

ARTICLE

Physiologically-based pharmacokinetics modeling to investigate formulation factors influencing the generic substitution of dabigatran etexilate

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Abstract

The exposure-response relationship of direct acting oral anti-coagulants (DOACs) for bleeding risk is steep relative to ischemic stroke reduction. As a result, small changes in exposure may lead to bleeding events. The overall goal of this project was to determine the effect of critical formulation parameters on the pharmacokinetics (PKs) and thus safety and efficacy of generic DOACs. In this first installment of our overall finding, we developed and verified a physiologically-based PK (PBPK) model for dabigatran etexilate (DABE) and its metabolites. The model was developed following a middle out approach leveraging available in vitro and in vivo data. External validity of the model was confirmed by overlapping predicted and observed PK profiles for DABE as well as free and total dabigatran for a dataset not used during model development. The verified model was applied to interrogate the impact of modulating the microenvironment pH on DABE systemic exposure. The PBPK exploratory analyses highlighted the high sensitivity of DABE exposure to supersaturation ratio and precipitation kinetics.

INTRODUCTION

In recent years, the US Food and Drug Administration (FDA) Agency has approved several direct-acting oral anticoagulants (DOACs) with favorable pharmacological

properties compared with warfarin, including predictable anticoagulant effect, rapid onset of action, and fewer drug-drug interactions.¹⁻³ The efficacy and safety profiles of DOACs are either improved or at least similar to those of warfarin.⁴⁻⁸ Dabigatran etexilate (DABE) was the first

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DOAC approved in the United States and other countries for prevention of stroke, embolism in patients with nonvalvular atrial fibrillation, venous thrombosis, and pulmonary embolism.⁹ DABE acts by directly inhibiting thrombin, whereas the pharmacological effect of apixaban, rivaroxaban, edoxaban, and betrixaban arises from inhibition factor Xa.

DABE is a double prodrug (which was generated by masking both carboxylate and amidinium moieties of dabigatran by ester and carbamate groups, respectively) that converts to the active form, dabigatran (DAB), a reversible competitive inhibitor of thrombin. There is currently no generic version of DABE available in the United States. The oral bioavailability of DABE is low due to its low solubility and P-glycoprotein (P-gp) mediated efflux in the gut. To improve the oral bioavailability, brand DABE capsules contain DABE coated pellets with an acidified inner core. The pellets dissolve only in the gut, creating an acidic microenvironment for DABE and thus improving its apparent solubility.^{10,11} Generic formulations may contain different excipients compared with those in the reference listed drug product, which may lead to different intraluminal concentration versus time profiles in comparison to the reference drug product.

Due to steep exposure-response relationship in risk of bleeding of DABE relative to ischemic stroke reduction (in subjects with normal renal function, yearly event rates are 0.73% and 1.86% for stroke and major bleed, respectively, following treatment with 150 mg of DABE),¹² there might be safety concern of generic DABE with variable pharmacokinetic (PK) profile. Therefore, the FDA product-specific guidance for DABE recommends that in addition to traditional average bioequivalence (BE) metrics, generic applicants should compare the resulting within-subject variability after oral administration of test and reference formulations in full replicated study designs.¹³ Thus, DOACs are representatives of a regulatory paradigm shift from traditional one-size-fits-all average BE approach toward product specific requirements (i.e., FDA guidance for industry), which incorporate a measurement of intrasubject variability in addition to the average BE approach with BE limits of 80%–125%.

In recent years, the application of *in silico* tools has become popular for the prediction of BE study outcomes, which is also referred to as virtual BE studies.^{14–16} Such virtual BE studies are generally based on *in vitro* *in vivo* extrapolation (IVIVE) of formulation performances using physiologically-based PK (PBPK) modeling. Virtual BE trials have been applied by different authors to inform formulation development, support clinically relevant drug product specifications by means of exploring potential design spaces, and anticipate formulation-dependent food effect, as well as investigate the feasibility of extrapolating BE decisions to special target populations.¹⁷

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

There is a paucity of physiologically-based pharmacokinetic (PBPK) models for dabigatran etexilate (DABE) and its metabolites with comprehensive mechanistic absorption. There are no PBPK models that have looked at critical formulation variables and their impact on bioequivalence (BE) criteria for future generic DABE products.

WHAT QUESTION DID THIS STUDY ADDRESS?

To what extent change in critical supersaturation ratio and precipitation rate constant would affect the pharmacokinetics (PKs) of DABE.

WHAT DOES THIS STUDY ADDS TO OUR KNOWLEDGE?

The inner core acidifying agent in the generic formulation may cause significant change in PK parameters of DABE. Generic DABE formulations with critical supersaturation ratio and precipitation rate constant between 18.5–215.8% and 55.9–174.8%, respectively, compared with the brand product would be predicted to be bioequivalent.

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

The results of this study demonstrate the impact of certain important formulation factors on the PK profiles of DABE. This PBPK model can be used to develop oral formulations of DABE that increase the likelihood of BE with reference DABE products.

We have collaborated with the Office of Generic Drugs at the FDA to develop a mechanism-based and risk-based strategy to evaluate reported postmarketing complaints about orally administered generic drugs not being equivalent to brand name drugs. As part of this collaboration, we developed a quantitative framework for metoprolol, which we intend to prospectively verify with drugs, such as DOACs, that are about to come off patent. To this end, we selected four representatives of DOACs: DABE, apixaban, rivaroxaban, and edoxaban to quantitatively predict the impact of change in active pharmaceutical ingredient particle properties of these products on both BE and clinical outcomes using our previously demonstrated PBPK-population PK (Pop-PK)-pharmacodynamic (PD) approach.^{18,19} Among the DOACs, DABE has a bioavailability of 3%–7%. It is consequently more likely to be affected by formulation design differences. The development of a PBPK model for DABE is

challenging due to: (a) being the only double prodrug in the class, (b) pharmacologically active primary metabolite, and (c) formulation-driven apparent solubilization enhancement. In this first part of our overall finding, we have quantitatively investigated the effect of inner core acidifying agent on the BE of DABE. To this end, we have developed a PBPK model that incorporates a mechanistic absorption model in conjunction with physiological distribution and elimination models for DABE and its metabolites.

METHODS

Modeling strategy

The general workflow for developing and verifying the PBPK model for DABE is outlined in Figure 1. Due to the unavailability of DABE PK reports following i.v. administration in humans, it was not possible to apply the stepwise modeling approach. Absorption and disposition models were thus developed concurrently. The absorption of DABE was characterized using the Advanced Dissolution, Absorption, and Metabolism (ADAM) model within the Simcyp simulator

(version 17; Certara, Sheffield, UK) by incorporating the DABE physicochemical properties, intestinal permeability, and efflux transport mediated by P-gp. The physiologically based disposition model was developed targeting the characterization of DABE (prodrug), dabigatran (DAB, active drug), and dabigatran glucuronide (DAB-G, active metabolite). The model was built using the observed plasma concentration data of DABE and total DAB following single administration of 150 mg oral DABE in healthy adults under fasting conditions.²⁰ It was subsequently verified using observed data following 100, 200, 300, and 400 mg oral administrations of DABE. Once developed and verified, a parameter sensitivity analysis (PSA) was carried out on both critical saturation ratio (CSR) and precipitation rate constant (PRC) to evaluate their impact on both maximum plasma concentration (C_{max}) and area under the concentration versus time curve from zero to infinity (AUC_{0-inf}) of total DAB.

Development of absorption model for DABE

Because oral absorption of immediate release formulations containing BCS class 2 drugs is limited by in vivo dissolution,²¹

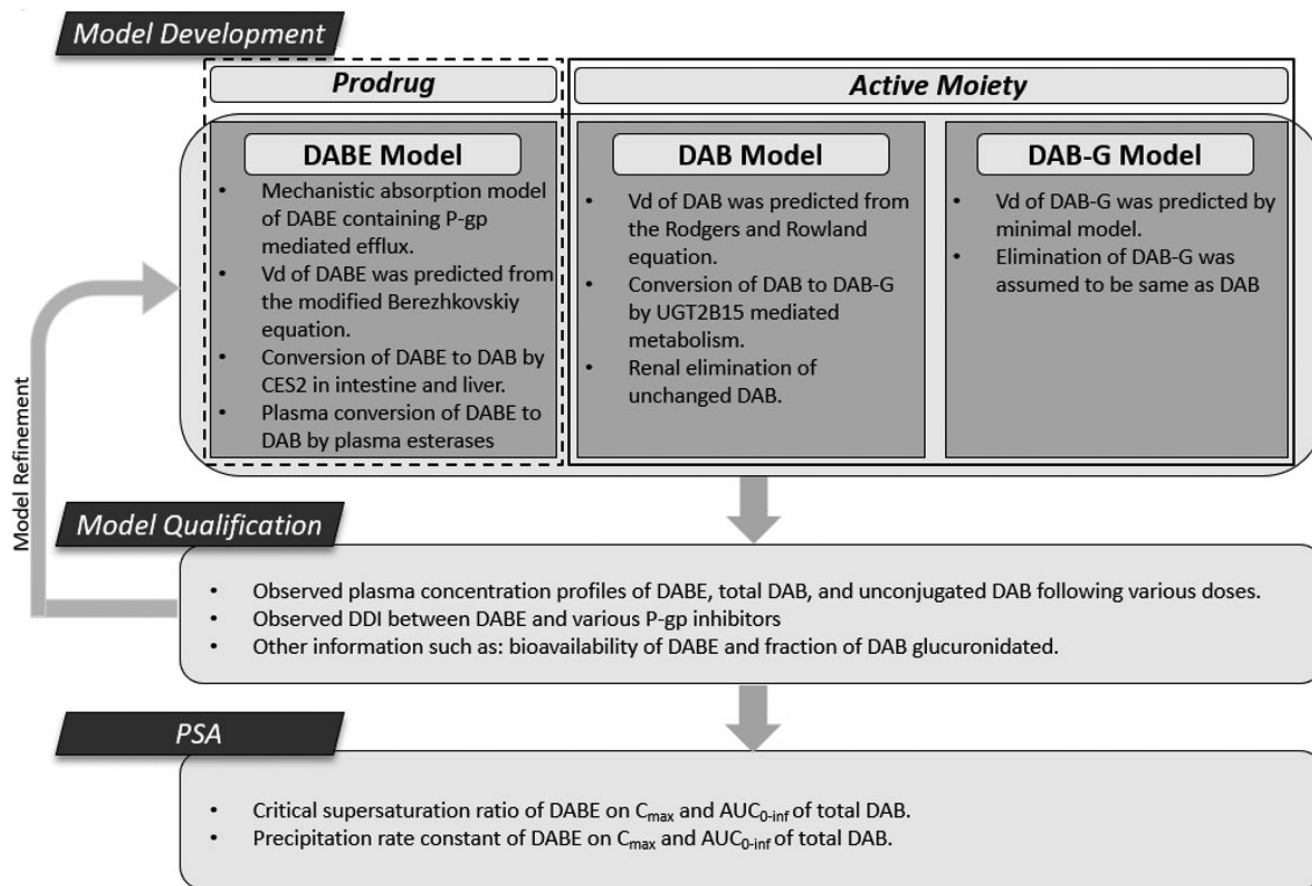


FIGURE 1 Overview of PBPK model development of DABE/DAB and its application on determination of effect of pH modifiers on PK of total DAB. AUC, area under the concentration versus time curve; C_{max} , maximum plasma concentration; DAB, dabigatran; DABE, dabigatran etexilate; PBPK, physiologically-based pharmacokinetic; PK, pharmacokinetic; PSA, parameter sensitivity analysis

TABLE 1 Parameters of DABE used in the PBPK model building

Parameter	Value	Source
MW	627.75	NDA for Pradaxa
logP	3.8	NDA for Pradaxa
Solubility, mg/ml	0.003 (@pH 7.4)	NDA for Pradaxa
pK _a	4 (Base), 6.7 (Base)	NDA for Pradaxa
f _{up} , %	0.063	Predicted (Simcyp)
B/P	1.26	Predicted (Simcyp)
P-gp CL _{int,T} , μl/min (assumed Caco 2, insert diameter = 0.33 cm ² , f _{uinc} = 1)	30	Fitted based on DDI literature (see Table S1)
CES2 CL _{int} , μl/min/mg protein	800	Fitted based on plasma curve of DAB and DABE
Plasma half-life, h	2	Estimated from data provided in Laizure et al. 2014
CSR	22.7	Derived from Chai et al. 2016
PRC, 1/h	1.43	
Duodenum, 10 ⁻⁴ cm/s	0.57	Scaled down values using MechPeff model
Jejunum I, 10 ⁻⁴ cm/s	1.13	
Jejunum II, 10 ⁻⁴ cm/s	0.79	
Ileum I, 10 ⁻⁴ cm/s	0.36	
Ileum II, 10 ⁻⁴ cm/s	0.36	
Ileum III, 10 ⁻⁴ cm/s	0.35	
Ileum IV, 10 ⁻⁴ cm/s	0.34	
Colon, 10 ⁻⁴ cm/s	0.23	

Abbreviations: B/P, blood to plasma ratio; CES, carboxylesterase; CL_{int,T}, transporter intrinsic clearance; CSR, critical saturation ratio; DAB, dabigatran; DABE, dabigatran etexilate; DDI, drug-drug interaction; f_{uinc}, fraction unbound in the incubation; f_{up}, fraction unbound in plasma; MechPeff, Mechanistic Permeability; MW, molecular weight; NDA, new drug application; PBPK, physiologically-based pharmacokinetics; PRC, precipitation rate constant.

a physiologically based absorption model was developed for DABE, incorporating drug-specific and formulation-specific properties in the ADAM model within the Simcyp simulator. The physicochemical parameters of DABE used in the development of the model are summarized in Table 1.

DABE has poor bioavailability (around 3%–7%) due to the combination of intestinal precipitation and efflux by P-gp in the gut wall. Consequently, the plasma concentrations of DABE are governed by these two simultaneously occurring processes. Precipitation of DABE was accounted for by using the “precipitation model 2” within the Simcyp software. Both

CSR (the ratio between critical supersaturation concentration and equilibrium concentration) and PRC were obtained from the literature reported in vitro two-phase dissolution profile of DABE (Table 1).²²

As the human intestinal effective permeability (P_{eff}) of DABE is unknown, the initial regional intestinal permeability values of DABE were estimated by optimizing the intrinsic transcellular permeability ($P_{trans,0}$) in the Mechanistic Permeability model. There are no literature reports of experimental values of P-gp transport kinetics (transporter intrinsic clearance [CL_{int,T}]) of DABE. Therefore, gut P-gp CL_{int,T} value was estimated utilizing the observed drug-drug interaction (DDI) between DABE and three P-gp inhibitors: (a) verapamil, (b) clarithromycin, and (c) ritonavir. The Simcyp library PBPK models for the aforementioned perpetrator drugs were used in DDI simulations. The intestinal P-gp CL_{int,T} was estimated by fitting the DAB AUC ratios between in the presence and absence of P-gp inhibitors. Table 1 shows the key input parameters.²³

Development of disposition model for DABE

The distribution of DABE was modeled using the full PBPK model within the Simcyp simulator, which included a total of 12 organs. The experimental values for blood to plasma ratio (B/P) and fraction unbound in plasma (f_u) of DABE could not be found in the public domain. Therefore, the B/P and f_u of DABE were predicted as 1.263 and 0.063, respectively, by Simcyp calculator based on DABE logP and pKa. The tissue-plasma partition coefficients (K_p) were calculated using the Poulin-Theil equation modified by Berezhkovskiy.²⁴ The predicted K_p values were then used to calculate the DABE steady-state volume of distribution (V_{dss}). Elimination of DABE is a complex process involving several intermediate metabolites, which converts to DAB by carboxylesterase (CES) enzymes. In this model, CES2 was added in both intestine and liver to describe the conversion of DABE to DAB. The CES2 enzyme kinetics was described by an intrinsic clearance (CL_{int}) component. Due to the high abundance of CES2 and the low bioavailability of DABE, enzyme saturation and, thus, nonlinear PK are unlikely. The CES2 CL_{int} value was scaled down from observed plasma concentrations of both DABE and DAB. In addition, conversion of DABE to DAB in plasma was also considered by including the literature-reported plasma esterase half-life of DABE into the model.²⁵

Development of PBPK model for DAB

Similar to DABE, a full PBPK model was developed for DAB using its physicochemical parameters (Table 2). Experimental values were used for all of the physicochemical parameters of

TABLE 2 Parameters of dabigatran and dabigatran glucuronide used in the PBPK model building

Molecule	Parameter	Value	Source
DAB	MW	471.52	NDA for Pradaxa
	logP	0.3	Calculated from logD
	Solubility, mg/ml	0.017	NDA for Pradaxa (ENV)
	pK _a	4.4 (acid)	NDA for Pradaxa
		12.4 (base)	
	f _{up} (%)	0.65	Blech et al. 2008
	B/P	0.784	ADMET Predictor 8.1 Prediction = 0.84. This value was reduced to 0.784 to fit V _{dss}
	UGT2B15 V _{max} , pmol/min/mg	700	Estimated
	UGT2B15 k _m , μM	512	Ebner et al. 2010
Renal CL, L/hr	7.44	Calculated based on total CL	
DAB-G	MW	647.65	ADMET Predictor v 8.1
	logP (or logD @ pH)	0.11	ADMET Predictor v 8.1
	Solubility, mg/ml	1.38	
	pK _a	4.14 (acid)	
		3.51 (base)	
	f _{up} (%)	0.225	
	B/P	0.89	
CL, L/h	7.44	Assumed same as DAB renal CL	

Abbreviations: B/P, blood to plasma ratio; CL, clearance; DAB, dabigatran; DABE, dabigatran etexilate; f_{up}, fraction unbound in plasma; MechPeff, Mechanistic Permeability; MW, molecular weight; NDA, new drug application; PBPK, physiologically-based pharmacokinetics; V_{dss}, steady-state volume of distribution; V_{max}, maximal rate of metabolism.

DAB except for logP and B/P.²⁶ LogP of DAB was estimated using the Simcyp PE calculator from experimentally determined logD value. The B/P value DAB was initially predicted as 0.84 using the ADMET predictor (version 8.1; Simulations Plus, Lancaster, CA) and then optimized to 0.784 to match to V_{dss} to the new drug application reported value (between 50 and 70 L). The tissue-plasma partition coefficients (k_p) were calculated using the Rodgers and Rowland equation.²⁷ Both liver metabolism and renal excretion were considered in the elimination of DAB. Phase II metabolism of DAB was accounted for by addition of UGT2B15 in the liver because UGT2B15 has been identified as the major enzyme responsible for glucuronidation of DAB.²⁸ The Michaelis Menten kinetics parameter, K_m for this process was taken from the literature and maximal rate of metabolism was estimated based on the fact that glucuronidation accounts for around 20% of the total clearance of DAB.²⁸ The renal clearance of DAB was calculated from the i.v. clearance following infusion of 5 mg DAB in the healthy subjects.²⁹

Development of PBPK model for DAB-G

Plasma concentrations of DAB is generally reported as total DAB (sum of unconjugated DAB and DAB-G).²⁹

Hence, a minimal PBPK model for DAB-G was developed. The experimental values for the physicochemical properties of DAB-G could not be found in the literature. Hence, ADMET predictor was used to predict these physicochemical properties (Table 2). The elimination parameters for DAB-G are not known. Therefore, we assumed them to be similar to DAB given that it is a structurally very similar but more polar compound. Formation of DAB-G is relatively slow as evident by the reported in vitro K_m of UGT2B15.²⁸ Therefore, DAB-G is likely to show formation rate limited kinetics. The elimination rate constant of DAB-G is consequently at least equal to that of DAB and was assumed to be the same in the model. The plasma profiles of DAB-G in healthy volunteers are not available in the public domain. Hence, the DAB-G model was indirectly verified by comparing the model predicted plasma profiles of total DAB with those of observations.

Model verifications

The model was externally verified with observed^{20,30–32} DABE and DAB data (Table 3). All of the simulations for model qualifications were done by simulating 10 trials enrolling 10 virtual subjects with similar demographics (age

Dose	Molecule	AUC _{0-inf} , μM*h		C _{max} , μM	
		Observed	Predicted	Observed	Predicted
100	T DAB	1.18	1.38	0.17	0.16
150	DABE	N/A	0.023	0.004	0.005
	T DAB	1.98	1.97	0.22	0.22
200	T DAB	2.43	2.29	0.33	0.29
300	U DAB	3.5	2.78	0.39	0.38
400	T DAB	5.15	4.25	0.71	0.56

Abbreviations: AUC_{0-inf}, area under the concentration versus time curve from zero to infinity; C_{max}, peak plasma concentration; DAB, dabigatran; DABE, dabigatran etexilate; N/A, not applicable; T DAB, total DAB; U DAB, unconjugated DAB.

TABLE 3 Observed^{20,30-32} and model predicted AUC_{0-inf} and C_{max} of DABE and DAB

range and sex ratio) to the observed data. The model was deemed appropriate if the ratio of the mean observed to predicted AUC_{0-inf} and C_{max} were within two folds. In addition, the estimated P-gp CL_{int,T} value was also verified by comparing virtual DDI studies between DABE and P-gp inhibitors (verapamil, clarithromycin, and ritonavir) with respective clinical observations.^{30,33,34}

Parameter sensitivity analysis

To identify the effect of formulation properties on the dissolution of DABE, a local PSA was carried out to investigate the impact of DABE CSR and PRC on systemic exposure of total DAB. The ranges for PSA on CSR and PRC were between 1.1–1.14 and 0.14–7.22/h, respectively. These ranges were selected to explore the failure edges and safe spaces (i.e., region within which changes in the critical quality attribute does not affect the BE conclusion). A second PSA was conducted to determine the impact of changes in stomach pH (e.g., as the result of achlorhydria or concomitant administration of proton pump inhibitors) on AUC_{0-inf} and C_{max}. Evaluated pH values ranged from 1.5 to 7.5. The PSA analysis for each of the parameters was conducted one at a time while fixing all the other parameters to their final value in the model.

RESULTS

Predictions of plasma concentration profiles

The developed model was able to simultaneously characterize the plasma concentration-time profiles of DABE, unconjugated DAB, total DAB, and DAB-G following oral administration of 150 mg DABE (Figure 2). Subsequently, the model was externally verified by comparing the predicted plasma concentration profiles for both unconjugated DAB and total DAB (combination of unconjugated DAB and amount of DAB in DAB-G) with observed data. Figures S1–S6 show

that the model can capture clinical data following oral administration of 100, 110, 200, 300, and 400 mg DABE reasonably well. As can be seen in Figures S1 and S6 the PBPK model can predict both central tendency and associated variability in plasma concentrations of DAB. The ratio of observed to predicted C_{max} for DABE was 0.88 following 150 mg oral capsule administration, as the reported DABE plasma concentrations profile only contains few data points. Therefore, the AUC_{0-inf} for DABE could not be calculated. The ratios of observed to predicted AUC_{0-inf} and C_{max} values of total DAB were within 0.86–1.21 and 1.05–1.27, respectively. In addition, the ratio of observed to predicted AUC_{0-inf} and C_{max} values of unconjugated DAB were within 1.25 and 1.02, respectively (Table 3). The model predicted the DABE fraction absorbed was 0.068, 0.063, 0.056, 0.055, and 0.052 following 100, 150, 200, 300, and 400 mg of oral administrations, respectively.

The P-gp kinetics parameter used in this model was verified by virtual DDI studies between DABE and P-gp inhibitors. The model was able to predict the fold change in AUC of DABE due to interaction with various P-gp inhibitors reasonably well. The observed versus predicted AUC_{0-inf} ratio was contained within 0.79–1.25. Among the simulated DDI scenarios, concomitant administration of 150 mg DABE with 120 mg verapamil showed the highest increase in DABE exposure (2.06-fold increase). On the other hand, concomitant administration of 150 mg DABE with 100 mg ritonavir at steady-state showed minimal increase in DABE exposure (1.19-fold increase; Table 4).

PSA on critical quality attributes of the formulation

In order to keep the C_{max} and AUC_{0-inf} of total DAB within 0.8–1.25 range compared with the brand product, the CSR of the generic DABE must be within 2–65 and 4.2–49, respectively. On the other hand, C_{max} and AUC_{0-inf} of total DAB would be within the 0.8–1.25 range of the brand product if PRC is within 0.7–2.6/h and 0.8–2.5/h, respectively

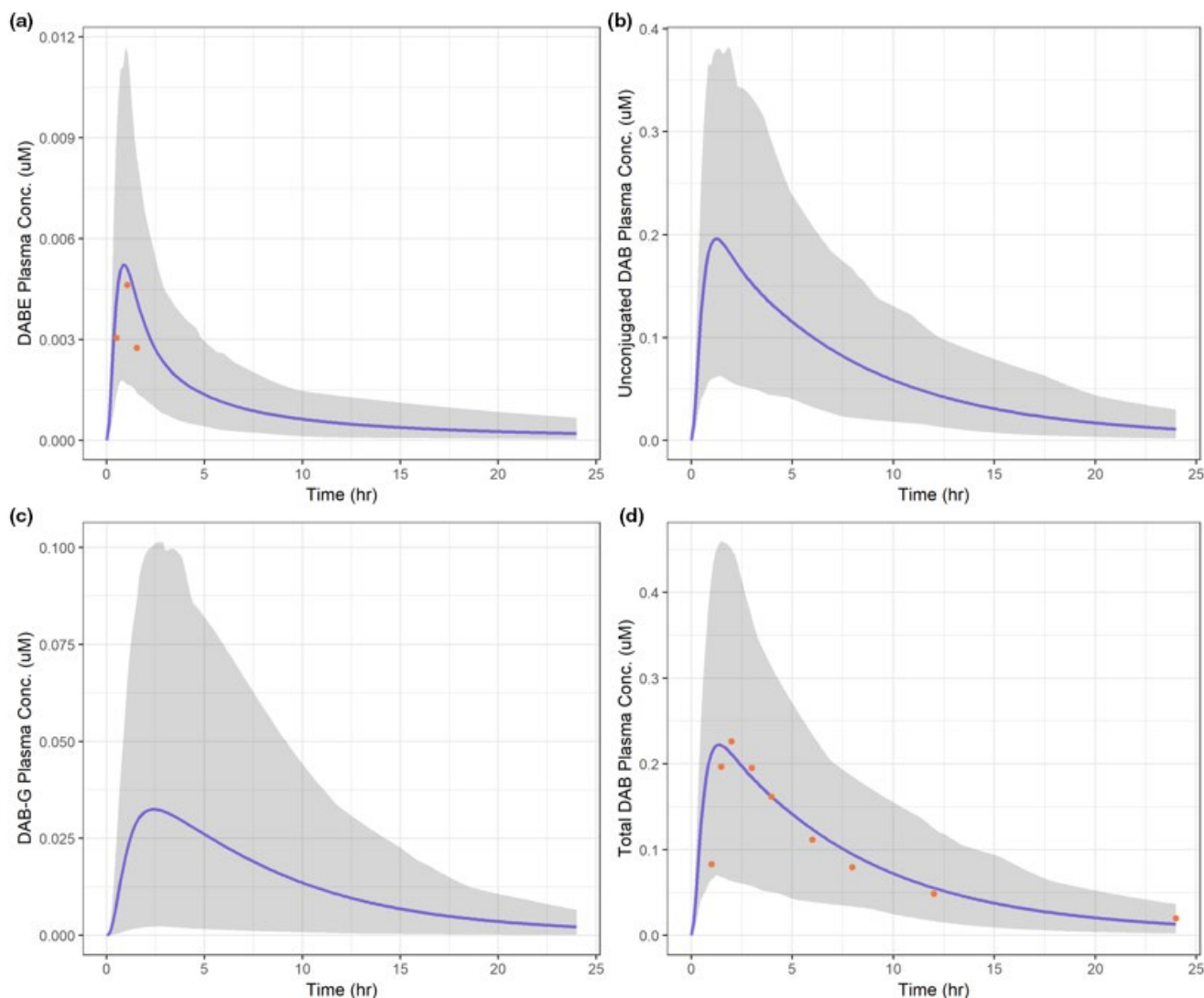


FIGURE 2 PBPK model predicted plasma concentrations time profiles following 150 mg of oral capsule administration. (a) DABE, (b) unconjugated DAB, (c) total DAB, and (d) DAB-G. The violet line represents the predicted concentrations, whereas the shaded area represents the 5th to the 95th prediction range. The orange dots represent the observed. C_{max} , maximum plasma concentration; DAB, dabigatran; DABE, dabigatran etexilate; DAB-G, dabigatran glucuronide; PBPK, physiologically-based pharmacokinetic

(Figure 3). Even though a downward trend was observed, neither C_{max} nor AUC_{0-inf} of total DAB is significantly affected by increase of stomach pH.

DISCUSSION

A few PBPK models for DABE has been published so far.^{31,35} Among these, the model developed by Moj et al. is comprehensive and can predict DABE, DAB, and DAB-G. However, that model used the salt solubility of DABE (1.8 mg/ml) to develop the absorption model. As a result, it did not account for the precipitation of DABE in the gastrointestinal (GI) track. We developed a comprehensive new PBPK model for DABE containing mechanistic absorption model. The objective of this research was to determine

quantitatively the effect of certain potentially critical formulation parameters on the PK of different formulations of DABE. Physiologically based models can be used to prospectively investigate the sensitivity of DABE systemic exposure to different formulation-specific critical quality attributes. Additionally, the clinical significance of variability in PK fluctuations can be addressed by combining Pop-PK-PD modeling to assess whether there could be a significant impact on safety and efficacy profiles. In the current study, we selected DABE as a case example to demonstrate to what extent failing to meet the BE criteria might be traced back to formulation-specific parameters. DABE is the first DOAC that was approved by the FDA in 2010, and the first generic DABE is expected to be available in the US market in the future. The dose-response curve of DABE is steeper for life-threatening bleed compared with that of prevention

DABE dosing regimen	Interacting drug dosing regimen	Simulated AUC _{0-inf} increase	Observed ^{30,33,34} AUC _{0-inf} increase
150 mg q.d.	Verapamil 120 mg administered concomitantly with DABE	206%	208%
150 mg q.d.	Verapamil 120 mg administered 1 h before DABE	193%	243%
150 mg q.d.	Verapamil 120 mg b.i.d. for 4 days then on fourth day morning dose 1 h before DABE dose	194%	154%
150 mg q.d.	Verapamil 120 mg b.i.d. for 8 days then on eighth day morning dose 2 h after DABE dose	115%	118%
300 mg q.d.	Clarithromycin 500 mg b.i.d. for 3 days and then fourth day morning clarithromycin was given concomitantly with DABE	148%	150%
150 mg q.d.	Ritonavir 100 mg q.d. for 26 days, and on day 26, ritonavir was administered concomitantly with DABE	119%	111%

TABLE 4 Predictions DDI between DABE and P-gp inhibitors

Abbreviations: AUC_{0-inf}, area under the concentration versus time curve from zero to infinity; DABE, dabigatran etexilate; DDI, drug-drug interaction.

of ischemic stroke. Therefore, a generic product with a slightly different formulation may lead to clinically relevant differences. Anticipating such an outcome, the FDA has published a product-specific guidance for DABE BE, advocating for not using the reference-scaled average BE approach to widen the BE limits for DABE, even though it is a highly variable drug as per current regulatory definitions.¹³

Development of a PBPK model for DABE is challenging due to its complex prodrug conversion and lack of publicly available experimental data. The absorption model for DABE was built utilizing the physicochemical data (Table 1) in Simcyp (version 17). The model predicted fraction DABE absorbed was 0.063 following 150 mg oral administration. Because the conversion of DABE to DAB is near 100%, thus the fraction absorbed of DABE can be interpreted as the bioavailability of DAB. Therefore, the model was able to capture the bioavailability of DABE, which is reported as 3%–7%. The model could predict the plasma concentrations of DABE, DAB, and DAB-G following oral administration of DABE (Figure 2, Figures S1–S4). In addition, plasma concentrations from recent work by Moj et al.³¹ were compared with our PBPK model simulations. As can be seen in Figures S5

and S6, the model could predict both the central tendency and the variabilities in plasma concentrations of DABE, DAB, and DAB-G following multiple dosing of 110 mg DABE (b.i.d.).

DABE is a double prodrug, which undergoes a presystemic two-step conversion to active product DAB. This two-step process involves generation of two intermediate metabolites (BIBR 1048 and BIBR 1087) by hydrolysis of DABE by intestinal CES1 and CES2 enzymes. As the subsequent conversion of these intermediate to DAB in the liver is almost complete and rapid, the plasma profiles of these intermediates are only partially reported. Besides, experimental physicochemical data for these intermediates could not be found in the literature. Previously, Laizure et al.²⁵ demonstrated that intestinal CES2 plays the major role in conversion of DABE to DAB. Hence, the PBPK model described here assumes that DABE is converted to an intermediate mainly by intestinal CES2 and the resultant intermediate is instantly converted to DAB in the liver. Therefore, the CES2 kinetic parameter (CL_{int}) estimated here does not represent a true physiological value, and it only describes the process of direct conversion of DABE to DAB.

P-gp efflux plays a significant role in the low bioavailability of DABE. In this model, the P-gp mediated transport

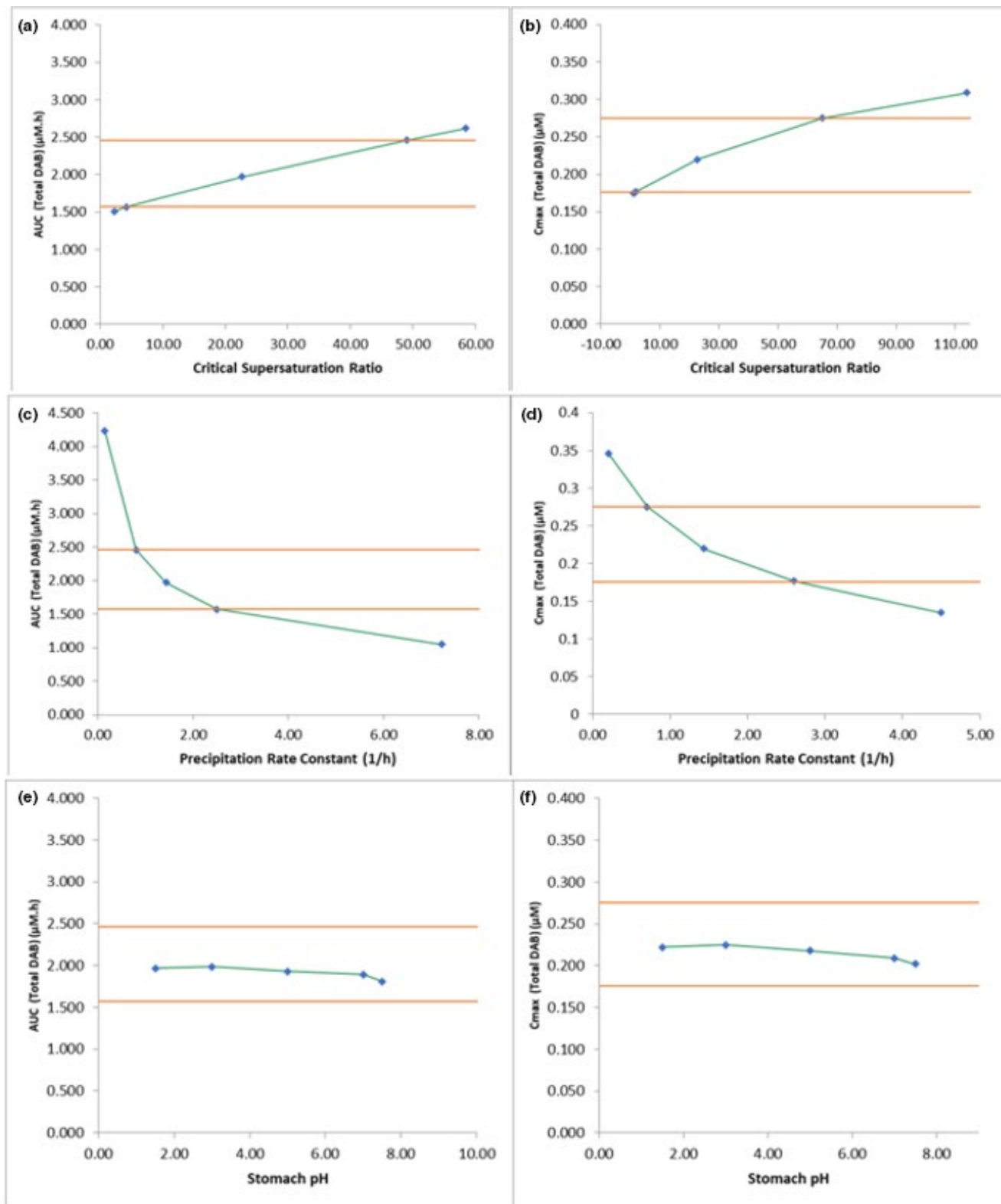


FIGURE 3 Parameter sensitivity analysis. (a) Effect of CSR on AUC_{0-inf} , (b) effect of CSR on C_{max} , (c) effect of PRC on AUC_{0-inf} , (d) effect of PRC on C_{max} , (e) effect of stomach pH on AUC_{0-inf} , (F) effect of stomach pH on C_{max} . The orange lines represent the 0.8–1.25 range for respective PK parameters compared with the reference product. AUC_{0-inf} , area under the concentration versus time curve from zero to infinity; C_{max} , maximum plasma concentration; CSR, critical saturation ratio; PRC, precipitation rate constant

kinetics was accounted by $CL_{int,T}$, assuming that due to very low bioavailability, DABE concentration in the enterocyte would not reach high enough to trigger nonlinearity. Finally, all of the parameters in DAB-G model are predicted, and it was assumed that the clearance (CL) of DAB-G is equal to the renal CL of DAB. Even with these limitations, the predicted exposure of DAB from DAB-G was around 16%, which is very close to the reported value of 19.5%. Apart from the P-gp efflux, potential pH mediated degradation of DABE in the GI tract can add an additional layer of complexity in its absorption. However, in vitro stability studies have demonstrated that minimal degradation of DABE in both acidic and basic environment.³⁶ Therefore, pH mediated degradation of DABE is not expected to play any role in its absorption.

Weak bases with low solubility are often formulated as high-energy salt to increase the rate and extent of their dissolution in the stomach. However, the supersaturated drug solution may start precipitating once it travels through the intestine where pH is much higher compared with the stomach. This may eventually result in poor and unpredictable absorption of drug across enterocyte membrane. Hence, gastric-coated pellets have been used in the reference formulation to bypass stomach dissolution and massive pH-shift precipitation after gastric emptying into the upper small intestine. Furthermore, an acidifying agent was added to the reference formulation to acidify the solid-liquid interface and surrounding microenvironment, maintaining a favorable pH condition, improving the apparent solubility, intestinal dissolution of the drug, and ultimately favoring intraluminal supersaturation, which may improve intestinal absorption. The caveat of using acidifying agents is to cause esophageal ulcer and dyspepsia.^{5,37,38}

In this research, PSAs were performed on both CSR and PRC as substitute of presence and absence of acidifying and enteric coating agents, respectively. CSR is the ratio between maximum solubility attainable kinetically (critical supersaturation concentration [CSC]) and equilibrium solubility (S_{eq}). At drug concentration above S_{eq} and below CSC, precipitation starts, which is governed by the first order PRC. CSR and PRC can be estimated using two-stage in vitro dissolution experimental designs, mimicking the gastric emptying of dissolved drugs into the duodenum. Nevertheless, in vitro PRC tends to overestimate the magnitude of the precipitated fraction in vivo, because it does not consider drug removal out of the intestinal lumen due to permeation across enterocyte membrane, which is an important counterforce to intestinal precipitation,³⁹ because “closed” in vitro apparatus are generally utilized. Therefore, “open” multistage in vitro dissolution apparatus or IVIVE-PBPK techniques have been applied to link in vitro to in vivo CSR and PRC values.⁴⁰

The PSA results demonstrated that the point estimates of the two PK parameters used in the bioequivalent test (C_{max} and AUC_{0-inf}) would be within the required range (0.8–1.25)

if CSR of a generic product is within 4.2–49. Because solubility of DABE in acidic media is greater than $> 50 \text{ mg/ml}^9$ and the PBPK model predicted solubility of DABE in the intestine is below 0.1 mg/ml (Figure S7), a CSR of 49 or more can indeed be achievable, by modulating apparent solubility via acidifying agents. For example, in the duodenum, the model predicted solubility of DABE is 0.07 mg/ml and if we consider the maximum solubility of DABE is 50 mg/ml, the corresponding CSR will be 714.28. On the other hand, point estimates of C_{max} and AUC_{0-inf} would be within the required range if PRC of the generic DABE is within 0.8–2.5/h. Precipitation kinetics faster than 2.5/h, plausible to occur in the absence of an effective enteric coating strategy triggering pH-shift precipitation, may pose challenges to meet the BE criteria. Therefore, enteric coating and core acidifying are likely to be critical quality attributes (CQAs) affecting the systemic exposure of total DAB.

In conclusion, a PBPK model was developed for DABE that can predict the PKs of DABE, DAB, and DAB-G. The effect of change in CSR and PRC due to change in inner core acidifying agent in DABE formulation was quantitatively explored. In order to better characterize the clinical relevance of these findings, and design adequate safe spaces for the two CQAs, we will translate the PK changes in clinical outcomes, targeting efficacy and safety end points, via integrated PBPK-Pop-PD modeling.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

N.F., R.C., and S.S. wrote the manuscript. L.F., L.J.L., and S.S. designed the research. N.F. and R.C. performed the research. N.F., R.C., S.B., S.K., K.L., S.J., L.J.L., J.D.B., and S.S. analyzed the data.

DISCLAIMER

The opinions expressed in this manuscript are those of the authors and should not be interpreted as the position of the US Food and Drug Administration.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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