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Research Article

Population Pharmacokinetics and Pharmacodynamics of Apixaban Linking Its Plasma Concentration to Intrinsic Activated Coagulation Factor X Activity in Japanese Patients with Atrial Fibrillation

Satoshi Ueshima,[1] Daiki Hira,[1,2] Chiho Tomitsuka,[1] Miki Nomura,[1] Yuuma Kimura,[1] Takuya Yamane,[1] Yohei Tabuchi,[2] Tomoya Ozawa,[3] Hideki Itoh,[3] Minoru Horie,[3,4] Tomohiro Terada,[2] and Toshiya Katsura[1,5]

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Abstract. Apixaban is used in the prevention and treatment of patients with deep vein thrombosis or pulmonary embolism, and in the prevention of stroke or systemic embolism in patients with nonvalvular atrial fibrillation (AF). In this study, we aimed to elucidate intrinsic factors affecting efficacy of apixaban by conducting population pharmacokinetic and pharmacodynamic analysis using data from 81 Japanese AF patients. The intrinsic FXa activity was determined to assess the pharmacodynamic effect of apixaban. The pharmacokinetic and pharmacodynamic profiles were described based on a one-compartment model with first-order absorption and a maximum inhibitory model, respectively. Pharmacokinetic and pharmacodynamic analysis was conducted using a nonlinear mixed effect modeling program. The population pharmacokinetic parameters of apixaban were fixed at the reported values in our recent study. The population mean of half-maximal inhibitory concentration (IC_{50}) of apixaban was estimated to be 45.3 ng/mL. The population mean IC_{50} decreased 27.7% for patients with heart failure, but increased 55% for patients with a medical history of cerebral infarction. In contrast, no covariates affected the population mean of baseline of intrinsic FXa activity (BASE) and maximum effect (I_{max}) value of apixaban. The population mean of BASE and I_{max} value were estimated to be 40.2 and 38.4 nmol/min/mg protein, respectively. The present study demonstrates for the first time that the co-morbidity of heart failure as well as the medical history of cerebral infarction are an intrinsic factor affecting the pharmacodynamics of apixaban.

KEY WORDS: apixaban; cerebral infarction; heart failure; population pharmacokinetics and pharmacodynamics.

INTRODUCTION

Apixaban is a selective, reversible, and direct inhibitor of activated coagulation factor X (FXa), which is positioned as a key enzyme at the confluence of intrinsic and extrinsic coagulation pathways (1). Apixaban is orally administered for the prevention and treatment of deep vein thrombosis and pulmonary embolism in patients (2–5), and for the prevention of stroke or systemic embolism in patients with nonvalvular atrial fibrillation (AF) (6,7). The ARISTOTLE and ARISTOTLE-J trials (7,8) demonstrated that in AF patients, apixaban was shown to have equivalent or better efficacy and safety than warfarin, which has been widely used in anticoagulant therapy. The recommended dose of apixaban stated in its package insert is dependent on age, body weight, and renal function of AF patients. However, it was shown that an increase in the number of concomitant drugs with apixaban would increase the risk of major bleeding and gastrointestinal bleeding probably due to drug-drug interactions (9). Additionally, it was recently reported that 32% of patients took inappropriately low or high dose of nonvitamin K anticoagulants including apixaban in real-world clinical practice (10). Therefore, characterization of pharmacokinetics and pharmacodynamics of apixaban in AF patients can help to determine safer and more effective therapeutic doses.

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[1] College of Pharmaceutical Sciences, Ritsumeikan University, 1-1-1 Noji-higashi, Kusatsu, Shiga 525-8577, Japan.
[2] Department of Pharmacy, Shiga University of Medical Science Hospital, Seta Tsukinowa-Cho, Otsu, Shiga 520-2192, Japan.
[3] Department of Cardiovascular Medicine, Shiga University of Medical Science, Seta Tsukinowa-Cho, Otsu, Shiga 520-2192, Japan.
[4] Center for Epidemiologic Research in Asia, Shiga University of Medical Science, Seta Tsukinowa-Cho, Otsu, Shiga 520-2192, Japan.
[5] To whom correspondence should be addressed. (e-mail: tkatsura@fc.ritsumei.ac.jp)

Apixaban is primarily metabolized by cytochrome P450 (CYP) 3A4/5 in the small intestine and liver, and is transported by the ATP-binding cassette multidrug transporters P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) in the small intestine, liver, and kidney (11–13). Several population pharmacokinetic analyses of apixaban have been conducted using data from phase I-III studies. These analyses suggested that the inter-individual variability of apixaban pharmacokinetics could be explained by renal function, sex, body weight, age, race, history of orthopedic surgery, and incidence of nonvalvular AF or acute coronary syndrome (14–17). Recently, we have conducted population pharmacokinetic and pharmacogenomic analysis and demonstrated that renal function, *ABCG2* 421A/A, and *CYP3A5**3 genotypes can explain the inter-individual variability in apixaban pharmacokinetics in AF patients in clinical practice (18).

Pharmacokinetic and pharmacodynamic studies demonstrated that the linear correlations between plasma concentrations of apixaban and prothrombin time (PT) or international normalized ratio of PT (PT-INR) were observed (19–21), although variability of apixaban pharmacodynamic profiles including PT and PT-INR appeared to be high. It was considered that, unlike warfarin, pharmacotherapy with apixaban does not require routine monitoring of PT or PT-INR. It was also demonstrated that there is a strong correlation between plasma concentrations of apixaban and anti-FXa activity (22,23). In these studies, however, the measured anti-FXa activity did not reflect the intrinsic FXa activity in plasma samples, as the anti-FXa activity was measured by adding plasma samples to FXa protein as a reagent. Furthermore, population pharmacokinetic and pharmacodynamic analysis of rivaroxaban (24) and edoxaban (25) indicated that the nonlinear relationship between these plasma concentrations and intrinsic FXa activity were observed. However, the inter-individual variability of apixaban pharmacodynamics including intrinsic FXa has not been clarified so far.

In this study, we conducted population pharmacokinetic and pharmacodynamic analysis of apixaban in Japanese AF patients and examined the effect of clinical laboratory data on the apixaban pharmacodynamic parameters.

METHODS

Ethics

This study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Review Board of Ritsumeikan University Biwako-Kusatsu Campus (approval number BKC-IRB-2014-021) and the Ethics Boards of Shiga University of Medical Science (approval number 26-116). All patients gave informed consent prior to enrollment.

Patients and Study Design

To conduct population pharmacokinetic and pharmacodynamic analysis of apixaban, whole blood and plasma samples were obtained from 81 Japanese adult patients with AF who were enrolled as described previously (18). Briefly, all patients were orally administered apixaban tablets (Eliquis®, Bristol-Myers Squibb, Princeton, NJ, USA, and Pfizer Inc., Groton, CT, USA) twice daily at a dose of 5–20 mg/day. For 23 in-patients, blood samples were collected at three time points during hospitalization (trough sampling, and serial sampling at 0.5–2 h and 9–12 h after last apixaban dose). For 32 out-patients, blood samples were collected at a single point during each hospital visit (0.3–16 h after last apixaban dose). For 26 patients, blood samples were collected during both hospitalization and hospital visit. Blood samples from patients were centrifuged at 2500*g* for 15 min at 4°C to collect plasma samples, which were stored at −80°C until analysis. The demographic and clinical laboratory data such as sex, age, body weight, serum creatinine (Scr), creatinine clearance (CLcr), aspartate amino transferase (AST), and alanine amino transferase (ALT) were retrospectively collected from electronic medical records. CLcr was calculated using the Cockcroft-Gault equation (26).

Apixaban Assay

Plasma concentrations of apixaban were determined by liquid chromatography-tandem mass spectrometry, according to our previous studies (27). The calibration curve of apixaban was linear from 2.5 to 500 ng/mL, and the lower limit of quantification was 2.5 ng/mL. When plasma concentration of apixaban was more than 500 ng/mL, the sample was diluted with the pooled normal human plasma containing sodium citrate (Kohjin Bio Co., Ltd., Sakado, Japan) and it was reanalyzed. The inter- and intra-day coefficient of variation values ranged from 2.0 to 8.2% and from 1.1 to 8.0%, respectively. The accuracy was calculated to be from 96.3 to 103.7% by dividing the measured quality concentrations (5, 50, and 400 ng/mL) of apixaban by its reference quality concentrations.

Intrinsic FXa Assay

The FXa activity in plasma was determined using chromogenic assay as described previously with some modifications (24,28). Briefly, the intrinsic FXa activity was measured using BIOPHEN® Factor X kit (HYPHEN BioMed, Oise, France) according to the manufacturer's instruction. During the incubation, hydrolysis of chromogenic FXa substrate by FXa released *p*-nitroaniline. After the incubation period, the mixture was then vortexed, and 120 μL of acetonitrile containing 150 μM 2, 4-dinitroaniline (Sigma-Aldrich, St. Louis, MO, USA) as an internal standard was added to 120 μL aliquots of the mixture to precipitate plasma proteins. After the mixture was vortexed and centrifuged at 13,000*g* for 5 min, 20 μL aliquots of the supernatant were injected into high-performance liquid chromatography (HPLC) system using ultraviolet (UV) detection. The HPLC system (Shimadzu Co., Kyoto, Japan) consisted of an LC-20AD pump, an SIL-20AC autosampler, a CTO-20AC column oven, and an SPD-20A UV detector. Chromatographic separations were conducted on a COSMOSIL® $5C_{18}$-MS-II Packed Column (4.6 × 150 mm, 5.0 μm; Nacalai tesque Inc., Kyoto, Japan) with a COSMOSIL® $5C_{18}$-MS-II Guard Column (4.6 × 10 mm, 5.0 μm) maintained at 40°C. Analytes were eluted under isocratic conditions at a flow rate of 1.0 mL/min with mobile

phase A: mobile phase B ratio of 60:40 *v*/*v*. Mobile phase A consisted of 20 mM phosphate buffer (pH 6.5) and mobile phase B was acetonitrile. The detection of *p*-nitroaniline was conducted with spectrometry at a wavelength of 229 nm. The calibration curve of *p*-nitroaniline was linear from 0.1 to 10 mM, and the lower limit of quantification was 0.1 mM. The inter- and intra-day coefficient of variation values ranged from 0.5 to 3.5% and from 0.04 to 1.8%, respectively. The accuracy was calculated to be from 95.3 to 101.6% by dividing the measured concentrations (0.25, 1.5, and 8 mM) of *p*-nitroaniline by its reference quality concentrations. The protein content was determined by the method of Bradford (29) using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). The intrinsic FXa activity was expressed as nanomoles of *p*-nitroaniline released per minute per milligram protein.

Population Pharmacokinetic and Pharmacodynamic Analysis

A population pharmacokinetic and pharmacodynamic analysis was conducted using a nonlinear mixed-effects modeling (NONMEM) program version 7.3.0 (Icon Development Solutions, Ellicott City, MD, USA) with the first-order conditional estimation method with interaction. The population pharmacokinetic and pharmacodynamic model of apixaban was developed sequentially: the population pharmacokinetic model was initially developed, and the intrinsic FXa activity-time profiles after oral administration of apixaban were then modeled with each individual Bayesian estimated pharmacokinetic parameters being calculated with the post hoc option in NONMEM (30,31). The population pharmacokinetic and pharmacogenomic model of apixaban was described using a 1-compartment model with first-order absorption, in which the population pharmacokinetic and pharmacogenomic parameters of apixaban were set to values previously reported (18).

The relationship between the observed and predicted intrinsic FXa activity was described by following an exponential error model, taking into account intra-individual variability (ε):

$$\mathrm{FXA}_{ij} = \overline{\mathrm{FXA}_{ij}} \cdot \exp(\varepsilon_{ij}) \tag{1}$$

where FXA_{ij} and $\overline{\mathrm{FXA}_{ij}}$ designate the observed and predicted intrinsic FXa activity in the *j*th record of *i*th patient, respectively. The $\overline{\mathrm{FXA}_{ij}}$ value is assumed to vary according to a direct inhibitory maximum effect model as follows:

$$\overline{\mathrm{FXA}_{ij}} = \mathrm{BASE}_i - \frac{\mathrm{I}_{\mathrm{max}_i} \cdot C_{ij}}{\mathrm{IC}_{50_i} + C_{ij}} \tag{2}$$

where the parameters IC_{50}, $\mathrm{I}_{\mathrm{max}}$, BASE, and C_{ij} designate a half-maximal inhibitory concentration, maximum effect, baseline of intrinsic FXa activity, and predicted plasma apixaban concentration according to Bayesian approach, respectively. The parameters IC_{50}, $\mathrm{I}_{\mathrm{max}}$, and BASE are then described using the population mean parameters (θ), as in following equations:

$$\mathrm{IC}_{50_i} = \theta_1 \cdot \exp(\eta_{1i}) \tag{3}$$

$$\mathrm{I}_{max_i} = \theta_2 \cdot \exp(\eta_{2i}) \tag{4}$$

$$\mathrm{BASE}_i = \theta_3 \cdot \exp(\eta_{3i}) \tag{5}$$

where η_1, η_2, and η_3 designate the intra-individual variabilities for IC_{50}, $\mathrm{I}_{\mathrm{max}}$, and BASE, respectively. Hereafter, the combination of these equations was selected as a basic model for the subsequent covariant analysis.

To develop the population pharmacodynamic model of apixaban, the effects of continuous covariates such as age, AST, ALT, Scr, and CLcr of patients on the parameters IC_{50}, $\mathrm{I}_{\mathrm{max}}$, and BASE were evaluated using the following equations:

$$P_i = \theta_4 \cdot \left(\frac{\mathrm{COV}_i}{\mathrm{COV}_{median}} \right)^{\theta 5} \tag{6}$$

$$P_i = \theta_4 \cdot \left(\frac{\mathrm{COV}_i}{\mathrm{COV}_{median}} \right) \tag{7}$$

where $\mathrm{P_i}$ is a pharmacodynamic parameter of the *i*th patients, $\mathrm{COV_i}$ and $\mathrm{COV_{median}}$ denote the covariate of the *i*th patients and median of the covariate, and θ_4 and θ_5 are population mean estimates. The effects of categorical covariates such as co-morbidities (hypertension, diabetes mellitus, heart failure, dyslipidemia, myocardial infarction, and cancer) and medical history of cerebral infarction were also evaluated using the following equation:

$$P_i = \theta_4 \cdot \theta_6^A \tag{8}$$

where θ_6 is the population mean estimate, and the dichotomous parameter *A* is equal to 1 if the categorical covariate is present, and if it was not present, *A* was set to 0. Covariates were added to the basic model using a forward stepwise inclusion method and considered statistically significant if the objective function value (OBJ) decreased more than 3.84 ($P < 0.05$ with 1 degree of freedom). Subsequently, covariates were removed from the full model using a backward stepwise deletion method and considered statistically significant if the OBJ increased in more than 6.63 ($P < 0.01$ with 1 degree of freedom).

Model Evaluation

The following goodness-of-fit plots were used to evaluate the final models: the relationship between observed (OBS) and population predicted value (PRED) or individual predicted value (IPRED), and the relationship between conditional weighted residuals (CWRES) and time after the last dose or PRED. The final model was also assessed using a visual predictive check (VPC) and nonparametric bootstrap analysis. In VPC analysis, a total of 1000 hypothetical data

sets were simulated by random sampling using the NONMEM program. The 50th percentile (median) and 90% prediction interval of the simulated intrinsic FXa activity were plotted using the observed intrinsic FXa activity. In the bootstrap analysis, a total of 1000 replication data sets were generated by random sampling using the Perl-speaks-NONMEM version 4.7.0 (32). The estimates of each population pharmacokinetic and pharmacodynamic parameters obtained using the final model were compared with the medians and 95% prediction intervals using the bootstrap analysis.

RESULTS

Patient Characteristics and Demographics

A total of 276 plasma apixaban concentrations and 276 intrinsic FXa activity measurements from 81 patients were included in analyses and the characteristics of the patients are summarized in Table I and Supplementary 1. The distribution of the genotype of *ABCG2* 421C>A and *CYP3A5**3 was previously reported (18).

Population Pharmacokinetic and Pharmacodynamic Modeling

In the preliminary analysis, the effect of inter-individual variability of population pharmacodynamic parameters on the OBJ value with the basic model was examined. The OBJ value was calculated with the basic model when the inter-individual variability of all parameters (IC_{50} value, I_{max} value, and BASE) was considered. As a result, the lowest OBJ value was calculated with the basic model in which the inter-individual variability of I_{max} value and BASE were removed from eqs. 4 and 5, respectively. Therefore, the inter-individual variability of I_{max} value and BASE in eqs. 4 and 5 were set equal to 0 for the pharmacokinetic and pharmacodynamic analysis of apixaban.

The results of the covariate analyses are summarized in Table II. A forward inclusion method revealed that co-morbidity of heart failure with AF and medical history of cerebral infarction had a significant impact on the IC_{50} value of apixaban. A backward elimination method did not exclude these covariates. Other covariate models including age, AST, ALT, Scr, CLcr of patients, and other co-morbidities (hypertension, diabetes mellitus, dyslipidemia, myocardial infarction, and cancer) did not affect the IC_{50} value of apixaban. The population pharmacodynamic parameter estimates of apixaban in Japanese AF patients are shown in Table III, and the NONMEM control stream for the final population pharmacokinetic and pharmacodynamic model is shown in Supplementary 2. The final population pharmacodynamic model for IC_{50} value was as follows:

$$IC_{50}(\mathrm{ng}/mL) = 45.3 \cdot 0.723^{\mathrm{HF}} \cdot 1.55^{\mathrm{CI}} \quad (9)$$

where the dichotomous parameter HF was equal to 1, if AF patients had heart failure; otherwise, it was set to 0, and the dichotomous parameter CI was equal to 1 if AF patients had a medical history of cerebral infarction; otherwise, it was set to 0. The population mean of IC_{50} value of apixaban was estimated to be 45.3 ng/mL. However, no covariates affected the population mean of I_{max} value of apixaban and BASE, in which the population mean of I_{max} value and BASE was estimated to be 38.4 and 40.2 nmol/min/mg protein, respectively. The population mean of IC_{50} value was decreased by 27.7% when AF patients had heart failure, while the population mean of IC_{50} value was increased by 55% when AF patients had medical history of cerebral infarction. Inter-individual variability for IC_{50} value was 26.1%, and the intra-individual variability was 30.5%.

Model Evaluation

The goodness-of-fit plots for the final pharmacokinetic and pharmacodynamic model are shown in Fig. 1. PRED was shown to reasonably correlate with OBS, and IPRED was shown to correlate well with OBS. No systematic deviation was observed in the relationship between CWRES and time after last dose or PRED. The final model was also evaluated by estimating the population pharmacodynamic parameters from 1000 bootstrap replicates. The median values of population pharmacokinetic parameters calculated from the bootstrap resampling were significantly similar to the population estimates in the final model (Table III). The final pharmacokinetic and pharmacodynamic model was further assessed using VPC analysis. As shown in Fig. 2, VPC analysis adequately resulted in a reasonable predictability of the final model. Figure 3 illustrates the simulated relationship profiles between apixaban concentrations and the intrinsic FXa activity calculated with the final population pharmacodynamic model. The profile for AF patients with medical history of cerebral infarction was shifted to the right, whereas the profile for AF patients with heart failure was shifted to the left relative to the profile for AF patients without either of these conditions.

DISCUSSION

In this study, we estimated the population pharmacodynamic parameters of apixaban in Japanese AF patients using population pharmacokinetic parameters (18), and examined the impact of clinical laboratory data on the pharmacodynamic parameters. The present results indicated that the nonlinear relationship between the intrinsic FXa activity and plasma concentrations of apixaban was described using an inhibitory maximum effect model. Previous population pharmacokinetic and pharmacodynamic studies of rivaroxaban and edoxaban showed that the nonlinear relationship between the intrinsic FXa activity and plasma concentrations of rivaroxaban and edoxaban was described using a maximum effect (E_{max}) model (24) and a dynamic binding model (25), respectively. Thus, similar to rivaroxaban and edoxaban, our pharmacodynamic model can explain the nonlinear relationship between the intrinsic FXa activity and plasma concentrations of apixaban.

The BASE in this study was estimated to be 40.2 nmol/min/mg protein (95% confidence interval; 34.0–46.4 nmol/min/mg protein), although no observed intrinsic FXa activity in AF patients before pharmacotherapy with apixaban was measured. In a preliminary experiment, the observed intrinsic FXa activity in the commercial pooled normal plasma used

Table I. Clinical Characteristics and Demographics of Patients with Atrial Fibrillation

Number of patients	81
Sex (male/female)	61/20
Age (years)	68.1 (40.5–84.9)
Body weight (kg)	65.0 (41.0–92.2)
Dosage of apixaban (mg/day)	10 (5–20)
Plasma concentration of apixaban (ng/mL)	157.9 (15.6–673.6)
Serum creatinine (mg/dl)	0.88 (0.41–1.34)
Creatinine clearance (mL/min)	69.8 (30.6–145.5)
Aspartate amino transferase (IU/l)	23 (13–97)
Alanine amino transferase (IU/l)	19 (5–115)
Intrinsic factor Xa activity (nmol/min/mg protein)	9.3 (2.6–32.8)
Number of patients with medical history of cerebral infarction	5
Number of patients with following co-morbidities	
Hypertension	36
Diabetes mellitus	34
Heart failure	20
Dyslipidemia	18
Myocardial infarction	10
Cancer	9

Data are presented as the number or median with the range in parentheses

for preparation of the calibration curve was calculated to be 31.1 nmol/min/mg protein (95% confidence interval; 29.7–32.5 nmol/min/mg protein). This value was comparable to the population mean of BASE. Thus, the BASE in this study seems to be adequately estimated. In the Japanese AF patients, the population mean IC_{50} decreased 27.7% for patients with heart failure, but increased 55% for patients with a medical history of cerebral infarction. Additionally, when plasma trough concentrations of apixaban in these AF patients were set to median value (137 ng/mL) as reported previously (27), the intrinsic FXa activity was decreased by 63.2–77.1% compared with BASE (Fig. 3). It has been demonstrated that the common therapeutic range of PT-INR in AF patients who took warfarin was from 2.0 to 3.0 (33). The intrinsic Factor X activity was reported to be less than 50% when PT-INR of subjects who took warfarin ranged from 2.0 to 3.0 (34). Therefore, many AF patients may exhibit the sufficient inhibitory effect of apixaban on FXa activity. As shown in Figs. 1 and 2 and in Table III, bootstrap and VPC analyses indicated that the final model provided a robust and unbiased fit to the data. Our results indicated for the first time the impact of medical history and co-morbidities on pharmacodynamic parameters of apixaban.

There is little information concerning the factors affecting FXa or factor X activity. The intrinsic FXa activity in subjects with chronic liver disease or stages 4 and 5 chronic kidney disease (CKD) was reported to be lower than that in healthy subjects (34–36). Additionally, it has also been reported in studies of healthy subjects that levels of prothrombin fragments 1 + 2 which were released from prothrombin by FXa increased with age (37). Therefore, the effects of age, AST, and ALT as indices of hepatic function, and Scr and CLcr as indices of renal function on intrinsic FXa activity were examined. In the present study, only 2 patients showed 3 times higher ALT levels than the upper limit of normal (ULN; 30 IU/L) according to Hy's law, which have been defined as drug-induced liver injury (38). Additionally, the minimum value of estimated glomerular filtration rate of the present study was higher than the upper limit of CKD stages 4 and 5 (30 mL/min/1.73 m^2), and no patients had stages 4 and 5 CKD. Therefore, the influence of AST and ALT on apixaban pharmacodynamics in AF patients may be considered to be negligible.

It was demonstrated that congestive heart failure, hypertension, age more than 75 years, diabetes mellitus, medical history of stroke, transient ischemic attack, or

Table II. Summary of the Tested Covariate Effects on OBJs

Tested covariates	ΔOBJ (1st selection)[a]	ΔOBJ (2nd selection)
Effect on IC_{50}		
AGE with Eq. 6	− 0.27	
AGE with Eq. 7	− 0.21	
AST with Eq. 6	− 0.02	
AST with Eq. 7	− 0.39	
ALT with Eq. 6	[b]	
ALT with Eq. 7	[b]	
Scr with Eq. 6	− 0.83	
Scr with Eq. 7	− 1.00	
CLcr with Eq. 6	− 1.35	
CLcr with Eq. 7	− 0.88	
Cerebral infarction	− 6.63	− 9.88
Hypertension	0.00	
Diabetes mellitus	− 4.65	− 1.66
Heart failure	− 9.03	Included
Dyslipidemia	− 2.28	
Myocardial infarction	− 0.33	
Cancer	− 3.64	

AST, aspartate amino transferase; *ALT*, alanine amino transferase; *Scr*, serum creatinine; *CLcr*, creatinine clearance

[a] The difference from the OBJ value was calculated using the basic model (OBJ value, 942.62), and it is expressed to two decimal places

[b] The OBJ value was not calculated

Table III. Population Pharmacodynamic Parameter Estimates for Apixaban in AF Patients

Parameters	Original data		1000 bootstrap sample data	
	Mean	95% confidence interval	Median	2.5th to 97.5th percentiles
$IC_{50}(\mathrm{ng}/mL) = \theta_1 \cdot \theta_4^{HF} \cdot \theta_5^{CI}$ [a, b]				
$\theta_1(\mathrm{ng}/mL)$	45.3	41.1–49.5	47.3	12.2–136.6
θ_4	0.723	0.568–0.878	0.724	0.522–0.868
θ_5	1.55	1.18–1.92	1.57	1.14–2.27
$I_{max}(\mathrm{nmol}/min/mg\ protein) = \theta_2$	38.4	30.4–46.4	37.3	25.0–95.4
$BASE\ (nmol/min/mg\ protein) = \theta_3$	40.2	34.0–46.4	38.7	25.5–98.2
Inter- and intra-individual variabilities [c, d]				
$\eta(\%)$	26.1 (32.1)	11.3–40.9	25.6	9.53–43.3
$\varepsilon(\%)$	30.5 (6.97)	26.6–34.4	30.1	26.3–34.0

IC_{50}, half-maximal inhibitory concentration; I_{max}, maximum effect; *BASE*, baseline of intrinsic coagulation factor Xa activity
[a] If AF patients had heart failure, then the dichotomous parameter HF was equal to 1; otherwise, it was set to 0
[b] If AF patients had a medical history of cerebral infarction, then the dichotomous parameter CI was equal to 1; otherwise, it was set to 0
[c] The η and ε values denote the inter-individual variability for IC_{50}, and the intra-individual variability, respectively
[d] Data are presented as the mean with the shrinkage (%) in parentheses

thromboembolism in AF patients were risk factors for stroke, and that an increase in the number of these factors tended to elevate the frequency of stroke (39). Therefore, we hypothesized that these co-morbidities might affect the activity including some coagulant factors such as FXa or factor X, and examined the effect of co-morbidities on pharmacodynamic parameters. As a result, co-morbidity of heart failure with AF and medical history of cerebral infarction affected

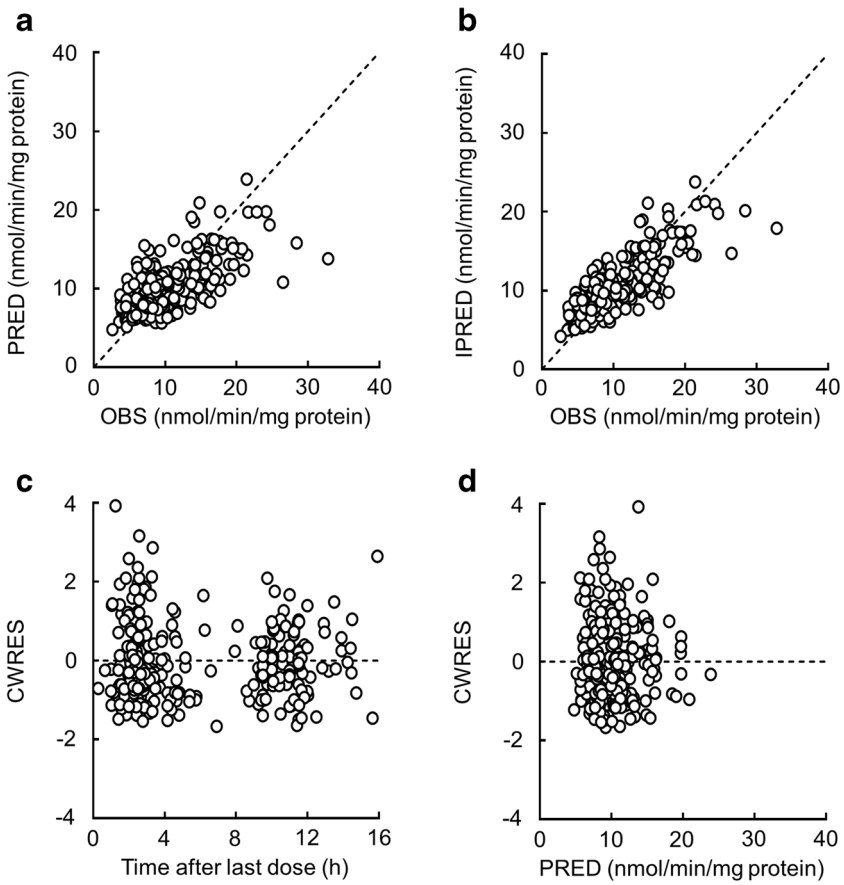

Fig. 1. Goodness-of-fit plots for the final pharmacokinetic/pharmacodynamic model of apixaban. Observed intrinsic FXa activity (OBS) versus population predictions (PRED; **a**) or individual predictions (IPRED; **b**). Conditional weighted residuals (CWRES) versus the time after last dose (**c**) or PRED (**d**). Open circles indicate the observed values. Each dotted line shows a line of identity

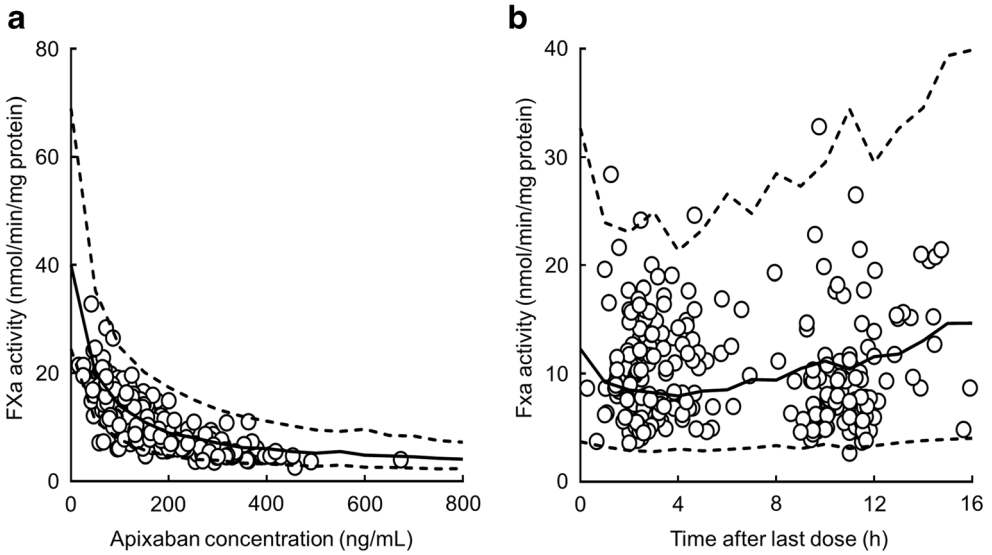

Fig. 2. Visual predictive check plots of intrinsic FXa activity with data obtained from the final PPK/PD model. Intrinsic FXa activity versus apixaban concentration (**a**) or the time after last dose (**b**). Open circles indicate the observed values. The top dotted, middle solid, and bottom dotted lines are the 95th, 50th, and 5th percentiles, respectively, calculated from 1000 simulated data sets

the IC_{50} value of apixaban. However, the mechanisms by which heart failure and cerebral infarction had impacts on pharmacodynamics of apixaban are not clear at this stage. So far, the association of intrinsic FXa or factor X activity with heart failure and cerebral infarction was not directly evaluated. Tissue factor pathway inhibitor (TFPI) has an anticoagulation effect by binding and inactivating FXa in an inhibitory complex, which further binds and inactivates coagulation factor VIIa and tissue factor (40). It has been reported that increase in the TFPI levels in plasma decreased FXa activity (41), and that the free TFPI levels in patients with ischemic stroke were lower than those in healthy subjects (42). These studies suggest that FXa activity in patients with ischemic stroke may be higher than those in healthy subjects. Additionally, it is possible that the relationship between TFPI and intrinsic FXa activity may explain the impact of cerebral infarction on IC_{50} value of apixaban.

The present study included some limitations. In the preliminary analysis, the inter-individual variability of I_{max} value and BASE was set equal to 0 for the pharmacokinetic and pharmacodynamic analysis of apixaban probably due to the limited data. Therefore, we could not identify the factors affecting I_{max} and BASE using NONMEM program. Additionally, a small number of AF patients with a medical history of cerebral infarction were included in this study. It was reported that the false positive rate (type 1 error inflation) of covariate effects tended to be high when the frequency of covariate was less than 20% (43). In the present study, the frequency of AF patients with a medical history of cerebral infarction was only 6.2% (Table I). The covariate effects of this disease on apixaban pharmacodynamics may be poorly estimated, although the goodness-of-fit plots, a visual predictive check and nonparametric bootstrap analysis, indicate that our population pharmacokinetic and pharmacodynamic model was adequately evaluated (Figs. 1 and 2, Table III). In addition, we could not evaluate the impact of changes in IC_{50} value of apixaban on the incidence of adverse events such as bleeding and thromboembolism due to the limited data. Therefore, the population pharmacokinetic and pharmacodynamic analysis, as well as exposure-response studies,

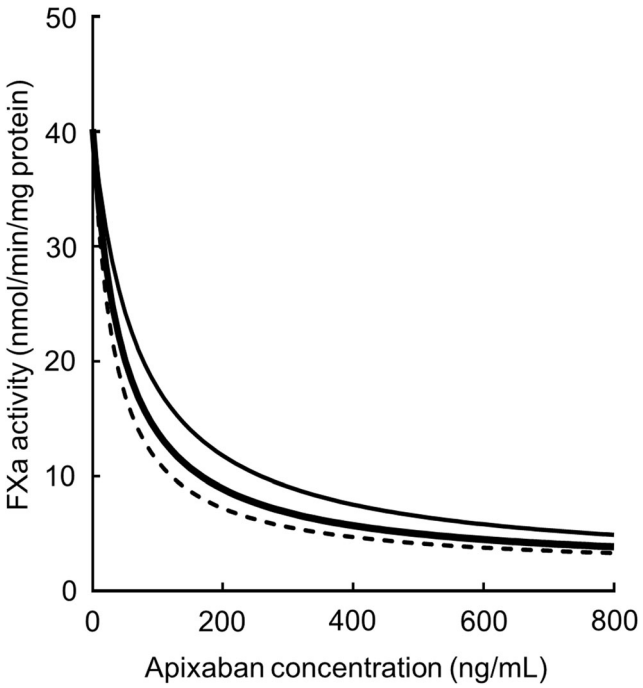

Fig. 3. Simulated relationship profiles between apixaban concentrations and the intrinsic FXa activity. The intrinsic FXa activity was calculated with the final model. The thick, thin, and thin dotted lines indicate the population mean estimates for a typical AF patient, for a typical AF patient with medical history of cerebral infarction, and for a typical AF patient with heart failure, respectively

of apixaban in a larger Japanese population will need to be examined in the future.

CONCLUSION

We successfully developed a population pharmacokinetic and pharmacodynamic model of apixaban in patients with AF. Our results suggest that co-morbidity of heart failure and medical history of cerebral infarction are considered to be significant predictors of apixaban pharmacodynamics. These findings may provide useful information for precision medicine of apixaban to prevent the risk of adverse reactions.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that they have no conflict of interest.

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