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ORIGINAL ARTICLE

Responses of prothrombin time and activated partial thromboplastin time to edoxaban in Japanese patients with non-valvular atrial fibrillation: characteristics of representative reagents in Japan (CVI ARO 7)

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Abstract
The aims of this study were to determine the distribution of plasma concentration of edoxaban (PC-Ed) with their 90% interval (on therapy range) and its correlation with anticoagulation markers in patients with non-valvular atrial fibrillation (NVAF). Consecutive 97 NVAF patients under edoxaban therapy were evaluated (60/30 mg dose, $n=48/49$; men/women, $n=71/26$; age, 69 years). $CHADS_2$ score 0, 1, and ≥ 2 were 27%, 44%, and 29%, respectively. The mean (90% interval) of PC-Ed by LC–MS/MS was 194.3 (49.4–345.3) and 17.0 (4.8–40.7) ng/mL at peak (2–4 h post-dose) and trough (pre-dose), respectively. Correlation of prothrombin time (PT) with PC-Ed was higher than that of activated partial thromboplastin time (aPTT). Among 6 PT reagents, Coagupia PT–N and Simplastin Excel S (both PT reagents) showed the highest predictive capability for the upper outlier of PC-Ed at peak and trough. Among 4 aPTT reagents, only Thrombocheck APTT measured at peak had a significant predictive capability. When using PT reagents, both peak and trough sampling showed a similar predictive capability for the upper outliers of PC-Ed with a high sensitivity, but a relatively low specificity. We demonstrated the distributions of plasma concentration, PT with 6 reagents, and aPTT with 4 reagents under edoxaban therapy in Japanese patients with NVAF, showing their 90% intervals. For predicting the upper outlier of PC-Ed, PT was more sensitive compared with aPTT, whereas predicting capability for the outliers of PC-Ed was mostly similar between peak and trough samplings among PT reagents (UMIN 000032492).

Keywords Atrial fibrillation · Anticoagulation · Edoxaban

Introduction

Edoxaban, one of the direct oral anticoagulants (DOACs), directly inhibits factor Xa activity [1]. Due to its predictable pharmacokinetic and pharmacodynamic profiles, monitoring is not primarily recommended. However, a sub-analysis of the phase III study of edoxaban revealed that some of the patients under labelling dosing had extremely high/low plasma concentrations which increased the incidence of bleeding/thrombotic events, respectively [2]. In this sense, extremely high/low plasma concentrations of edoxaban (PC-Ed) should be properly evaluated in daily clinical practice, especially in patients with high risks of bleeding or in patients at an emergency state, including severe bleeding, symptomatic thromboembolic events, or emergent invasive procedure [3, 4]. For this purpose, the basic information of adequate PC-Ed in daily clinical practice is necessary.

Recently, an "on therapy range" of plasma concentration of DOACs has been proposed [5, 6], which is defined as 90% intervals of a distribution of plasma concentration in a particular cohort under a DOAC treatment. Its concept is to obtain information on the adequate plasma concentration

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under a DOAC treatment easily without accumulating a huge number of data sets with plasma concentration and patient outcomes.

It is common knowledge that routine coagulation tests, i.e., prothrombin time (PT) and activated partial thromboplastin time (aPTT), generally do not provide an accurate assessment of the anticoagulant effects of edoxaban. In contrast, the latter can be measured via anti-Xa assays [5]. However, in Japan, anti-Xa assays are not still covered by insurance, and therefore, until now, many physicians in Japan attempt to judge the intensity of edoxaban by PT or aPTT. Although it has been already demonstrated that edoxaban produced a concentration-dependent prolongation of PT and aPTT and that the magnitude of concentration-dependent increase was different according to the reagents [4, 7, 8], these data were obtained in in vitro studies using plasma from healthy volunteers. The correlation of PT and aPTT with edoxaban concentration in patients with non-valvular AF (NVAF) has been reported recently from Italy [9]; however, only a single reagent for PT and aPTT was used. Thus, data in daily clinical practice on the reagent-specific distribution of PT and aPTT under edoxaban therapy in Japanese patients with NVAF are still lacking.

The objectives of this study were to show the distribution of PC-Ed and to determine the relationship between PC-Ed and commonly used anticoagulant test (PT and aPTT) in Japanese NVAF patients.

Methods

Objectives

This was a prospective observational study, registering Japanese patients with NVAF under treatment with edoxaban (UMIN Clinical Trials Registry: UMIN 000032492). The primary objective of this study was to show the distribution of PC-Ed and to determine the relationship between PC-Ed and prevalent anticoagulation markers of PT and aPTT in Japanese patients with NVAF.

Ethics and informed consent

This study was performed in accordance with the ethical norms based on the Declaration of Helsinki (revised in 2013) and Ethical Guidelines for Medical and Health Research Involving Human Subjects (Public Notice of the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labour and Welfare, Japan, issued in 2017). Written informed consent was obtained from all participants. The study protocol was reviewed by the Institutional Review Board of the Cardiovascular Institute.

Study population

Patients with NVAF under edoxaban therapy for at least 2 weeks with the aim of prevention of ischemic stroke that visited the Cardiovascular Institute were eligible in the present study. The exclusion criteria were as follows: (1) receiving dual anti-platelet therapy; (2) inadequate dosage of edoxaban at registration; (3) edoxaban hypersensitivity; (4) patients who are bleeding; (5) patients with acute bacterial endocarditis; (6) renal dysfunction (creatinine clearance < 30 mL/min); (7) liver dysfunction with clotting disorders; (8) patients who had admitted with cardiovascular disease (stroke, myocardial infarction, percutaneous coronary intervention, and heart failure) or bleeding requiring hospitalization within 1 month before the registration; (9) patients who did not give written informed consents for this study; and (10) patients who are judged by the researchers as inadequate for this study.

In total, 106 patients eligible for the inclusion criteria were registered between June 26, 2017 and March 27, 2018 (Fig. 1). Excluding 2 patients who withdrew the informed consent, remaining 104 patients were study population in the present study. For these patients, baseline data were obtained

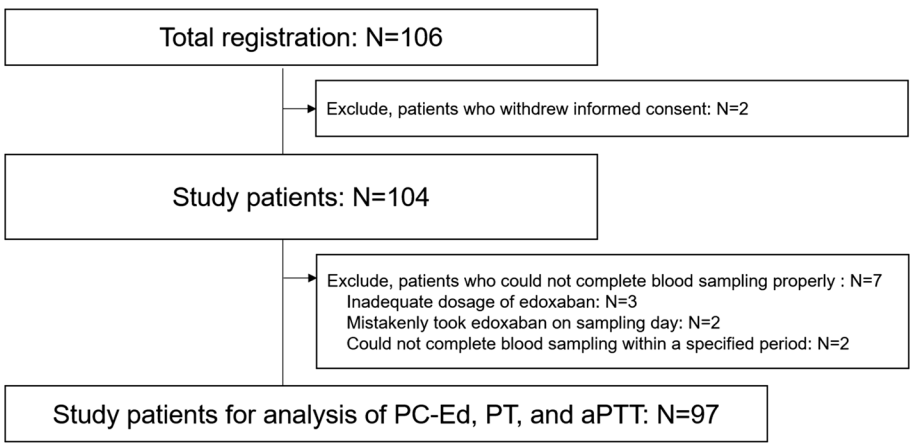

Fig. 1 Flow chart

including patient profiles (age, sex, height, weight, edoxaban dose, and AF types), risk factors (history of smoking and drinking, hypertension, diabetes mellitus, dyslipidemia, history of cerebral infarction or transient ischemic attack, chronic obstructive pulmonary disease, aortic disease, history of cerebral bleeding, bleeding disease, history of major bleeding, active cancer, and use of CYP3A4 inhibitors or P-glycoprotein inhibitors), and organic heart disease (heart failure, mitral regurgitation, aortic regurgitation, aortic stenosis, tricuspid regurgitation, history of myocardial infarction, history of percutaneous intervention, history of coronary artery bypass graft, hypertrophic cardiomyopathy, and dilated cardiomyopathy). Thereafter, they were followed up for maximum 6 months.

Furthermore, blood sampling was attempted twice [at peak (2–4 h post-dose) and trough (pre-dose)] to the 104 study patients at outpatient clinic during the 6 month period. At each timepoint, PC-Ed, PT, aPTT, and serum creatinine were measured, and creatinine clearance (CCr) was calculated by the Cockcroft–Gault equation [10]. Among them, proper blood samplings could not be obtained in 7 patients due to following reasons: 3 patients had inadequate dosage of edoxaban due to a temporal decline of CCr, 2 patients mistakenly took edoxaban on the sampling day (very-elderly patients aging of 86 and 87 years), and 2 patients could not complete blood sampling within a specified period. Among the 2 patients who could not complete blood sampling, 1 patient was admitted to another hospital with ischemic stroke after the registration, but before the blood sampling. Accordingly, after excluding the 7 patients above, the remaining 97 patients who properly completed the blood sampling both at peak and trough were the study patients for the analysis of PC-Ed, PT, and aPTT.

Plasma concentration and PD measurement

PC-Ed was measured using a validated chromatographic assay with liquid chromatography–tandem mass spectrometry (LC–MS/MS) at Shin Nippon Biomedical Laboratories (Tokyo, Japan) [11]. All plasma samples were stored at – 30 to – 10 °C and analyzed within 6 months after sampling. It was confirmed that the analyte was stable for this time period. Isotope-labeled edoxaban was used as an internal standard. The calibration range of the procedure was from 1 ng/mL (lower limit of quantification: LLOQ) to 500 ng/mL. Quality control samples at concentrations of 2, 25, and 400 ng/mL were determined with an accuracy of 96.8–105.2%, 95.6–106.0%, and 95.8–103.0%, respectively.

PT and aPTT measurements were performed using multiple reagents which are commonly used in Japan [12] and/or known to have a good response to edoxaban [8]. In PT measurement, the different thromboplastin reagents used were Thromborel S® and Thrombocheck PT® (Sysmex, Kobe, Japan); HemosIL RecombiPlasTin 2G® (IL Japan, Tokyo, Japan); Neoplastin Plus® (Diagnostica Stago, Asnières, France); Coagupia PT–N® (Sekisui Medical, Tokyo, Japan); and Simplastin Excel S® (Kyowa Medex, Tokyo, Japan). Thrombocheck PT, Neoplastin Plus, and Coagupia PT–N are derived from rabbit brain. Simplastin Excel S is derived from rabbit thromboplastin. HemosIL RecombiPlasTin 2G is recombinant human thromboplastin, whereas Thromborel S is derived from human placenta. Clotting time of plasma samples using Thromborel S or Thrombocheck PT was measured with a CS-2000i® coagulometer (Sysmex, Kobe, Japan); HemosIL RecombiPlasTin 2G was measured with a ACL-TOP® coagulometer (IL Japan, Tokyo, Japan); Neoplastin Plus was measured with a STA-compact® coagulometer (Diagnostica Stago, Asnières, France); Coagupia PT–N was measured with a CP-3000® coagulation analyzer (Sekisui Medical, Tokyo, Japan); and Simplastin Excel S was measured with a COAGTRON-180® coagulation analyzer (Kyowa Medex, Tokyo, Japan).

In aPTT measurement, the thromboplastin reagents used were Datafi APTT®, Thrombocheck APTT®, and Thrombocheck APTT SLA® (Sysmex, Kobe, Japan), and Platelin LS II® (Kyowa Medex, Tokyo, Japan). Datafi APTT and Thrombocheck APTT are derived from rabbit brain, whereas Platelin LS II is derived from swine and domestic fowl. Thrombocheck APTT SLA is synthetic phospholipids. Clotting time of plasma samples using Datafi APTT, Thrombocheck APTT, and Thrombocheck APTT SLA was measured with a CS-2000i® coagulometer (Sysmex, Kobe, Japan), and Platelin LS II was measured with a COAGTRON-180® coagulation analyzer (Kyowa Medex, Tokyo, Japan).

Adverse events

During the follow-up period of 6 months, adverse events including all-cause death, stroke, systemic thromboembolism, bleeding requiring hospital admission, and cardiovascular events requiring hospital admission (acute myocardial infarction, unstable angina pectoris, percutaneous coronary intervention, and heart failure) were monitored.

Statistical analysis

Categorical and consecutive data are presented as number (%) and mean ± standard deviation, respectively. For PC-Ed, PT, and aPTT, 90% interval, minimum value (min), and maximum value (max) were described. The correlation between PT/aPTT and PC-Ed was analyzed by Pearson's coefficient of correlation. The upper outlier of PC-Ed was defined as > 90% interval (higher than the value of upper 5%). The predictive capability of PT and aPTT for the outlier of PC-Ed was assessed by the receiver operating curve (ROC) analysis [13]. With the cut-off value obtained by

Youden index [14], sensitivity and specificity were calculated for each reagent. The cut-off value, sensitivity, and specificity for each reagent were calculated when the AUC was statistically significant. Statistical analyses were performed using SAS for Windows version 9.4 (SAS Institute Inc., North Carolina, US). In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results

Patient characteristics

Patient characteristics are shown in Table 1. Among the 97 patients, the standard dose (60 mg) and reduced dose (30 mg) were prescribed for 48 and 49 patients, respectively. The patient population included 71 (73.2%) men, and the subjects had a mean age of 69 years. $CHADS_2$ scores 0, 1, and ≥ 2 were 26.8%, 44.3%, and 28.9%, respectively (Table 2).

Table 1 Patient characteristics

$N = 97$	Mean	SD
Age (years)	69	10
Height (cm)	165.1	9.6
Weight (kg)	65.2	15.2
BMI (kg/m^2)	23.7	4.0
Serum creatinine (mg/mL)	0.9	0.2
Creatinine clearance (mL/min)	75.1	29.6
	N	%
Male	71	73.2
Female	26	26.8
Edoxaban dose		
60 mg once daily	48	49.5
30 mg once daily	49	50.5
Types of atrial fibrillation		
Paroxysmal	59	60.8
Persistent	15	15.5
Permanent	23	23.7
Risk factors		
Age ≥ 75 years	62	63.9
Weight ≤ 60 kg	41	42.3
Creatinine clearance < 50 mL/min	22	22.7
History of smoking	10	10.3
History of drinking	60	61.9
Hypertension	56	57.7
Diabetes mellitus	15	15.5
Dyslipidemia	34	35.1
Heart failure	11	11.3
History of cerebral infarction or transient ischemic attack	6	6.2
Aortic disease	0	0.0
History of cerebral bleeding	1	1.0
Bleeding disease	6	6.2
History of major bleeding	2	2.1
Chronic obstructive pulmonary disease	1	1.0
Active cancer	0	0.0
Use of CYP3A4 inhibitors	4	4.1
Use of P-glycoprotein inhibitors	7	7.2

Distributions of plasma concentration, PT, and aPTT

The distributions of PC-Ed, PT, and aPTT values are displayed in Table 3. The mean PC-Ed (90% interval, min/max) were 194.3 (49.4–345.3, 38.5/462.0) and 17.0 (4.8–40.7, 2.3/70.6) ng/mL at peak and trough, respectively.

The mean PT values (90% interval) at peak were 24.7 (17.4–32.8) s and 16.0 (12.8–19.5) s in assays using Simplastin Excel S and HemosIL RecombiPlasTin 2G, respectively, which were the highest and the lowest among PT values measured using the 6 reagents. Similarly, the mean PT values (90% interval) at trough were 15.2 (13.3–17.6) s and 12.3 (11.0–13.9) s in assays using Simplastin Excel S and HemosIL RecombiPlasTin 2G, respectively, which were the highest and the lowest among PT values measured using the 6 reagents.

The mean aPTT values (90% interval) at peak were 47.1 (36.7–58.7) s and 38.5 (32.2–48.9) s in assays using Thrombocheck APTT and Thrombocheck APTT SLA, respectively, which were the highest and the lowest among aPTT values measured using the 4 reagents. Similarly, the mean aPTT values (90% interval) at trough were 36.1 (31.2–45.0) s and 32.6 (27.7–38.5) s in assays using Thrombocheck APTT and Thrombocheck APTT SLA, respectively, which were the highest and the lowest among aPTT values measured using the 4 reagents.

Correlations between plasma concentration and PT or aPTT

In Fig. 2, the correlations of PT (2A–2F) and aPTT (2G–2J) with PC-Ed are shown. The correlation coefficients between PC-Ed and PT measured with Thromborel S, Thrombocheck PT, HemosIL RecombiPlasTin 2G, STA Neoplastin Plus, Coagpia PT–N, and Simplastin Excel S were 0.909, 0.949, 0.911, 0.931, 0.928, and 0.943 (all, $p < 0.001$), respectively. Meanwhile, the correlation coefficients between PC-Ed and aPTT were much lower than those for PT, which were 0.773, 0.779, 0.543, and 0.516 in Datafi APTT, Thrombocheck APTT, Thrombocheck APTT SLA, and Platelin LS II, respectively (all, $p < 0.001$).

Table 2 Distribution of risk scores

Risk scores	$N=97$	%
CHADS2 score		
0	26	26.8
1	43	44.3
2	16	16.5
3	8	8.2
4	4	4.1
≥5	0	0.0
CHA2DS2-VASc score		
0	14	14.4
1	16	16.5
2	29	29.9
3	25	25.8
4	8	8.2
5	3	3.1
6	2	2.1
≥7	0	0.0
HAS-BLED score		
0	17	17.5
1	29	29.9
2	39	40.2
3	12	12.4
≥4	0	0.0

Predictive capability of PT and aPTT for an upper outlier of PC-Ed

At peak sampling, the upper outlier of PC-Ed defined as over the upper range of 90% interval was > 345.3 ng/mL. Whether PT and aPTT can predict the upper outlier of PC-Ed was assessed by the ROC analysis (Table 4). The areas under the ROC curve (AUCs) with the 6 different PT reagents ranged within relatively high values: Coagupia PT–N showed the highest AUC of 0.859 (95% CI 0.717–1.000; $P<0.001$), whereas HemosIL Recombi-PlasTin 2G showed the lowest AUC of 0.738 (95% CI 0.420–1.000; $P=0.142$). Meanwhile, the AUCs with the 4 aPTT reagents ranged in relatively lower values: only Thrombocheck APTT showed a statistically significant AUC of 0.808 (95% CI, 0.667–0.948; $P<0.001$).

At trough sampling, the upper outlier of PC-Ed was > 40.7 ng/mL. A similar ROC analysis was performed (Table 4). The AUCs with the 6 different PT reagents ranged within relatively high values: Simplastin Excel S showed the highest AUC of 0.869 (95% CI 0.745–0.992; $P<0.001$), whereas Thrombocheck PT showed the lowest AUC of 0.769 (95% CI 0.590–0.947; $P<0.001$). Meanwhile, the AUCs with the 4 aPTT reagents ranged in lower values and were not statistically significant.

With the cut-off values of PT and aPTT for predicting the upper outlier of PC-Ed in each reagent, there was a mostly consistent tendency that the sensitivity was high, and the specificity was relatively low.

Adverse events

Among the 97 patients analyzed on PC-Ed, all-cause death, stroke, systemic embolism, bleeding requiring hospitalization, and cardiovascular events requiring hospitalization were not observed during the observation period. As described in "Methods", among the 7 patients excluded from the analysis of PC-Ed, PT, and aPTT due to failing to obtain proper blood samplings, 1 patient was admitted with ischemic stroke after registration but before blood sampling.

Discussion

We determined the distribution of plasma concentration, PT with 6 reagents, and aPTT with 4 reagents under edoxaban therapy in Japanese patients with NVAF, showing their 90% intervals ("on therapy range"[5, 6]). Among 6 PT and 4 aPTT reagents prevalent in Japan, the correlation coefficient exceeded 0.9 in all PT reagents, while in aPTT reagents, the correlation coefficient ranged below 0.8. The predictive capability for the upper outlier of PC-Ed at peak and trough was the highest in Coagupia PT–N and Simplastin Excel S, both were PT reagents, whereas that were the lowest in Platerin LS II and Datafi APTT, both were aPTT reagents, respectively. Among PT reagents, both peak and trough sampling showed a similar predictive capability for the upper outlier of PC-Ed, with high sensitivity, but with modest specificity.

Distribution of plasma concentration of edoxaban

Our data demonstrated a distribution of PC-Ed in Japanese patients with NVAF, with the mean value of 194.3 (90% interval 49.4–345.3) ng/mL and 17.0 (90% interval 4.8–40.7) ng/mL at peak and trough, respectively. As a sub-analysis of ENGAGE-AF, a distribution of PC-Ed sampled at trough was reported, where the median (inter quartile range) of 60 mg and 30 mg with dose reduction was 36.1 (19.4–62.0) ng/mL and 27.0 (14.6–44.6) ng/mL, respectively [2]. In the report of Japanese NVAF patients with a small population, median (minimum–maximum) pre-dose PC-Ed in patients with normal-to-mild renal impairment under 30 mg and 60 mg dosing were 9.66 (3.88–23.2) and 18.2 (6.86–29.5) ng/mL, respectively, whereas 1–3 h post-dose PC-Ed under similar dosing were 75.7 (7.2–243.0) and 170 (16.7–537.0) ng/mL, respectively [15]. Thus, PC-Ed by edoxaban dosing and by the

Table 3 Plasma concentration of edoxaban by LC–MS/MS method, prothrombin time, and activated partial thromboplastin time under edoxaban therapy at peak and trough

Reagents			Number of samples	Mean	90% intervals		Min	Max
Plasma concentration of edoxaban (ng/mL)		Peak	97	194.3	49.4	345.3	38.5	462.0
		Trough	97	17.0	4.8	40.7	2.3	70.6
PT (s)	Thromborel S	Peak	97	16.4	13.2	19.9	11.9	22.1
	(Reference value: 9.8–12.1 s)[a]	Trough	97	12.6	11.3	14.3	10.8	14.8
	Thrombocheck PT	Peak	97	16.9	13.2	20.5	12.2	23.2
	(Reference value: 10.8–13.5 s)[b]	Trough	97	12.5	11.3	13.7	10.9	14.1
	HemosIL RecombiPlasTin 2G	Peak	97	16.0	12.8	19.5	11.9	21.8
	(Reference value: 9.4–12.5 s)[b]	Trough	97	12.3	11.0	13.9	10.6	14.4
	Neoplastin Plus	Peak	97	19.2	15.1	24.0	13.7	27.8
	(Reference value: 11.2–17.0 s)[b]	Trough	97	13.6	12.3	15.2	11.8	16.0
	Coagupia PT–N	Peak	96[d]	21.3	15.8	27.3	14.1	31.5
	(Reference value: < 14 s)[b]	Trough	97	13.9	12.4	16.1	11.8	17.1
	Simplastin Excel S	Peak	97	24.7	17.4	32.8	15.3	35.3
	(Reference value: 12.6–15.7 s)[c]	Trough	97	15.2	13.3	17.6	12.7	18.8
aPTT (s)	Datafi APTT	Peak	97	43.1	34.3	54.7	32.7	62.3
	(Reference value: 24–34 s)[e]	Trough	97	33.3	27.0	39.2	25.7	44.3
	Thrombocheck APTT	Peak	97	47.1	36.7	58.7	34.1	79.1
	(Reference value: 25–35 s)[e]	Trough	97	36.1	31.2	45.0	27.3	48.7
	Thrombocheck APTT SLA	Peak	97	38.5	32.2	48.9	30.8	69.4
	(Reference value: 24–32 s)[e]	Trough	97	32.6	27.7	38.5	25.0	48.4
	Platelin LS II	Peak	97	39.4	33.2	55.4	30.0	63.0
	(Reference value: 25–40 s)[e]	Trough	97	33.6	28.0	40.1	27.1	53.3

[a]The reference value of Thromborel S was the 95% interval of the range of PT measurement with control blood plasma written on the product label

[b]The reference values of ThromboCheck PT, HemosIL RecombiPlasTin 2G, Neoplastin Plus, and Coagupia PT–N were the range of PT measurement with control blood plasma written on the product label

[c]The reference value of Simplastin Excel S was the range of PT measurement in 80 healthy subjects by Kyowa Medex (not written on the product label)

[d]One sample in PT by Coagupia PT–N at peak could not be measured because of chyle

[e]The reference values of Datafi APTT, ThromboCheck APTT, ThromboCheck APTT SLA, and Platelin LS II were the range of aPTT measurement with control blood plasma written on the product label

timing of blood sampling (peak or trough) was mostly similar in the present study and the previous studies.

In this study, our data proposed the 90% intervals of PC-Ed in patients with NVAF using a relatively small Japanese cohort. No stroke, systemic embolism, and bleeding requiring hospitalization occurred during the observation period, which partially indicates that the range of PC-Ed in the present study would be safe. However, of course, we should be aware that it is not an absolute "on therapy range", but just one of the examples. We should further accumulate the data set of plasma concentration and patient outcome with NVAF patients under edoxaban therapy in different patient characteristics.

Several clinical factors that affect the PC-Ed would be speculated, including renal function, body weight, and coadministration of p-glycoprotein inhibitors, which should be confirmed in pharmacokinetic analysis [16–21].

Correlations of PT and aPTT with edoxaban plasma concentration

In the present study, the correlation coefficient exceeded 0.9 in PT reagents which ranged from 0.909 in Thromborel S to 0.949 in Thrombocheck PT. Meanwhile, in aPTT reagents, the correlation coefficient was relatively low (ranging from 0.516 in Platelin LS II to 0.779 in Thrombocheck APTT). So far, there have been several reports of reagent-specific responses of PT and aPTT to edoxaban in plasma from healthy volunteers [4, 7, 8], but data of NVAF patients in daily clinical practice are scarce. A recent report from Italy demonstrated that the PT with Neoplastin CI Plus and the aPTT with Cephascreen showed correlation coefficients of 0.93 and 0.70, respectively [9]. These values were comparable to our data, indicating that the values does not contradict each other.

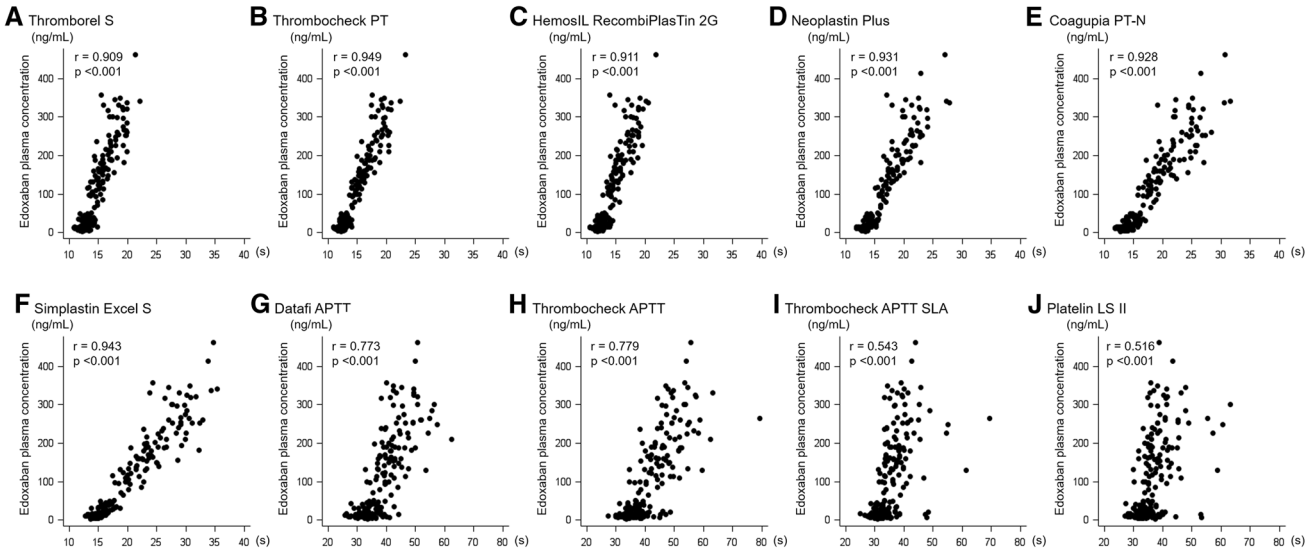

Fig. 2 Correlation of prothrombin time (**a**–**f**) and activated partial thromboplastin time (**g**–**j**) with PC-Ed. Data at peak and trough were combined. The data points in each reagent were 194, except for Coagupia PT-N, where 1 sample at peak could not be measured because of chyle.

Table 4 Receiver operating curve analysis for prediction of upper outlier of edoxaban plasmaconcentration by prothrombin time and activated partial thromboplastin time at peak and trough

	Reagents		AUC	95% confidence intervals		*P* value	Cut-off	Sensitivity	Specificity
PT (s)	Thromborel S	Peak	0.755	0.552	0.959	0.014	17.8	0.80	0.74
		Trough	0.827	0.698	0.957	< 0.001	12.7	1.00	0.61
	Thrombocheck PT	Peak	0.841	0.718	0.964	< 0.001	17.6	1.00	0.65
		Trough	0.768	0.590	0.947	0.003	12.7	0.80	0.74
	HemosIL RecombiPlasTin 2G	Peak	0.738	0.420	1.000	0.142	–	–	–
		Trough	0.777	0.619	0.935	< 0.001	12.3	1.00	0.55
	Neoplastin Plus	Peak	0.746	0.488	1.000	0.062	–	–	–
		Trough	0.832	0.645	1.000	< 0.001	14.3	0.80	0.85
	Coagupia PT-N	Peak	0.859	0.717	1.000	< 0.001	22.2	1.00	0.67
		Trough	0.790	0.640	0.941	< 0.001	14.6	0.80	0.76
	Simplastin Excel S	Peak	0.826	0.650	1.000	< 0.001	24.3	1.00	0.54
		Trough	0.868	0.745	0.992	< 0.001	15.5	1.00	0.64
aPTT (s)	Datafi APTT	Peak	0.675	0.468	0.882	0.097	–	–	–
		Trough	0.539	0.280	0.798	0.789	–	–	–
	Thrombocheck APTT	Peak	0.808	0.667	0.948	< 0.001	53.6	0.80	0.85
		Trough	0.483	0.237	0.728	0.899	–	–	–
	Thrombocheck APTT SLA	Peak	0.729	0.476	0.983	0.076	–	–	–
		Trough	0.363	0.182	0.544	0.173	–	–	–
	Platelin LS II	Peak	0.638	0.412	0.864	0.230	–	–	–
		Trough	0.430	0.167	0.694	0.640	–	–	–

AUC area under curve, *PT* prothrombin time, *aPTT* activated partial prothrombin time

The cut-off value, sensitivity, and specificity for each reagent were calculated when the AUC was statistically significant (the *P* value was < 0.05)

Estimating outlier of edoxaban plasma concentration by PT and aPTT

In the present study, the outlier of PC-Ed was defined as above the upper borderline of the 90% intervals (≥345.3 ng/mL and ≥40.7 ng/mL at peak and trough, respectively), and the cut-off values of PT or aPTT estimating the outlier of PC-Ed were determined by the ROC analysis.

Among PT reagents, the predicting ability by AUC was the highest in Coagupia PT–N (0.859) at peak, while at trough, it was the highest in Simplastin Excel S (0.869). It is of note that the cut-off values in each PT reagent mostly demonstrated a high sensitivity (0.8–1.0), but the specificity was mostly modest (0.5–0.8). It means that, when judging the outlier of PC-Ed by PT, false negative is rare, but false positive would be relatively frequent.

Among aPTT reagents, only Thrombocheck APTT measured at peak had a statistically significant AUC. Otherwise, aPTT did not have sufficient predictive ability to discriminate the outlier of PC-Ed.

Distribution of PT values at peak and trough

The adequate timing of measurement of PT (peak or trough) has often been discussed, but there are both advantages and disadvantages to measurement at peak and trough [22]. When PT is measured at the presumptive peak, the wide distribution allows discrimination of high and low responses, but the plasma concentration changes rapidly around the true peak time, leading to fluctuation between measurements. Conversely, when PT is measured at the trough, the time variation of plasma concentration is small, but narrow distribution limits discrimination of high and low responses. However, in the present study, the predictive capability of PT for an upper outlier of PC-Ed was similar between measurement at peak and trough, although a small difference can be observed by reagents. Therefore, in terms of discrimination of upper outliers with high PC-Ed, measurement at peak and trough does not seem to make a difference. So far, evidence showing the association between upper outliers with high PC-Ed and bleeding events has been scarce, but at least, the results from phase II and III trials of edoxaban in NVAF patients [2, 23] suggested that PC-Ed at trough sampling may well discriminate upper outliers of PC-Ed who would actually encounter bleeding events.

Future perspective

As these reagents are commonly used in daily clinical practice in Japan, we believe that the information helps the judgement of attending physicians who prescribe edoxaban for NVAF patients in Japan. However, at the same time, as the statement has been pointed out [5], we should aware that the accuracy of the judgement of overdosing of edoxaban by commonly used anticoagulation tests including PT and aPTT is limited, and the spread of more accurate methods including anti-Xa assays should be urgent [3, 24].

Limitations

Our study had several limitations. First, the number of patients included in the study was relatively small. Second, our data were derived from a single cardiovascular hospital. These factors may affect 90% interval of PC-Ed and the cut-off values of PT and aPTT for detecting outliers, and therefore, caution is necessary when extrapolating our data to other cohorts.

Conclusions

We determined the distribution of plasma concentration, PT with 6 reagents, and aPTT with 4 reagents under edoxaban therapy in Japanese patients with NVAF, showing their 90% intervals (namely, "on therapy range"). For predicting upper outliers of PC-Ed, PT was more sensitive compared with aPTT, and among PT reagents, the predicting capability for outlier of PC-Ed was mostly similar between peak and trough samplings. However, the accuracy of such anticoagulation tests to assess the anticoagulant effects of edoxaban is limited, and the spread of more accurate methods including anti-Xa assays would be urgent.

Compliance with ethical standards

Conflict of interest This work was financially supported by Daiichi Sankyo. Dr. Suzuki received research funding from Daiichi Sankyo and Mitsubishi-Tanabe. Dr. Yamashita received research funding from Boehringer Ingelheim and Daiichi Sankyo, and remuneration from Boehringer Ingelheim, Daiichi Sankyo, Bayer Healthcare, Pfizer, Bristol-Myers Squibb, Eisai and Ono Pharmaceutical. Dr. Morishima and Mr. Takita are employees of Daiichi Sankyo. The management of this work was supported by Cardiovascular Institute Academic Research Organization (CVI ARO) [25–34].

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