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Original Article

The influence of residual apixaban on bleeding complications during and after catheter ablation of atrial fibrillation

Yutaro Mukai [a,c], Kyoichi Wada [a,c], Koji Miyamoto [b], Kazuki Nakagita [a,c], Mai Fujimoto [d], Kouichi Hosomi [d], Takeshi Kuwahara [e], Mitsutaka Takada [c,d,*], Kengo Kusano [b], Akira Oita [a]

[a] Department of Pharmacy, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan
[b] Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan
[c] Division of Cardiovascular Drugs, Therapy, Kindai University Graduate School of Pharmacy, Higashi-osaka, Japan
[d] Division of Clinical Drug Informatics, Kindai University School of Pharmacy, Higashi-osaka, Japan
[e] Department of Pharmacy, National Center for Global Health and Medicine, Tokyo, Japan

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ABSTRACT

Background: The periprocedural protocol for atrial fibrillation (AF) ablation commonly includes anticoagulation therapy. Apixaban, a direct oral anticoagulant, is currently approved for clinical use; however, little is known about the effects of residual apixaban concentration on bleeding complications during/after AF ablation. Therefore, we measured residual apixaban concentration by using mass spectrometry and examined the anticoagulant's residual effects on bleeding complications.

Methods: Fifty-eight patients (Mean age of 64.7 ± 12.5 years; 31 males, 27 females) were enrolled and administered apixaban twice daily. We analyzed trough apixaban concentration, activated clotting time (ACT), heparin dose, and bleeding complications during/after AF ablation. Apixaban concentrations were directly measured using mass spectrometry.

Results: Bleeding complications were observed in 19 patients (delayed hemostasis at the puncture site, 16; hematuria, 3; hemosputum, 1). No patient required blood transfusion. The mean trough apixaban concentration was significantly lower in patients with bleeding complications than without (152.4 ± 73.1 vs. 206.8 ± 98.8 ng/mL respectively, $P=0.037$), while the heparin dose to achieve ACT > 300 s was significantly higher in patients with bleeding complications (9368.4 ± 2929.0 vs. 7987.2 ± 2135.2 U/body respectively, $P=0.046$). Interestingly, a negative correlation was found between the trough apixaban concentration and the heparin dose to achieve ACT > 300 s ($P=0.033$, R=-0.281).

Conclusions: Low residual plasma apixaban is associated with a higher incidence of bleeding complications during/after AF ablation, potentially because of a greater heparin requirement during AF ablation.

1. Introduction

Catheter ablation (CA) is an effective treatment for patients with atrial fibrillation (AF). During CA in patients with AF, bleeding at the puncture site can be a significant problem because periprocedural anticoagulation therapy is continued to prevent thromboembolism and stroke. Some large clinical studies reported that uninterrupted warfarin therapy just before CA is a safe strategy, because intravenous unfractionated heparin (UFH) bridging is not required [1–3]. Therefore, in recent years, a strategy of uninterrupted warfarin therapy was frequently selected for warfarin users to avoid thromboembolism and stroke during the CA periprocedural period [4–7].

Furthermore, UFH has been used to prevent catheter-induced thrombosis during CA, with a recommended activated clotting time (ACT) of over 300 s. This approach seems to decrease the amount of UFH required to achieve the target ACT. Some studies report an inverse relationship between the international normalized ratio of prothrombin time (PT-INR) value and UFH dosage in warfarin users during the CA periprocedural period. Therefore, the required UFH dosage was decreased in patients with higher PT-INR values, reducing the risk of hemorrhagic complications [8,9].

Since dabigatran was approved in 2011 in Japan, direct oral anticoagulants (DOACs) have often been used for anticoagulation

* Corresponding author at: Division of Clinical Drug Informatics, Kindai University School of Pharmacy, 577-8502, 3-4-1, Kowakae, Higashi-osaka, Osaka 577-8502, Japan. Fax: +81 6 6730 1394.
E-mail addresses: mukai.yutaro.hp@ncvc.go.jp (Y. Mukai), kwada@ncvc.go.jp (K. Wada), koji3koji3@gmail.com (K. Miyamoto), okimono223@ncvc.go.jp (K. Nakagita), fujimoto-m@phar.kindai.ac.jp (M. Fujimoto), hosomi@phar.kindai.ac.jp (K. Hosomi), tkuwahara@hosp.ncgm.go.jp (T. Kuwahara), takada@phar.kindai.ac.jp (M. Takada), kusanokengo@ncvc.go.jp (K. Kusano), oitaa8354@ncvc.go.jp (A. Oita).

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therapy in patients with AF. Several studies reported that uninterrupted administration of apixaban (a blood coagulation factor Xa inhibitor) was beneficial in preventing thromboembolism and stroke during the periprocedural period when CA is used to treat AF [10–13]. Notably, excessive anticoagulation during the CA periprocedural period could increase the risk of bleeding, and inadequate anticoagulation would be expected to increase the risk of stroke. However, the relationship between plasma apixaban concentration and the UFH dosage required during CA is unclear.

The purpose of this study was to examine the relationship between serum apixaban concentration and the UFH dosage required during CA in AF patients, and to determine the optimum plasma apixaban concentration to minimize the risk of thromboembolism and bleeding complications.

2. Materials and methods

2.1. Subjects

AF patients who were treated with apixaban and CA between April 2015 and March 2016 at the National Cerebral and Cardiovascular Center (NCVC) were included in the study. Neither the type of AF (paroxysmal, persistent, or permanent) nor the technology for AF ablation (radiofrequency CA or cryoballoon ablation) was taken into account in case selection. Only patients who were treated with 5 mg/day or 10 mg/day for at least 21 days before CA, and who did not receive apixaban on the morning the CA procedure took place, were included in the study. Informed consent was obtained from all patients, and clinical data including drug dosage and the results of laboratory examinations were obtained from patient records. All patients were administered UFH 100 U/kg IV before transseptal access, and a continuous UFH infusion of 1000 U/h was started at the time of, or just after, transseptal access. This was followed by an additional bolus of UFH approximately every 30 min to maintain an ACT over 300 s during CA. Protamine was not routinely used prior to the removal of femoral sheaths. The relationship between the bolus UFH dosage and the development of bleeding complications at the puncture site was examined in the study. Delayed hemostasis at the puncture site was defined as a condition that required astriction and prolonged hospitalization. Creatinine clearance (Ccr) was calculated by the Cockcroft-Gault equation [14].

2.2. Measurement of plasma apixaban concentration

Apixaban trough concentrations were analyzed on the day of CA by high-performance liquid chromatography (HPLC, Nexera ×2®; Shimadzu Co., Kyoto, Japan) and mass spectrometry (MS, LCMS-8040®; Shimadzu Co., Kyoto, Japan). Plasma was pretreated by the SCLAM-2000® (Shimadzu Co., Kyoto, Japan) for sample processing. Apixaban was obtained from Funakoshi Co., Ltd (Tokyo, Japan). The SCLAM-2000® was programmed to perform protein precipitation using acetonitrile followed by filtration and sample collection. The mobile phase of the HPLC consisted of mobile phase A (0.1% formic acid/water) and mobile phase B (0.1% formic acid/methanol) and was pumped at a rate of 0.4 mL/min. The concentration of mobile phase B was increased from 5% to 100% during the first 4 min, maintained at 100% for 1 min, and then decreased to 5% initially and equilibrated for the following 3 min. A Mastro® C18 column (50 mm×2.1 mm×3 μm Shimadzu GLC Ltd., Kyoto, Japan) was used for HPLC. Apixaban was ionized with electrospray ionization (ESI)-positive mode, and monitored by the multiple reaction monitoring (MRM) mode with an *m/z* transition of 460.20–443.20.

Table 1
Characteristics of the study patients.

Number of patients	58
Age (yr)[a]	64.6 ± 12.5
Body weight (kg)[a]	61.8 ± 12.2
Body length (cm)[a]	162.2 ± 10.0
Body mass index (kg/m^2)[a]	23.3 ± 2.9
Sex (male/female)[a]	31 / 27
Apixaban dose/kg (mg/kg/day)[a]	0.154 ± 0.033
Previous history	
Congestive heart failure	6
Hypertension	24
Diabetes mellitus	6
Stroke	2
Low dose aspirin use	2
Other antiplatelet therapy	0
$CHADS_2$ score[a]	0.91 ± 1.01
HAS-BLED score[a]	1.09 ± 0.94
Bleeding complication	
Major bleeding	0
Minor bleeding	19
Delayed hemostasis at the puncture site	16
Hematuria	3
Hemosputum	1

[a] Mean ± SD

2.3. Statistical analysis

All continuous variables are expressed as the mean ± standard deviation (SD). Categorical variables are expressed as frequencies (percentages). A Student's *t*-test was used to compare continuous variables. The optimal apixaban cutoff concentration that predicted bleeding complications was calculated using receiver operating characteristic (ROC) curve analysis. A P value < 0.05 was considered statistically significant. All data were analyzed using JMP 9.0.0 (SAS Institute Inc., Cary, North Carolina, USA).

The adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated by multivariate regression analysis. Body weight, sex, and plasma trough apixaban concentration were included in the multivariate logistic regression model.

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committees of the NCVC [M27-085] on December 1, 2015.

3. Results

Characteristics of the study patients are presented in Table 1. A total of 58 patients (31 males and 27 females) were included in the study. The mean age of the study patients was 64.6 ± 12.5 years old. Eight patients were treated with low-dose apixaban (5 mg/day). Of them, 2 patients met dose reduction criteria (age ≥ 80 years and body weight ≤ 60 kg). The remaining 6 patients did not completely meet the approved dose reduction criteria (5 patients < 60 kg, 3 patients > 75 years old). No patient developed stroke, thromboembolism, or major bleeding such as brain bleeding or cardiac tamponade, and no patient required blood transfusion. There were 16 patients with delayed hemostasis at the puncture site, 3 patients with hematuria, and 1 patient with hemosputum. Of the 16 patients with delayed hemostasis at the puncture site, two patients were diagnosed with hematoma by ultrasonic echo. Hematuria and hemosputum occurred in patients with higher apixaban concentrations. These patients displayed normal renal functions, and the cause of bleeding could not be specified.

Table 2
Comparison of patients with (+) and without (−) bleeding complications.

	Bleeding complication (−)	Bleeding complication (+)	*P*
Number of patients	39	19	
Age (yr) [a]	64.4 ± 12.4	65.1 ± 13.0	0.850
Body weight (kg) [a]	60.9 ± 12.3	63.8 ± 12.1	0.402
Body length (cm) [a]	161 ± 10.2	164.7 ± 9.6	0.194
Body mass index (kg/m^2) [a]	23.3 ± 3.2	23.3 ± 2.3	0.975
Sex (male/female)	17 / 22	14 / 5	0.031[b]
Apixaban dose (mg/kg/day) [a]	0.15 ± 0.04	0.15 ± 0.03	0.666
Serum trough apixaban concentration (ng/mL) [a]	206.8 ± 98.8	152.4 ± 73.1	0.037[b]
Average time from blood collection to entrance to catheter room (min) [a]	236.0 ± 158.2	244.7 ± 154.4	0.843
Dosage of UFH to achieve ACT > 300 s (U/body) [a]	7987.2 ± 2135.3	9368.4 ± 2929.0	0.046[b]
Protamine use during CA procedure	0	1	0.148
Technology for ablation			
Radiofrequency catheter ablation / Cryoballoon ablation	31 / 8	15 / 4	0.962
Previous history			
Congestive heart failure	4	2	0.975
Hypertension	16	8	0.938
Diabetes mellitus	3	3	0.355
Stroke	1	1	0.608
Low dose aspirin use	1	1	0.608
Other antiplatelet therapy	0	0	NC
$CHADS_2$ score [a]	0.85 ± 0.93	1.05 ± 1.18	0.472
HAS-BLED score [a]	1.16 ± 1.07	1.16 ± 1.07	0.690
Laboratory data			
Total BIL (mg/dL) [a]	0.74 ± 0.48	0.81 ± 0.45	0.574
Direct BIL (mg/dL) [a]	0.19 ± 0.11	0.20 ± 0.10	0.799
AST (U/L) [a]	24.3 ± 7.2	25.3 ± 10.8	0.684
ALT (U/L) [a]	21.4 ± 8.7	23.4 ± 26.4	0.671
LDH (U/L) [a]	206.3 ± 38.8	212.3 ± 77.1	0.697
CK (U/L) [a]	115.1 ± 50.5	110.0 ± 59.1	0.734
eGFR (mL/min/1.73 m^2) [a]	66.5 ± 11.9	71.8 ± 19.8	0.209
Ccr (mL/min) [+]	75.4 ± 21.9	83.9 ± 41.3	0.312
SCr (mg/dL) [a]	0.79 ± 0.16	0.82 ± 0.17	0.643
RBC (×10^6/μL) [a]	4.48 ± 0.51	4.51 ± 0.48	0.786
HGB (g/dL) [a]	13.7 ± 1.4	14.3 ± 1.6	0.165
HCT (g/dL) [a]	41.1 ± 4.2	42.4 ± 4.2	0.274
PLT (×10^3/μL) [a]	209.5 ± 52.6	188.1 ± 29.3	0.105

ACT: activated clotting time, CA: catheter ablation, BIL: bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, CK: creatine kinase, eGFR: estimated glomerular filtration rate, Ccr: creatinine clearance, SCr: serum creatinine, RBC: red blood cell count, HGB: hemoglobin, HCT: hematocrit, PLT: platelet count
Ccr was calculated by the Cockcroft-Gault equation.
NC: not compared

[a] Mean ± SD.
[b] $P < 0.05$.

Comparison between patients with and without bleeding complication is presented in Table 2. A significant difference was detected between sex ($P=0.031$), the plasma trough apixaban concentration and the dosage of UFH to achieve ACT > 300 s. The plasma trough apixaban concentration was higher in patients without than with bleeding complications (206.8 ± 98.8 vs. 152.4 ± 73.1 ng/mL respectively, $P=0.037$). The dosage of UFH to achieve ACT > 300 s during CA was lower in patients without than with bleeding complications (7987.2 ± 2135.2 vs. 9368.4 ± 2929.0 U/Body, $P=0.046$).

The ROC curve analysis was performed using apixaban trough concentrations measured on the day of CA, and the optimal cutoff value to predict bleeding complications was 160 ng/mL (Fig. 1). Area under the curve (AUC) of the ROC curve was 0.665. Patients were divided into 2 groups; those with high plasma trough apixaban concentrations (≧160 ng/mL, $n=29$), and those with low concentrations (< 160 ng/mL, $n=29$) (Table 3). Of the 16 patients with delayed hemostasis at the puncture site, 14 patients were in the < 160 ng/mL group and 2 patients were in the ≧160 ng/mL group. The four patients with hematuria or hemosputum were in the ≧160 ng/mL group. There was a significant

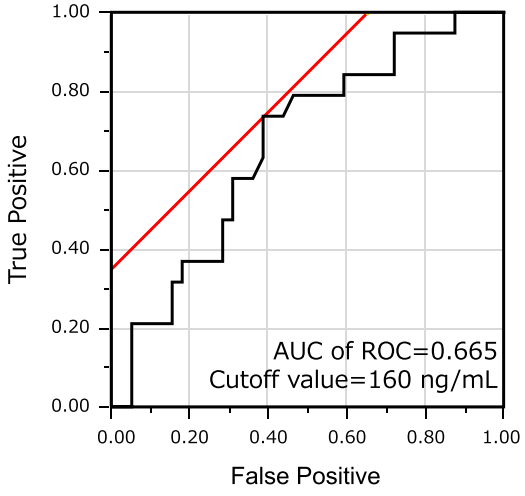

Fig. 1. ROC curve analysis using apixaban trough concentrations measured on the day of CA and bleeding complications. AUC of ROC=0.665, Cutoff value=160 ng/mL. ROC: receiver operating characteristic, CA: catheter ablation, AUC: area under the curve.

Table 3
Comparison of patients with plasma trough apixaban concentrations ≧160 ng/mL and with plasma trough apixaban concentrations < 160 ng/mL.

Trough apixaban concentration (ng/mL)	≧160	< 160	*P*
Number of patients	29	29	1.000
Age (yr)[a]	67.2 ± 10.3	62.1 ± 14.1	0.126
Body weight (kg)[a]	58.9 ± 10.9	64.7 ± 13.0	0.069
Body length (cm)[a]	159.7 ± 9.2	164.7 ± 10.4	0.056
Body mass index (kg/m^2)[a]	23 ± 3.0	23.7 ± 2.8	0.369
Sex (male/female)[a]	13 / 16	18 / 11	0.188
Apixaban dose (mg/kg/day)[a]	0.161 ± 0.034	0.146 ± 0.030	0.084
Protamine use during CA procedure	1	0	NC
Technology for ablation			
Radiofrequency catheter ablation / Cryoballoon ablation	22 / 7	24 / 5	0.516
Previous history			
Congestive heart failure	4	2	0.389
Hypertension	11	13	0.594
Diabetes mellitus	3	3	1.000
Stroke	2	0	0.092
Low dose aspirin use	1	1	1.000
Other antiplatelet therapy	0	0	NC
$CHADS_2$ score[a]	1.03 ± 1.14	0.79 ± 0.86	0.369
HAS-BLED score[a]	1.17 ± 0.97	1.00 ± 0.93	0.491
Laboratory data			
Total BIL (mg/dL)[a]	0.77 ± 0.55	0.75 ± 0.39	0.847
Direct BIL (mg/dL)[a]	0.2 ± 0.12	0.19 ± 0.10	0.715
AST (U/L)[a]	23.9 ± 8.1	25.3 ± 8.9	0.511
ALT (U/L)[a]	19 ± 9.5	25.1 ± 21.1	0.161
LDH (U/L)[a]	207.9 ± 42.7	208.7 ± 63.7	0.954
CK (U/L)[a]	102.5 ± 39.6	124.3 ± 62.4	0.118
eGFR (mL/min/1.73m^2)[a]	65.7 ± 15.2	70.9 ± 14.5	0.190
Ccr (mL/min)[a]	69.9 ± 19.3	86.4 ± 35.6	0.033[b]
SCr (mg/dL)[a]	0.8 ± 0.18	0.797 ± 0.14	0.839
RBC (×10^6/μL)[a]	3.95 ± 0.41	4.19 ± 0.52	0.052
HGB (g/dL)[a]	12.5 ± 1.4	13.0 ± 1.4	0.207
HCT (%)[a]	36.8 ± 4.2	38.5 ± 4.0	0.113
PLT (×10^3/μL)[a]	212.8 ± 54.5	192.1 ± 36.4	0.095
Bleeding complication			
Major bleeding			
Brain bleeding	0	0	NC
Cardiac tamponade	0	0	NC
Required blood transfusion	0	0	NC
Minor bleeding	5	14	0.012[b]
Delayed hemostasis at the puncture site	2	14	< 0.001[b]
Hematuria	3	0	0.075
Hemosputum	1	0	NC

BIL: bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, CK: creatine kinase, eGFR: estimated glomerular filtration rate, Ccr: creatinine clearance, SCr: serum creatinine, RBC: red blood cell count, HGB: hemoglobin, HCT: hematocrit, PLT: platelet count
Ccr was calculated by the Cockcroft-Gault equation.
NC: not compared

[a] Mean ± SD.
[b] $P < 0.05$.

difference in the incidence of delayed hemostasis at the puncture site between the two groups ($P < 0.001$). In addition, the group with an apixaban concentration of < 160 ng/mL showed a significantly lower creatinine clearance than that with > 160 ng/mL apixaban (69.9 ± 19.3 vs. 86.4 ± 35.6 mL/min, respectively, $P=0.033$). There were no significant differences in age, body weight, body mass index, sex, apixaban dose, estimated glomerular filtration rate, serum creatinine, and hemoglobin or platelet counts between the two groups.

The correlation of plasma trough apixaban concentration and dosage of UFH to achieve ACT > 300 s is shown in Fig. 2. A significant inverse correlation between plasma trough apixaban concentration and the dosage of UFH to achieve ACT > 300 s was observed ($P=0.033$, Fig. 2).

The dosage of UFH to achieve ACT > 300 s of the low (< 160 ng/mL) apixaban concentration group was higher than that of the high (≧160 ng/mL) apixaban concentration group (8948.3 ± 2515.5 U/body vs. 7931.0 ± 2389.4 U/body, respectively). However, no statistically significant difference was observed between these two groups ($P=0.060$).

The results of multivariable logistic regression analysis showed that a higher plasma trough apixaban concentration was significantly associated with a decreased risk of bleeding, (adjusted OR: 0.992, 95% CI: 0.983–0.999, $P=0.049$) and that male sex was significantly associated with an increased risk of bleeding (adjusted OR: 8.56, 95% CI: 1.423–67.992, $P=0.027$) (Table 4).

4. Discussion

The study showed that the plasma trough apixaban concentration was significantly lower in patients with bleeding complications than without. Furthermore, the dosage of UFH to achieve ACT > 300 s during CA was higher in patients with bleeding complications. In addition, a significant negative correlation was observed between the plasma trough apixaban

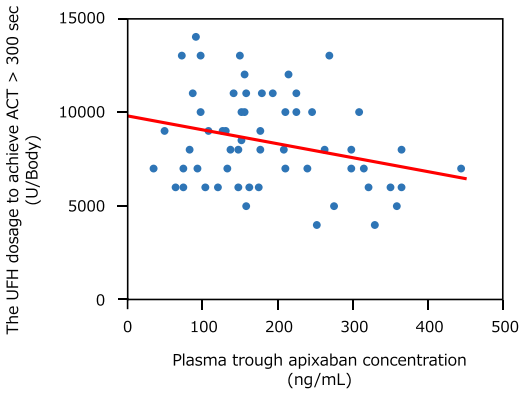

Fig. 2. Correlation between the plasma trough apixaban concentration and the dosage of UFH to achieve ACT > 300 s. y=−7.42x+9842.6 P=0.033, R=-0.281 UFH: unfractionated heparin, CA: catheter ablation, ACT: activated clotting time.

Table 4
Multivariate logistic regression analysis of bleeding risk.

	OR	Lower 95% CI	Upper 95% CI	*P*
Body weight	0.946	0.867	1.018	0.164
Sex (male)	8.560	1.423	67.922	0.027[a]
Trough apixaban concentration	0.992	0.983	0.999	0.049[a]

OR: odds ratio, CI: confidence interval

[a] $P < 0.05$.

concentration and the dosage of UFH required to achieve ACT > 300 s during CA. These results suggested that residual apixaban in the blood on the day of CA had anticoagulation activity, and that a higher trough apixaban concentration could reduce the UFH dosage required to achieve ACT > 300 s during CA. The dosage of UFH may be critical in determining the risk of bleeding, and a higher plasma trough apixaban concentration may diminish this risk by reducing the dosage of UFH required to achieve ACT > 300 s during CA.

Hamam et al. reported that a higher baseline PT-INR during warfarin therapy reduced the dosage of UFH required to achieve an ACT > 300 s, and a linear correlation was observed between baseline PT-INR and ACT [9]. Sandeep et al. reported that the mean ACT during the CA procedure was > 350 s in patients with PT-INR≧2.0; in contrast, the mean ACT in patients with PT-INR < 2.0 remained < 350 s at most time points during the procedure [8]. In addition, the total heparin requirement for the procedure was higher in patients with PT-INR < 2.0 than in those with PT-INR≧2.0 [8]. These findings suggested that residual anticoagulation activity of oral anticoagulants affects UFH dosage during CA.

Comparison of apixaban and warfarin therapy for CA revealed that patients treated with apixaban required a higher dose of UFH, but maintained a lower ACT during CA [10]. This may reflect the difference in half-life of anticoagulation activity between warfarin and apixaban. Antifactor Xa activity, which is responsible for the anticoagulation activity of apixaban, is strongly correlated with plasma apixaban concentration [15]; however, the effect of plasma apixaban concentration on UFH dosage during CA is unknown.

In this study, a significant difference was observed in the incidence of minor bleeding and delayed hemostasis at the puncture site between the group with apixaban concentration < 160 and the group with ≧160 ng/mL. In addition, multivariate logistic regression analysis of bleeding risk indicted that a higher apixaban concentration could reduce the risk of bleeding complications. These findings suggested that an apixaban trough concentration of over 160 ng/mL may be beneficial in preventing bleeding complications during CA. The multivariate logistic regression analysis also shows that male sex was significantly associated with an increased risk of bleeding. Mean UFH dosage of male patients was significantly greater than that of female patients (9580.7 ± 2668.0 U/body vs. 7129.6 ± 1411.4 U/body respectively, $P < 0.01$). Increased UFH dosage in male patients may be associated with the increased risk of bleeding. In addition, there was a difference in mean apixaban trough concentration between male and female patients. (169.0 ± 65.8 ng/mL vs. 212.0 ± 115.8 ng/mL respectively, $P=0.082$). Although the difference was not statistically significant, this marginal decrease in apixaban concentration might increase UFH dosage in male patients, resulting in increased risk of bleeding.

In this study, a significant difference was observed in Ccr between patients with low (< 160 ng/mL) and high (≧160 ng/mL) apixaban concentrations. It has been reported that a lower Ccr increases apixaban exposure based on the area under the concentration-time curve (AUC) [16]. Age and sex alone did not affect the pharmacokinetics and pharmacodynamics of apixaban in clinical use [17]. Furthermore, in patients administered apixaban, a modest change in body weight alone did not influence the AUC of apixaban; however, additional factors such as severe renal impairment that could increase apixaban exposure should be noted [18]. Ccr is one of the factors affecting plasma apixaban concentration. In this study, patients with a lower Ccr had a higher plasma apixaban concentration (≧160 ng/mL) and required a lower UFH dosage.

Although routine coagulation assays such as activated partial thromboplastin time (APTT) and prothrombin time (PT) show apixaban concentration-dependent time prolongation in patients administered apixaban, they are not sufficiently sensitive to accurately estimate the pharmacodynamic effects of apixaban [19,20]. Therefore, APTT and PT are not used to indicate apixaban-related bleeding complications. However, the plasma trough apixaban concentration may be a useful indicator to avoid bleeding complications during CA, because the apixaban concentration is strongly correlated with antifactor Xa activity (the factor responsible for the anticoagulation activity of apixaban) [15,19,21,22]. Although the UFH dosage must be adjusted during CA, the plasma trough apixaban concentration may be a beneficial index for avoiding bleeding complications during CA.

Eight patients were treated with low-dose apixaban (5 mg/day) in our study. Of these, two patients completely met the dose reduction criteria (age ≥ 80 years and body weight ≤ 60 kg), while the remaining six patients did not (five patients < 60 kg, three patients > 75 years old). We focused on the relationship between bleeding complications and plasma apixaban concentrations. In addition, it was expected that apixaban dose would be reflected in the plasma concentration. Therefore, these eight patients were included in the study. Indeed, there was no significant difference in mean concentration of apixaban between the low-dose and normal-dose patients (175.6 ± 100 ng/mL vs. 191.1 ± 93.9 ng/mL respectively, $P=0.669$). The mean age of patients treated with low-dose apixaban was greater than that of patients treated with normal-dose (76.4 ± 5.0 years vs. 62.8 ± 12.3 years respectively, $P < 0.01$). In addition, the patients treated with low-dose apixaban had lower body weight (48.4 ± 8.9 kg, 64.0 ± 11.3 kg, $P < 0.01$) and lower Ccr values (53.5 ± 13.2 mL/min, 82.1 ± 29.6 mL/min, $P < 0.01$) compared to the patients treated with normal-dose apixaban.

Several potential limitations need to be considered when interpreting the results of the present study. First, this was an observational study with a retrospective design and was performed at a single, highly specialized, national center that treats cardiovascular diseases. Therefore, the available sample size in our study was small. It was not appropriate to include all variables in the multivariate logistic regression model of our study. Consequently, only body weight, sex, and the plasma trough apixaban

concentration were included in the model. Second, different diagnostic criteria may affect the incidence of delayed hemostasis at the puncture site. In the present study, delayed hemostasis at the puncture site required interventions including hemostasis and was associated with a prolonged hospitalization period. In addition, other minor bleeding complications were not considered. Furthermore, asymptomatic ischemic stroke, thromboembolism, cardiac tamponade, and brain bleeding were not evaluated because these complications were not identified. Third, laboratory values, including APTT, PT, PT-INR, fibrin degradation products (FDP), and D-dimer, were not determined during the CA or bleeding complications. Finally, for unknown reasons, hematuria and hemosputum were observed only in the group with apixaban concentration $\geqq$160 ng/mL, and not in the group with a concentration $<$ 160 ng/mL. Despite these limitations, the plasma trough apixaban concentration was significantly associated with the risk of bleeding.

This study revealed that low residual plasma apixaban concentration, especially $<$ 160 ng/mL, is associated with an increased risk of bleeding complications during/after CA. Monitoring of apixaban concentration may be helpful in controlling ACT levels by dose adjustment of UFH, resulting in a reduced risk of bleeding complications. However, higher apixaban concentrations may be also associated with bleeding complications. An appropriate range of apixaban concentration during/after CA has not been determined, and therefore further studies are required to yield this information.

5. Conclusions

In this study, patients who developed bleeding complications during/after CA for AF had a lower plasma trough apixaban concentration and required a higher UFH dosage to achieve the target ACT level (ACT $>$ 300 s). These findings suggested that a low concentration of residual plasma apixaban, especially $<$ 160 ng/mL, is associated with an increased risk of bleeding complications during/after CA. Monitoring of apixaban concentration may be helpful in controlling ACT level by dose adjustment of UFH, resulting in a reduced risk of bleeding complications. Further studies are required to elucidate the association between apixaban concentration and the risk of bleeding complications.

Conflict of interest

All authors declare no conflict of interest related to this study.

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