-S-rivaroxaban)  | FXa-FVa complex | 360                           | 756                  |      |
| Apixaban           | FXa             | 0.0774                        | 12.6                 | 8    |
|                    | FXa-FVa complex | 162.72                        | 40.68                | 15   |

$^a$Data was provided by M. Amaral and J. Bomke (Merck-Serono, personal communication).

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**Figure 6** The simulated profiles of rivaroxaban (red lines) and 5-R-rivaroxaban (green lines) after 30 mg b.i.d. treatments.

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sensitive to model parameters and the concentration of species. This is reasonable when we consider that PT and aPTT are global assessments of coagulation, such that the parameter values and species conditions have more direct/indirect impacts on the predicted PT and aPTT profiles, whereas the impact on FXa activity results almost directly from the inhibition of FXa. In addition, the drug properties, including the BKs for targeting free FXa (especially $k_{on}$), and drug concentrations themselves were identified to influence the clinical coagulation behaviors. An interesting finding is that the BKs between drug and FXa-FVa complex seem to have no influence. It might be due to the binding of the drug to the FXa before the formation of the FXa-FVa complex, because the complex is at the downstream of free FXa. However, significant changes in the profiles of PT, aPTT, and FXa inhibition were observed when we were trying to remove this component from the systems model. A possible reason might be the distribution of drug across free and bound FXa. The drug concentration at the site of free FXa was lower than the overall free drug plasma concentration. It suggests that the FXa-FVa complex binding of rivaroxaban might act as a “sink” for free drug plasma concentration. This illustrates that a deeper understanding of the interaction of drug molecules with their targets is essential for the prediction of clinical drug effects. Apart from these drug and target-related properties, the biological system-related parameters and species, as depicted in Figure 4, are also strong determinants of drug effect. As such, this might provide insights in the behavior of the system as a whole.

In addition, reversal agents are very important in anticoagulation treatments. However, until now, a specific antidote for Factor Xa inhibitors was not available. Because of high plasma protein binding, rivaroxaban and apixaban are not expected to be dialyzable. Protamine sulfate and vitamin K are not expected to affect the anticoagulant activity of FXa inhibitors. Partial reversal of PT prolongation has been seen only after administration of prothrombin complex concentrates in healthy volunteers. The use of other procoagulant reversal agents, like activated prothrombin complex concentrate or recombinant factor VIIa (rFVIIa), has not been evaluated. Andexanet alfa, a potential first-in-class recombinant, modified Factor Xa molecule is being developed as an antidote for patients receiving a Factor Xa inhibitor. However, some important information for modeling and simulation (i.e., the interaction of drug and Andexanet) are currently not available, which need to be included for model prediction.

In summary, a systems pharmacology model for blood coagulation has been developed based on publicly available data. The model includes the PKs of FXa inhibitors, drug BKs, and coagulation cascades, and predicts clinical pharmacodynamics (PT, aPTT, and FXa activity). The current model is a good starting point and has potential for future development, in particular to evaluate drug outcome under disease conditions or the inclusion of adverse event analyses.

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Conflict of Interest. The authors are employees of Janssen.
