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PHARMACOKINETIC DYNAMIC RELATIONSHIPS

Factor Xa inhibition by rivaroxaban in the trough steady state can significantly reduce thrombin generation

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Keywords nonvalvular atrial fibrillation, plasma concentration, rivaroxaban, tissue factor pathway inhibitor, trough steady state

AIMS

The aim of the present study was to demonstrate evidence of reduced thrombin generation at the trough plasma rivaroxaban concentration.

METHODS

A single-centre, prospective, nonrandomized, drug-intervention, self-controlled study was conducted in 51 anticoagulation therapy-naïve patients with nonvalvular atrial fibrillation. Plasma rivaroxaban concentration was measured by liquid chromatography tandem mass spectrometry (LC–MS/MS) and the anti-factor Xa chromogenic assay. Partial thrombin time (PT), protein C activity, and protein S antigen, prothrombin fragment 1 + 2 (F1 + 2), D-dimer, thrombomodulin (TM), thrombin–antithrombin complex (TAT), plasminogen activator inhibitor-1 (PAI-1) and tissue factor pathway inhibitor (TFPI) levels were also measured at the trough steady state after 4 weeks of rivaroxaban treatment and compared with baseline.

RESULTS

Plasma concentrations obtained by the LC–MS/MS and anti-Xa assays were correlated ($r = 0.841$, $P < 0.001$). The mean concentration of rivaroxaban at the trough steady state was 23.6 ng ml^{-1}, at which F1 + 2, TAT and D-dimer had decreased from the baseline values ($P < 0.0001$, $P = 0.029$ and $P < 0.005$, respectively). PT was prolonged (+0.59 s, $P < 0.0001$). TFPI increased from baseline to the trough steady state in the first to third quartile groups (+0.79 pg ml^{-1}, $P = 0.048$). By contrast, PAI-1, protein C activity, protein S antigen and TM remained within the normal range at the trough steady state.

CONCLUSIONS

Residual plasma rivaroxaban at the trough steady state may explain the antithrombin effect of rivaroxaban in patients with nonvalvular atrial fibrillation.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The preventive effect of once-daily rivaroxaban, which has a short half-life, on stroke and/or systemic embolism is equal or superior to that of warfarin.

WHAT THIS STUDY ADDS

- Residual plasma rivaroxaban at the trough steady state can be traced.
- Factor Xa inhibition by rivaroxaban in the trough steady state can downregulate and reduce thrombin generation.
- Rivaroxaban's antithrombin effect at the trough steady state may play a role in the prevention of thromboembolic events in patients with nonvalvular atrial fibrillation.

Introduction

Once-daily oral administration of **rivaroxaban**, a direct factorXa inhibitor, demonstrates an equal or superior prophylactic effect on cerebral infarction and systemic embolism compared with adjusted-dose warfarin treatment. This was shown in the Rivaroxaban-Once-daily, oral, direct factor Xa inhibition Compared with vitamin K antagonism for prevention of stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF) trial, in which the target international normalized ratio (INR) was 2.0–3.0 and the mean percentage of time in the therapeutic range (TTR) was 55.2% [1, 2]. It was also demonstrated in the J ROCKET AF trial, with adjustment of the standard dosage from 20 mg in the ROCKET AF trial to 15 mg for Japanese patients using pharmacokinetic modelling data [3] and a lower anticoagulation target in elderly people in the Japanese guideline [4], with a target INR of 2.0–3.0 in patients aged <70 years and a TTR of 51.8%; the reduced INR was1.6–2.6 in patients aged ≥70 years and the TTR was 74% [5]. This effect of rivaroxaban this was not found to be associated with increased haemorrhagic events in patients with nonvalvular atrial fibrillation (NVAF) in the ROCKET AF and J ROCKET AF trials. Currently, the direct oral anticoagulants dabigatran, rivaroxaban, apixaban and edoxaban are available for clinical use. Although the half-life of the serum concentration of these drugs is almost 12 h and the serum concentrations display peaks and troughs, the numbers of administrations and dosages are not necessarily consistent [6]. With regard to rivaroxaban, phase II trials in venous thromboembolism [oral direct factor Xa inhibitor BAY59-7939 in patients with acute symptomatic proximal deep vein thrombosis (ODIXa-DVT)] [7] revealed that efficacy did not differ among once-daily (40 mg), twice- (10, 20 and 30 mg) daily and enoxaparin followed by a vitamin K antagonist, and safety was superior with once-daily dosing to twice-daily dosing or to enoxaparin followed by a vitamin K antagonist for 3 months. Similarly, the Einstein-DVT dose-ranging study [8] demonstrated that efficacy and safety did not differ between three different dosing groups (20, 30 or 40 mg once daily) and a low-molecular-weight heparin or vitamin K antagonist group after 3 months of treatment. Thus, the phase III large-scale ROCKET AF worldwide randomized trial was conducted using the standard fixed dose of 20 mg rivaroxaban once daily in patients with NVAF.

Thrombin generation may be almost completely inhibited at the maximum concentration of rivaroxaban in terms of the peak phase. By contrast, the rivaroxaban plasma concentration drops nearly zero at 20–24 h after administration, in terms of the trough steady state [3, 6]. Thus, the mechanism whereby once-daily administration of rivaroxaban is effective at the trough phase remains to be clarified. Yasaka [9] explained this mechanism on the basis of the hypothesis of activation and/or maintenance of physiological anticoagulation factors such as tissue factor pathway inhibitor, antithrombin, protein C, protein S and the fibrinolytic system during trough phase. However, changes in coagulation, anticoagulation and fibrinolysis markers have never been evaluated in the trough steady state after rivaroxaban treatment in anticoagulation therapy-naïve patients with NVAF.

The present study was conducted to test the hypothesis that the residual plasma rivaroxaban at the trough steady state plays a role in reducing thrombin generation, which also involves tissue factor pathway inhibitor (TFPI) at the trough steady state compared with those before treatment in anticoagulation therapy-naïve patients.

Patients and methods

Study design and patient selection

This was a single-centre, prospective, nonrandomized, drug-intervention, self-controlled study aimed at identification of the antithrombin effect of the trough steady state of rivaroxaban treatment in anticoagulation therapy-naïve patients. Newly diagnosed patients with NVAF, aged >20 years and referred to Dokkyo Medical University Hospital to initiate oral anticoagulant treatment with rivaroxaban, were considered eligible for the study. Patients who met any of the following criteria were excluded from the study: less than 6 months after the onset of acute myocardial infarction, unstable angina or arteriosclerosis obliterans; within 6 months after surgery; having acute phase congestive heart failure, as defined by the National Institute for Health and Care Excellence (NICE) guideline [10]; taking medication with dual-antiplatelet therapy; having concomitant chronic kidney disease [creatinine clearance (CCr) (Cockcroft–Gault) <30 ml min^{-1}], having a malignant tumour, rheumatic disease, uncontrollable hypertension or infection; or considered ineligible to participate by the attending physician. All patients provided written informed consent, and baseline

medical history, examination and laboratory tests were performed. Subjects who entered the study received rivaroxaban once daily. All subjects were followed without any changes to the medications during the study period. The study complied with the ethical principles of the Helsinki Declaration and was approved by the Ethics Committee of Dokkyo Medical University (approval number: 26 047; approval date: 9 September 2014). Patients were recruited from 1 June 2015 to 31 May 2016.

Blood sample collection and rivaroxaban dosing

Physicians at our hospital enrolled patients from Monday to Friday. After obtaining informed consent, baseline characteristics and peripheral blood samples were collected between 9:00 AM and 10:00 AM on the enrolment day. Patients were prescribed rivaroxaban from enrollment and took the drug every morning after breakfast between 8:00 AM and 9:00 AM. After at least 4 weeks of treatment, to measure the plasma concentration of rivaroxaban in the trough phase, patients visited our hospital at 8:30 AM before taking the drug in the morning, and peripheral blood samples were collected between 8:30 AM and 9:30 AM. The drug was administered thereafter. Blood samples were divided into a citrate-containing tube and a lithium heparin tube. After centrifugation at 1600 *g* for 10 min, platelet-poor plasma was collected, and it was quickly frozen and stored at −80°C until measurement of the rivaroxaban concentration. Rivaroxaban dosing was determined by CCr (Cockcroft–Gault formula). The rivaroxaban dose was 15 mg once daily when CCr was more than 50 ml min^{-1}, and 10 mg once daily when CCr was 30–49 ml min^{-1}.

Blood sample assay

Haemostasis, coagulation and physiological anticoagulation markers assay. Partial thrombin time (PT) (normal range: 9.4–12.5 s) was measured using the coagulation turbidity method by HemosIL RecombiPlasTin 2G (Instrumentation Laboratory, Bedford, MA, USA). Protein C activity (normal range: 64–135%) and protein S antigen levels (normal range: 60–127%) were also determined by the synthetic chromogenic assay and antigenic immunoassay, respectively, using the ACL TOP analyser (Instrumentation Laboratory, Bedford, MA, USA). Prothrombin fragment 1 + 2 (F1 + 2) (normal range: 69–229 pmol l^{-1}) was measured by the enzyme-linked immunosorbent assay (ELISA), using the BEP® III analyser (Siemens Healthcare, Erlangen, Germany). D-dimer (normal range: <0.72 μg ml^{-1}) was measured by enzyme immunoassay using the LPIA ACE DD dimer II on the LPIA-NV7 analyser (LSI Medience Corporation, Tokyo, Japan). TM (normal range: 8.7–22.7 U ml^{-1}) and thrombin–antithrombin complex (TAT) (normal range: <3.0 ng ml^{-1}) were determined by chemiluminescent enzyme immunoassay using STACIA CLEIA TM and STACIA CLEIA TAT, respectively (LSI Medience Corporation). Plasminogen activator inhibitor-1 (PAI-1) (normal range: <50 ng ml^{-1}) was also measured by the chemiluminescent latex agglutination assay using the LPAI tPAI test on the STACIA analyser (LSI Medience Corporation). TFPI levels (normal range: 7.5–41.2 ng ml^{-1}) were measured by the quantitative sandwich ELISA using monoclonal antibody specific for human TFPI (Quantikine® ELISA, Human TAPI Immunoassay, R&D Systems, Inc., Minneapolis, MN, USA). Patients were also divided into quartile groups based on the pretreatment TFPI levels, and the differences (trough TFPI − baseline TFPI) in each group were analysed.

Liquid chromatography tandem mass spectrometry (LC–MS/MS) assay. Plasma concentrations of rivaroxaban were measured by Swiss BioQuant AG (Reinach, Switzerland) using LC–MS/MS validated in accordance with the US Food and Drug Administration guidance for Industry, Bioanalytical Method Validation [11].

Anti-factor Xa chromogenic assay. The anti-factor Xa chromogenic assay for the measurement of rivaroxaban plasma concentrations using rivaroxaban calibrators and controls (STA®-Liquid Anti-Xa with STA®-Rivaroxaban Calibrator and Control, Diagnostica Stago, Asnières, France) calibrated rivaroxaban plasma concentrations, expressed in ng ml^{-1}, and this working range was from 20 ng ml^{-1} to 500 ng ml^{-1} [12].

Clinical events

The observation period for the incidence of clinical events was 3 months after administration of rivaroxaban. Stroke, systemic embolism and all bleeding events were included in the clinical events.

Stroke and systemic embolism definitions

Stroke was defined as an abrupt episode of a focal neurological deficit generally in the distribution area of a single brain artery, lasting at least 24 h. Systemic embolism was defined as an abrupt episode of arterial insufficiency associated with clinical or radiological evidence of arterial occlusion. In the presence of atherosclerotic peripheral arterial disease, the diagnosis of embolism required angiographic demonstration of abrupt occlusion.

Definitions of bleeding

Major bleeding was defined according to the International Society on Thrombosis and Haemostasis criteria [13]. Nonmajor bleeding was defined as acute or subacute clinically overt bleeding that did not satisfy the criteria for major bleeding and led to hospital admission for bleeding. When nonmajor or minor bleeding was observed, patients were instructed to visit the hospital the following morning without taking rivaroxaban.

Statistical analysis

The results are presented as mean ± standard deviation or median [the lowest (Q1) – third (Q3) quartiles] for continuous data, and numbers and percentages for categorical data. The data are represented visually by box plots to identify outliers and compare distributions. The primary endpoint was changes in coagulation, anticoagulation and fibrinolysis markers at the trough steady state after 4 weeks of rivaroxaban treatment. Comparison of two groups was tested by the paired *t*-test or Wilcoxon signed-rank test on the basis of the data distribution with and without normality. Factors that reached significant differences and plasma concentrations were analysed by dosage. The correlation coefficient

was calculated for paired data and Bland–Altman analysis was performed to determine the agreement between the measurements. Statistical significance was considered at a level of $P < 0.05$. All statistical calculations were performed using JMP software 10.0 (SAS Institute, Cary, NC, USA).

Nomenclature of targets and ligands

Key ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [14].

Results

A total of 51 patients were enrolled in the study. The clinical characteristics are shown in Table 1. Mean age, weight, $CHADS_2$, CHA_2DS_2-VASc scores and CCr were 67.4 ± 11.0 years, 63.7 ± 12.7 kg, 1.33 ± 1.05, 2.43 ± 1.55 and 63.2 ± 18.9 ml min^{-1}, respectively, and 38 patients (75%) were male. Forty-one patients (80%) received rivaroxaban at a dose of 15 mg. According to the dose reduction criteria of rivaroxaban (CCr < 50 ml min^{-1}), ten patients received rivaroxaban at a dose of 10 mg (20%). When compared by dosage, patients who received rivaroxaban at a dose of 10 mg were significantly older, and had higher CHADS2 and CHA2DS2-VASc scores and lower weight and CCr than those who received 15 mg (Table 1).

Haemostasis and coagulation markers

PT was slightly but significantly prolonged at the trough steady state of rivaroxaban compared with that before treatment [+0.59 s, $P < 0.0001$ (10 mg: +1.07 s, $P = 0.0133$; 15 mg: +0.48 s, $P < 0.0004$); Figure 1A]. F1 + 2 significantly decreased and returned to the normal range at the trough steady state of rivaroxaban compared with that before treatment [−94.2 pg ml^{-1}, $P < 0.0001$ (10 mg: −57.7 pg ml^{-1}, $P = 0.0323$; 15 mg: −103.3 pg ml^{-1}, $P < 0.0001$); Figure 1B]. TAT returned to the normal level at the trough steady state of rivaroxaban compared with that before treatment [−4.7 ng ml^{-1}, $P = 0.0285$ (10 mg: −1.9, $P = 0.0133$; 15 mg: −5.4, $P = 0.0398$); Figure 1C]. D-dimer also declined to the normal level at the trough steady state of rivaroxaban compared with that before treatment [−0.9 μg ml^{-1}, $P < 0.0050$ (10 mg: −0.7 μg ml^{-1}, $P = 0.0505$; 15 mg: −0.9 μg ml^{-1}, $P = 0.0131$); Figure 1D]. The PAI-1 level did not change (Table 2). Thus, there were no significant differences in these values between the two doses.

Physiological anticoagulation markers

Protein C activity, protein S antigen, TM and TFPI levels did not change, but maintained a normal range at the trough steady state of rivaroxaban compared with the values before treatment (Table 2). However, TFPI levels were significantly increased at the trough steady state when the baseline TFPI was at the level of Q1 to Q3 (mean difference = +0.79 pg ml^{-1}, $P = 0.048$), and TFPI levels were decreased in the upper quartile (Q4) (mean difference = −6.62 pg ml^{-1}, $P = 0.032$) when patients were divided in quartile groups on the basis of the baseline TFPI levels, as shown in Figure 2.

Plasma rivaroxaban concentrations measured by the LC–MS/MS and anti-factor Xa chromogenic assays at the trough steady state

The mean plasma rivaroxaban concentration measured by the LC–MS/MS assay was 23.6 ± 15.4 ng ml^{-1} [range: 3.2–74.0 ng ml^{-1} (Q1: 3.2–12.3; Q2: 12.3–20.8; Q3: 20.8–31.8; Q4: 31.8–74.0); Figure 3]. The plasma concentrations measured by the LC–MS/MS assay did not differ between

Table 1

Baseline clinical characteristics of patients

	Overall ($n = 51$)	15 mg OD ($n = 41$)	10 mg OD ($n = 10$)	P value 15 mg *vs.* 10 mg
Male/female	38/13	33/8	5/5	0.100
Age, years	67.4 ± 11.0	65.2 ± 10.1	76.2 ± 10.3	0.004
Congestive heart failure	18	13	5	0.474
Hypertension	21	15	6	0.302
Diabetes mellitus	7	7	0	0.184
Stroke	3	2	1	0.496
$CHADS_2$ score	1.33 ± 1.05	1.20 ± 1.03	1.90 ± 0.99	0.036
CHA_2DS_2-VASc score	2.43 ± 1.55	2.19 ± 1.45	3.40 ± 1.65	0.030
Weight	63.7 ± 12.7	65.8 ± 12.0	55.0 ± 12.1	0.026
CCr (Cockcroft–Gault; ml min^{-1})	63.2 ± 18.9	67.3 ± 17.2	44.4 ± 16.6	0.001
30–49 ml min^{-1}	10	0	10	-
Child–Pugh grade B or C	0	0	0	-

CCr, creatinine clearance; $CHADS_2$, congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, stroke [double weight]; CHA_2DS_2-VASc, congestive heart failure, hypertension, age ≥75 years [double weight], diabetes mellitus, stroke [double weight], vascular disease, 74 > age ≥ 65 years, sex category (female); OD, once daily treatment

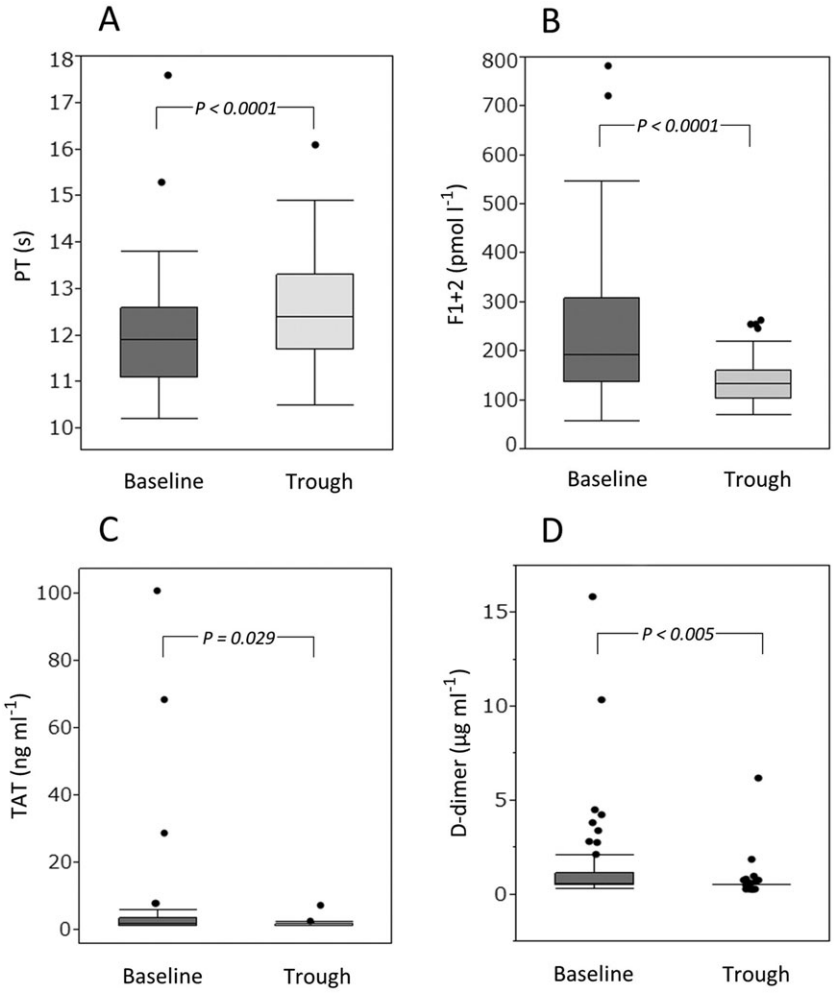

Figure 1

(A) Partial thrombin time at baseline and the trough steady state after rivaroxaban treatment. Box plots are shown; the bottom and top of the box are the first and third quartiles, and the band inside the box is the second quartile (the median). PT, prothrombin time; trough = trough steady state at 4 weeks of rivaroxaban treatment. (B) Prothrombin fragment 1 + 2 at baseline and the trough steady state after rivaroxaban treatment. F1 + 2, prothrombin fragment 1 + 2; trough = trough steady state at 4 weeks of rivaroxaban treatment. (C) Thrombin–antithrombin complex at baseline and the trough steady state after rivaroxaban treatment. TAT, thrombin–antithrombin complex; trough = trough steady state at 4 weeks of rivaroxaban treatment. (D) D-dimer at baseline and the trough steady state on rivaroxaban treatment. Trough = trough steady state at 4 weeks of rivaroxaban treatment

Table 2

Feedback inhibition markers

	Baseline	Trough	*P* value
Feedback inhibition markers			
PAI-1 (ng ml^{-1})	23.50 ± 18.96	24.40 ± 17.74	0.802
Protein C (%)	103.2 ± 19.61	103.8 ± 18.88	0.533
Protein S (%)	92.48 ± 18.67	92.44 ± 18.07	0.805
TM (FU ml^{-1})	15.33 ± 4.53	15.90 ± 3.97	0.192
TFPI (pg ml^{-1})	27.25 ± 9.45	26.77 ± 8.37	0.684

Values are presented as mean ± standard deviation.
PAI-1, plasminogen activator inhibitor-1; TM, thrombomodulin; TFPI, tissue factor pathway inhibitor

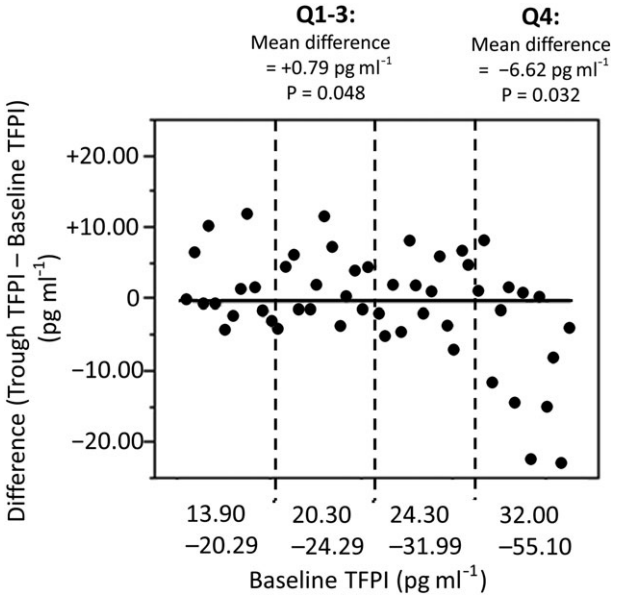

Figure 2

Differences in tissue factor pathway inhibitor (TFPI) values between baseline and the trough steady state on rivaroxaban treatment (Altman plot analysis) in quartiles based on the baseline TFPI value. Patients were divided into quartiles (from Q1 to Q4) on the basis of the baseline TFPI levels, and the differences (trough TFPI − baseline TFPI) in all groups were analysed. Q1: baseline concentration of TFPI was 13.90 pg ml^{-1} to 20.29 pg ml^{-1}. Q2: Baseline concentration of TFPI was 20.30 pg ml^{-1} to 24.29 pg ml^{-1}. Q3: Baseline concentration of TFPI was 24.30 pg ml^{-1} to 31.99 pg ml^{-1}. Q4: baseline concentration of TFPI was 32.00 pg ml^{-1} to 55.10 pg ml^{-1}. Q1–Q3: within the range from Q1 to Q3

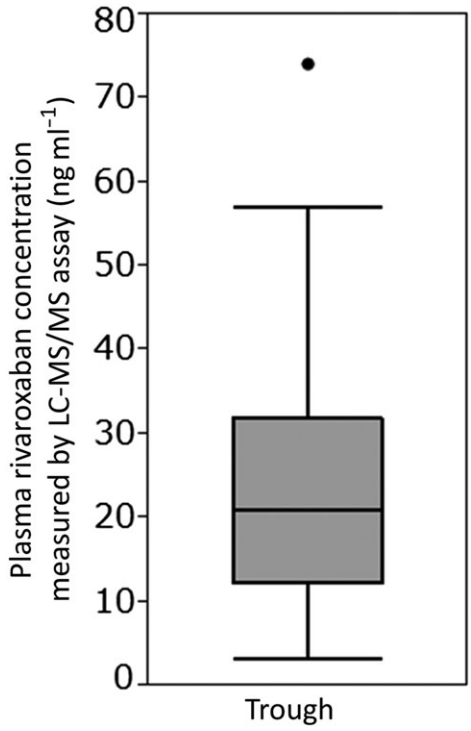

Figure 3

Plasma rivaroxaban concentration measured by the liquid chromatography tandem mass spectrometry (LC–MS/MS) assay at the trough steady state after rivaroxaban treatment. Box plots are shown; the bottom and top of the box are the first and third quartiles, and the band inside the box is the second quartile (the median). Trough = trough steady state at 4 weeks of rivaroxaban treatment

the 10 mg (27.6 ± 20.2 ng ml^{-1}; range: 3.2–74.0 ng ml^{-1}) and 15 mg (22.0 ± 13.7 ng ml^{-1}; range: 4.2–56.9 ng ml^{-1}) dosages. By contrast, the plasma rivaroxaban concentration measured by the anti-Xa assay was 28.9 ± 14.3 ng ml^{-1} (range: 7.8–87.0 ng ml^{-1}). The plasma concentrations measured by the anti-Xa assay also did not differ between the 10 mg (35.3 ± 23.9 ng ml^{-1}; range: 11.4–87.0 ng ml^{-1}) and 15 mg (27.2 ± 10.9 ng ml^{-1}; range: 7.8–57.2 ng ml^{-1}) dosages. However, a significant correlation was observed between plasma concentrations measured by the LC–MS/MS and anti-Xa assays (r = 0.854, $P < 0.0001$), and the Bland–Altman analysis revealed that the plasma concentrations measured by the LC–MS/MS assay were significantly lower than those measured by the anti-Xa assay (differences: −5.67 ± 7.73 ng ml^{-1}; $P < 0.0001$; Figure 4). Thus, the calibrated rivaroxaban plasma concentrations measured by the anti-factor Xa chromogenic assay were slightly overestimated compared with those measured by the LC–MS/MS assay at relatively low concentrations at the trough steady state.

PT and plasma rivaroxaban concentration measured by the LC–MS/MS assay

Although PT weakly correlated with plasma concentration measured by the LC–MS/MS assay (r = 0.448), it showed high degrees of individual variability. Thus, a normal value of PT cannot be used to exclude the existence of plasma

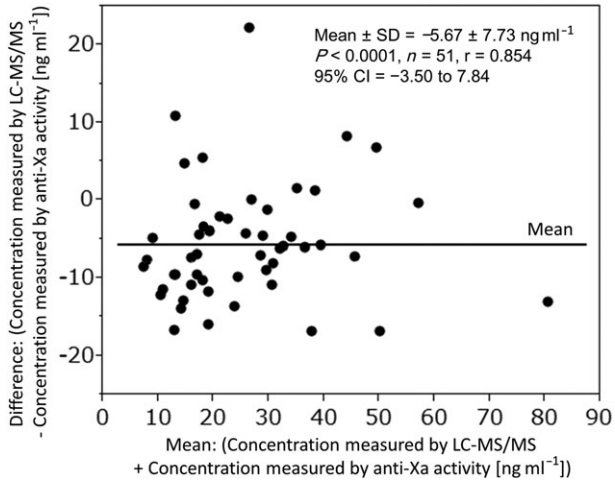

Figure 4

Bland–Altman comparison of the concentrations measured by the liquid chromatography tandem mass spectrometry (LC–MS/MS) assay and anti-Xa activity at the trough steady state after rivaroxaban treatment. SD, standard deviation, CI, confidential interval

rivaroxaban, especially in the trough phase, and PT can be used only to obtain a crude estimate.

Incidence of events

No events of stroke, systemic embolism or major bleeding were observed in any patients but a few nonmajor haemorrhagic events were observed. One patient ($CHADS_2$ score of 2, CHA_2DS_2-VASc score of 4) who received rivaroxaban 15 mg and aspirin 100 mg developed haematuria after 4 weeks of treatment, and the trough steady-state plasma concentration of rivaroxaban measured by the LC–MS/MS assay was 74.0 ng ml^{-1}. Another patient ($CHADS_2$ score of 1, CHA_2DS_2-VASc score of 3) who received rivaroxaban 10 mg and had a Child–Pugh score A for liver cirrhosis associated with a reduction in the number of platelets developed progressive subcutaneous haemorrhage after 4 weeks of treatment, and the trough plasma concentration was 16.4 ng ml^{-1}. Another patient ($CHADS_2$ score of 1, CHA_2DS_2-VASc score of 2) who received rivaroxaban 15 mg developed haemosputum after 4 weeks of treatment, and the trough plasma concentration was 41.7 ng ml^{-1}.

Discussion

The present study demonstrated that plasma rivaroxaban could be detected at the trough steady state by both the LC–MS/MS and the anti-factor Xa chromogenic assay. Although the residual plasma concentrations were distributed over a wide range, residual rivaroxaban could downregulate and almost completely reduce the process of thrombin generation, which was demonstrated by normalization of F1 + 2, TAT and D-dimer without over-suppression of the feedback inhibition system such as the protein C–TM–thrombin-activated protein C system. TFPI was also upregulated at the trough steady state when the baseline TFPI was included in Q1–Q3, and was downregulated in Q4 if patients were divided into quartiles on the basis of the pretreatment TFPI levels. The overall results showed that PT was slightly yet significantly prolonged in our study.

In the present study, thrombin generation in response to rivaroxaban administration was assessed indirectly by measuring F1 + 2, an indicator of prothrombin activation [15]. TAT is also formed when thrombin cleaves antithrombin to bind with it [16], and a high value of TAT means excessive thrombin generation. D-dimer, which is a very stable coagulation marker, reflects the amount of thrombin formation and endogenous turnover of fibrin as well as activation of fibrinolysis. Anticoagulant therapy significantly suppresses the D-dimer level, which is an independent marker that can predict stroke or systemic embolism, cardiovascular mortality and bleeding [13]. Thus, the present study suggested that rivaroxaban effectively attenuates activation of the haemostatic system, even at the trough steady state, compared with the natural state in patients with NVAF who have never used anticoagulant therapy before.

The mechanism whereby rivaroxaban may play a role to prevent thrombotic events at the trough steady state is under speculation.

Firstly, Perzborn *et al*. [17] reported that the plasma concentration of rivaroxaban is below the half maximal inhibitory concentration (IC_{50}) of 21 nM (9.15 ng ml^{-1}) of endogenous human plasma factor Xa activity in an *in vitro* study. Thus, the residual plasma rivaroxaban may exert a persistent anticoagulation action because the mean plasma concentration (23.6 ng ml^{-1}) was comparable with the IC_{50} of factor Xa activity in our study. Gerotziafas *et al*. [18] also reported inhibition of thrombin generation by rivaroxaban with an IC_{50} of 2.1 nM (0.92 ng ml^{-1}) using a prothrombin assay of the reconstituted prothrombinase complex on platelets. Rivaroxaban also prolonged the initial phase of thrombin generation (represented by the lag time) after activation of the tissue factor pathway. Levels of approximately 20 nM (8.7 ng ml^{-1}) and 10 nM (4.4 ng ml^{-1}) of rivaroxaban induced a twofold increase in the lag time of F1 + 2 formation in whole blood, and thrombin generation was also observed in platelet-rich plasma. During the propagation phase of thrombin generation, the IC_{50} values for the rate of F1 + 2 formation in whole blood and thrombin generation in platelet-rich plasma were 60 nM (26.1 ng ml^{-1}) and 10 nM (4.4 ng ml^{-1}), respectively. In fact, the minimum plasma concentration of rivaroxaban at the trough steady state was 7.3 nM (3.2 ng ml^{-1}) in our study. Thus, even at this minimum concentration, rivaroxaban may inhibit thrombin generation and delay the initial and/or propagation phase. Graff *et al*. [19] also reported that factor Xa activity and endogenous thrombin potential triggered by collagen or tissue factor return to the baseline, but prothrombinase-induced clotting time remained prolonged at 24 h after a single rivaroxaban dose of 5 mg in healthy subjects. These small amounts of rivaroxaban, which had an almost undetectable effect on factor Xa activity, could inhibit prothrombinase-induced thrombin generation via factor Xa. The authors also speculated that active drug remained bound to platelets and maintained the inhibition of thrombin generation at 24 h after once-daily treatment of rivaroxaban at 5 mg, which caused an almost undetectable plasma concentration of rivaroxaban. However, this mechanism seems doubtful because a small amount of rivaroxaban in the plasma could be traced in our study after 24 h of repeated administration of 10 mg or 15 mg of rivaroxaban for 4 weeks in patients with NVAF.

Secondly, the anticoagulation effect of rivaroxaban at the trough steady state may be implemented by maintained physiological anticoagulation factors such as appropriate levels of protein C activity, protein S antigen and TM. Vitamin K is necessary for synthesizing protein C and protein S, which both include a Gla domain. The vitamin K antagonist warfarin prevents the creation of the Gla domain by inhibiting the gamma-carboxylation of glutamic acid. Thus, a vitamin K antagonist causes a decline in protein C activity and protein S levels [20, 21]. By contrast, rivaroxaban did not affect the protein C activity or protein S level. This finding is in agreement with that of a previously published study [22]. TM binds to the excessively produced thrombin, altering its substrate affinity and activating protein C. This pathway is one of the important feedback inhibition mechanisms of the blood coagulation cascade. However, this protein C-TM-thrombin-activated protein C system may not continue to prevent the antithrombotic effects of

rivaroxaban as, in the present study, this system did not change from before to after rivaroxaban treatment.

Thirdly, TFPI is also an anticoagulation protein and a dual inhibitor, binding to the tissue factor/factor VIIa complex to prevent it from acting on the factor IX and factor X substrates, and inhibiting factor Xa directly. Therefore, factor Xa exerts negative feedback on its own production. TFPI is the principal regulator of the initial phase of thrombin generation [23]. Heparin and low-molecular-weight heparin derivatives release TFPI from the vascular endothelium, which may contribute primarily to the antithrombotic effect [24]. Approximately 80–85% of the total body TFPI is associated with the endothelial cell surface and 15–20% is circulating in the plasma, of which 80% is bound to lipoproteins and the rest is in the free form. The free TFPI in the plasma is the physiologically active anticoagulant part of TFPI [25]. TFPI may be ineffective at tissue factor concentrations below the threshold, which is comparable with Q1–Q3 in our study, but if the tissue factor concentration exceeds the threshold that is comparable with Q4, the production of TFPI may be upregulated to be almost equivalent to thrombin generation at the baseline condition [23, 26, 27]. The R&D System ELISA kit detects both full-length and truncated isoforms, and the normal range is from 7.5 ng ml^{-1} to 41.2 ng ml^{-1}, with a mean of 22.6 ng ml^{-1} [28, 29]. This value almost corresponds to the range of Q1–Q3. Thus, TFPI may be upregulated after rivaroxaban treatment if the baseline value is in the normal range. This effect also may contribute to the antithrombotic effect of rivaroxaban at the trough steady state.

In the present study, minor haemorrhagic events were observed in a few patients with a relatively high plasma concentration of rivaroxaban and prolongation of PT compared with the mean of all patients in the trough steady state, while patients with an underlying disease prone to haemorrhage were excluded, similarly to previously reports of edoxaban [30]. However, a much larger sample size would be beneficial to confirm this association in a future study.

Limitations

Although the present study demonstrated evidence of reduced thrombin generation even at the trough steady state of rivaroxaban, it is not fully understood whether or not this reduced thrombin generation is associated with the prevention of cerebral infarction and systemic embolism. The study did not evaluate the peak concentration of rivaroxaban while this was influencing anticoagulation markers in the trough steady state. However, it was difficult to measure the actual peak concentration as this had a greater individual variability than that of the trough concentration. Although antithrombin was not measured, the antithrombin activity assay based on thrombin inhibition has previously been shown not to be affected by rivaroxaban [31]. To date, it has not been possible to evaluate the pharmacodynamics of rivaroxaban treatment. Other limitations were the small number of patients and short observational period for analysis of clinical events, and the present lack of observation of stroke or embolic events.

Conclusions

In conclusion, residual plasma rivaroxaban significantly reduced thrombin generation at the trough steady state, which may play a role in the prevention of thromboembolic events in patients with NVAF.

Competing Interests

This study has received research grants from Bayer Yakuhin, Ltd. S.H. has received honoraria for lectures from Bayer Yakuhin, Ltd. There are no other potential conflicts of interest relevant to this article.

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